

**PHARMACOGNOSTICAL, PHYTOCHEMICAL
PHARMACOLOGICAL EVALUATION OF JUSTICIA GLAUCA**

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26108664**

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MADURAI MEDICAL COLLEGE
MADURAI – 625 020**

CHAPTER - 1

INTRODUCTION ^[1-4]

Herbal medicine referred to as herbalism or botanical medicine is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic qualities.

Plants produce and contain a variety of chemical substances that act upon the body. A herbolist uses the leaves, flowers, stems, berries and root of plants to prevent, relieve and treat illness. From a scientific perspective many herbal treatments are considered experimental. The reality is however, that herbal medicine has a long and respected history. Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today science has isolated the medicinal properties of a large number of botanicals and their healing components have been extracted and analysed. Many plant components are now synthesized in large laboratories for used in pharmaceutical preparations.

World health organization (WHO) has estimated that 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary healthcare. Herbal medicine is a major component in all indigenous peoples traditional medicine and a common element in Ayurvedic, Homeopathic, Naturopathic, traditional oriental and native American Indian medicine.

WHO notes that of 119 plants derived pharmaceutical medicines i.e. about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forest and other places for their potential medicinal value.

Rather than using a whole plant, a pharmacologist identifies, isolates, extracts and synthesizes individual components thus capturing the active properties. This can create problems because in addition to active ingredients, plants also contain minerals, vitamins, volatile oil, glycosides, alkaloids, bioflavonoids and other substances that are important in supporting particular herbs medicinal properties.

Doctors and government agencies want to see scientific studies before recognizing the effectiveness of plant as medicine. Yet even though substantial research is being done in other countries, drug companies and laboratories in the United States so far have not chosen to put much money or resources into botanical research. The result is that herbal medicines do not have the same place of importance or level of acceptance in US as it does in other countries.

HEART FAILURE

Interruption of blood supply to a part of the heart causing heart cells to die. This is commonly due to occlusion of coronary artery following the rupture of a vulnerable atherosclerotic plaque, which is an unstable collection of lipids and white blood cells in the wall of an artery. The resulting ischemia and ensuing oxygen shortage, if left untreated for a sufficient period of time can cause damage or death of heart muscle tissue. Classical symptoms of acute myocardial infarction are sudden chest pain, shortness of breath, nausea, vomiting, palpitation, sweating, anxiety.

Etiology

A). Depressed ejection fraction:

1. Coronary artery disease – myocardial infarction, myocardial ischemia.
2. Chronic pressure overload – hypertension, obstructive valvular disease.
3. Chronic volume overload – regurgitant valvular disease, intracardiac shunting.

4. Non ischemic dilated cardiomyopathy – familial genetic disorder, infiltrative disorder toxic or drug induced damage, metabolic disorder, viral, chronic disease.
4. Disorder of rate and rhythm – chronic bradyarrhythmia chronic tachyarrhythmia.
- B). Preserved hypertrophy – infiltrative disorders (Amyloidosis, sarcoidosis) storage disease(haemochromatosis). Paste from padma book

CARDIOTOXICITY

Cardiotoxicity is a condition in which the damage of heart muscle occur and the heart may not be able to pump the blood throughout the body. Severe cardiotoxicity leads to cardiomyopathy

CARDIOTOXIC AGENTS

A variety of pharmacologic agents may damage the myocardium acutely, producing a pattern of inflammation(myocarditis) or may even lead to chronic damage as seen in dilated cardiomyopathy. Certain drugs may produce ECG abnormalities alone while others may precipitate CHF and death. Some of the cardiotoxic agents includes doxorubicin, daunorubicin, cyclophosphamide, phenothiazine, chloroquine, cocaine, tratuzumab etc.

Doxorubicin is one of the most active cytotoxic agents in current use. It has proven efficacy in various malignancies either alone or combined with other cytotoxic agents.

It produces severe cardiomyopathy and heart failure in doxorubicin treated cancer patients hence its use is being limited. It causes lipid peroxidation of the myofibre membranes resulting in myofibre swelling, vacuolization, fatty changes, cytolysis, systolic dysfunction and ventricular tachyarrhythmic occurs in a dose dependent manner.

CARDIOPROTECTIVE HERBS

The cardioprotective herbs have been extensively investigated chemically and evaluated clinically for its beneficial cardiovascular properties. The cardio protective herbs support the heart due to the high content of bioflavonoid. It increases the body's ability to utilize oxygen, and the heart's ability to utilize calcium.

Most of the cardio tonic herbs produced bradycardia, significant decrease of arterial blood pressure both in normotensive and hypotensive rats and antiarrhythmic activity in all experimental models of arrhythmia. The cardio tonic herbs also show strong negative chronotropic, positive inotropic and coronary dilating effects.

Several herbs offer potential for cardiovascular conditions including venous insufficiency, intermittent claudicating, hyperlipidemia, hypertension and congestive heart failure. *In-vitro* evidence suggest garlic reduces blood pressure by inhibiting platelet NO synthase. The mechanism of *Gingko biloba* improving vascular health include free radical scavenging, antiplatelet actions, anti-inflammatory actions, vasodilations and decrease blood viscosity.

Howthorn is a spiny shrub and contains flavonoids and oligomeric procyanthins which are used for antioxidants, inotropic, vasodilatory and antihyperlipidemic actions and as well as decrease capillary permeability. Positive inotropic action may also be due to inhibition of myocardial Na^+/K^+ ATPase which is an integral membrane enzyme that maintain cardiac resting potential. It is also decreases blood pressure which results in an increase in exercise tolerance during the early stage of congestive heart failure.

Bioflavonoids of herbs used for dilating both peripheral and coronary blood vessels leading to its use in angina. Procyanidins contents is claimed to support the

vasorelaxant effects. Glycoside component of Hawthorn's has also been reported to increase vagal tone of the heart. *Ma Huang (Ephedra sinica)* is a natural source of ephedrine and has potent sympathomimetic activity. *Dang-Gui Buxue tang* enhance myocardial mitochondrial as well as red blood cell glutathione status, increasing resistance to oxidative stress. *Terminalia arjuna* is a reputed heart friendly herb that has been in use for over 2500 B.C. It is useful in hypertension, angina, heart attacks, hypercholesterolemia and hyperlipidemia.

There are lots of potential of using plants as cardioprotective agents against toxic substances, and anticancer agents etc. Hence an attempt is made to study whether the little known herb *Justicia glauca* will be useful for treating the above diseases.

CHAPTER - 2

REVIEW OF LITERATURE

PHARMACOGNOSY

Sanchez E *et al.*, (1985) have studied the pharmacognostical parameters in several medicinal plants namely *Artemisia abortamun*, *Calend officinalis*, *Cymbopogan citratus*, *Justicia pectoralis*, *Ocimum basilica*, *Rhoeo spathaceae* and *Ruta graveolens*.^[5]

Anuradha U *et al.*, (1988) have studied six species namely *Glossocardia bosvallea*, *Justicia procumbens*, *Haplanthus verticillaris*, *Oldenlandia corymbosa*, *Peristrophebicaly cultata* and *Rrungia repenes* used as 'pittapapada' by local people of pune and neighboring districts.^[6]

La serna B *et al.*, (1989) have reported water content (81%), total nitrogen (2.5%) and chemical constituents of the different extracts of the plant. Decoction of the plant is widely used as sedative in folk medicine.^[7]

PHYTOCHEMISTRY

Ghosal S *et al.*, (1979) have isolated three known lignans sesamin, asarinin and sesamol and new lignan simplexolin. Screening tests of the lignans conducted on laboratory animals indicated significant CNS activity.^[8]

Olaniyi A *et al.*, (1980) have isolated three lignans namely helioxanthin, justicinol and (+)-isolariciresinol from leaves of *Justicia flava*.^[9]

Ghosal S *et al.*, (1981) have isolated a new triterpenoid saponin, justiciasaponin-I from the petroleum ether extract of the whole plant and identified as oleanolic acid -3-o- β -D-glucopyranosyl-4-o-ferulate.^[10]

Rao B et al., (1984) have isolated several compounds belonging to the class of steroids, terpenes, coumarins, glucosides, alkaloids and flavonoids from a number of Indian medicinal plants *Ipomoea digitata*, *Ipomoea kentrocaulos*, *Artabotrys odoratissimus*, *Michelia champaca*, *Justicia glauca*, *Premna integrifolia*, *Premna foetida*, *Euphorbia splendens*.^[11]

Joseph H et al., (1988) have isolated justicidin B, a 1-aryl-2,3-naphthalide lignan from ethanolic extract of whole plants of *Justicia pectoralis*.^[12]

Trujillo J M et al., (1990) have isolated two new lignans namely J1, J2 from leaves of *Justicia hyssopifolia*. The latter as a β -D-glucoside together with the known lignans helixanthin, justicidin E, gadain, cubebin and sesamin.^[13]

Chen CC et al., (1996) have isolated nine known aryl-naphthalide lignans, Neolignans A (1), justicidin B (2), justicidin A (3), taiwanin E methyl ether (4), neojusticidin B (5), chinensinaphthol methyl ether (6), Taiwan E (8), chinensinaphthol (9), and diphyllin (10), and a new aryl-naphthalide lignans.^[14]

Chen C C et al., (1998) have reported the isolation of diarylbutane lignans from *Justicia procumbens* namely Justicidin A, B, C. The structures were established by spectral analysis.^[15]

Rajasekar D et al., (2000) have isolated prostaticidin D, a new aryl-naphthalide lignan from *Justicia diffusa* var. *prostata*. Prostaticidin D (1) and seven were isolated from *Justicia diffusa* var. *prostata* and were identified by direct comparison with authentic samples.^[16]

Perez JA et al., (2004) have described the isolation of a novel p-quinone-lignan derivatives from *Justicia hyssopifolia*. An unknown, previously unreported p-quinone

lignan compound called justicidone along with known savinin from *Justicia hyssopifolia* (Acanthaceae).^[17]

Srinivastava S K et al., (2007) have reported the isolation of β -sitosterol from petroleum ether extract and choline from the ethanolic extract of *Justicia aurea* (Acanthaceae)^[18]

PHARMACOLOGY

Mills J et al., (1986) have reported aqueous and organic extracts of *Justicia pectoralis* influenced the wound healing process. Coumarin isolated from the little plant showed attenuated inflammatory process and significantly enhanced wound healing in the experimental rats.^[19]

Mruthyunjayaswamy BHM et al., (1998) have screened the alcoholic extract of *Justicia procumbens* for *in-vitro* anti-inflammatory activity in albino rats at a dose of 100mg/kg body weight. The activity has been attributed to the presence of steroids and flavonoids in the extract^[20]

Day SH et al., (1999) have done the cytotoxic activity against several cell lines. Justicidin A showed potent cytotoxic effect against T-24, caski, sitta, HT-3, PLC/PRF/5 and cells *in-vitro*. Two new naturally occurring 1-aryl-2,3 aphthalide lignans, cilinaphthalide A (C₂₂H₂₀O₇, m.p 221-2°C) and cilinaphthalide B (C₂₃H₂₂O₇, m.p. 202.5-203.5°C) and nine compounds were isolated from the whole plants of *Justicia ciliata*^[21].

Day SH et al., (2000) have evaluated the anti-inflammatory effect of (two new lignan glycosides 4-O- $\{\alpha$ -arabinosyl-1) (1-2)- β -D-xylopyranosyl-(1-5)- β -D-

apiofuranosyl) diphyllin(1) named ciliatoside A (1), 4-O-(β -D-apiofuranosyl-(1-3)- α -L-arabinopyranosyl (1-2) β -D-xylo pyranosyl (1-5)- β -D-apiofuranosyl) diphyllin (2) named ciliatoside B (2) isolated from *Justicia ciliate*^[22]

Mohaghezadesh A et al., (2002) have reported aryl-naphthalene lignans from *in-vitro* cultures of *Linum austrianum*. Lignans and their derivatives find application in cancer chemotherapy. Callus suspension and normal and hairy root cultures of *Linum austrianum* produced a new aryl-naphthalene dioxy-2, 7-cycloligna-7, 7'-dieno-9,9'-lactone together with Justiciadin B^[23]

Day SH et al., (2002) have studied the potent cytotoxic effects against number of cancer cells *in-vitro* by using new lignan glycoside. 4-O- α -L-arabinosyl-(1-2)- β -D-apiofuranosyl-diphyllin (2) named procumbenoside A and 11 known compounds were isolated from methanolic extract of the air dried whole plant of *Justicia procumbens*^[24].

Telefo PB et al., (2003) have reported the oestrogenicity and effect on hepatic metabolism of the aqueous extract of leaf mixture of *Aloe buttneri*, *Dicliptera ventricullata*, *Hibiscus macranthus* and *Justicia insularis*. The aqueous extracts of leaf mixture showed the induced effect on ovarian and uteri weight as well as serum and ovarian oestrodial. Significant decrease in liver of aminopyrin N-demethylase activity was noticed in treated animals^[25]

Woradulayapinij W et al.,(2005) have studied the *in-vitro* HIV type 1 reverse transcriptase inhibitory activities of Thai medicinal plants and *Canna indica* L. rhizomes. Water and 80% ethanol extract of 20 Thai medicinal plants used to treat AIDS were tested for their HIV type 1 reverse transcriptase inhibitory activity. The

water extracts of *Ipomea carnea* subsp. *Fistulosa* (aerial parts), *Justicia gendarussa* (aerial parts) showed HIV-1 RT inhibitory ratio (% IR) higher than 90% at a 200µg/mL concentration [26]

Ratnasooriya WD et al., (2007) have reported that the aqueous leaf extract of *Justicia gendarussa* (Acanthaceae) significant antinoceptive action when tested in mice. The aqueous leaf extract also showed strong antioxidant activity [27]

Bedoya L M et al., (2008) have reported the Guatamalan plant extracts as virucides against HIV -1 infection. Three Guatamalan plants extracts *Justicia reptans* (Acanthaceae), *Neurolaene lobata* (Astraceae) and *Pouteria viridis* (Sapotaceae) were used for classical antiviral assay and found to inhibit HIV replication. The active fractions were non-toxic *in-vitro* and also inhibited other enveloped virus [28]

Dwivedi et al., (2008) have reported the use of *Justicia adhatoda* in traditional herbal remedies from the Vindhaya region of Madhya Pradesh in treatment of viral hepatitis [29]

Paval J et al., (2009) have compared the anti-arthritis activities of the plants *Justicia gendraussa* Burm. F and *Withania somnifera* Linn in arthritis induced in male albino rats using Freund's complete adjuvant and bovine type II collagen [30]

Sathianarayanan S et al., (2009) have studied the antimicrobial activity of various extracts of *Justicia transquarberiensis* against Gram positive, Gram negative bacteria, *streptococci aureus*, *Basillus subtilis*, *E. coli* and antifungal activity was also studied [31]

Umar S *et al.*, (2010) have studied hepatoprotective activities of two Ethiopian medicinal plants namely *Justicia schimperiana* and *Verbascum sinaiticum* which are used in Ethiopian traditional medicinal practices for the treatment of liver diseases^[32]

CHAPTER - 3

AIM AND SCOPE OF THE PRESENT STUDY

The ethno medical information reveals that the species of *Justicia* possessed a wide spectrum of activity which included anticancer, antirheumatic, antisyphilitic, diuretic, antitubercular, antimalarial, laxative, and sedative, cardio protective, antifungal and antibacterial activities. According to Siddha system of medicine, leaves of *Justicia adathoda* possess cardio protective property.

The ethno medical information also revealed that the fruits of *Justicia glauca* were found to possess antibacterial and antifungal activities. The phytochemical studies on the leaves have reported for the presence of flavonoids, tannins, alkaloids, sterols and carbohydrates.

The cardioprotective, anticancer, antibacterial, antifungal and antioxidant have not yet been reported so far on the whole plant of *Justicia glauca*

Hence the present work has been designed to carry out the following studies on the leaves *Justicia glauca* Rottler.

1. Pharmacognostical studies on the whole plant.
2. Preliminary phytochemical screening on the extracts of *Justicia glauca*
3. Quantitative estimation of secondary metabolites.
4. Phytochemical evaluation of whole plant ethanolic extract by TLC and HPTLC studies.

5. Screening of the ethanolic extract of the whole plant for following

pharmacological activities.

- *In vitro* antioxidants activity
 - DPPH radical scavenging activity
 - Nitric oxide scavenging activity
 - Phosphomolybdenum method
- Cardioprotective effect of leaf extract of *Justicia glauca* on doxorubicin induced cardiotoxicity in rats.
- Antibacterial activity
- Antifungal activity

CHAPTER - 4

PLANT PROFILE

BOTANICAL SOURCE : *Justicia glauca* Rottler

FAMILY : Acanthaceae

Justicia glauca is an undershrub found in the foothills of Peninsular India. The leaves are elliptic-ovate, 3-5 cm long and 2-3 cm wide. The flowers appear during July-December.

SYSTEMATIC POSITION ^[33,34]

Domain : Eukaryota
Kingdom : Plantae
Subkingdom : Viridaeplantae
Phyllum : Tracheophyta
Subphylum : Euphyllophytina
Infraphyllum : Radiatopses
Class : Vagnoliposida
Subclass : Asteridae
Super order : Lamianae
Order : Lamiales
Family : Acanthaceae
Subfamily : Acanthoideae
Tribe : Ruellieae
Subtribe : Justicinae
Genus : *Justicia*
Specific epithet : *Glauc*-Rottler
Division : Magnoliophyta
Genies : *Justicia*
Species : *Glauc*

SYNONYM:

Galucous justicia

GEOGRAPHICAL DISTRIBUTION:

Justicia glauca grows in waste lands and slopes. It is very common near foothills to 1000 (1750) m.

HABIT AND HABITAT:

Justicia glauca is an undershrub found in the foothills of India.

CHAPTER - 5

PHARMACOGNOSTICAL STUDIES

A detailed pharmacognostical evaluation gives valuable information regarding the morphology, microscopical and physical characteristics of crude drugs. They can be used for the botanical identification of the plant at a future date. Many pharmacognostic studies have been carried out on many important drugs, and the resulting observations have been incorporated in various herbal pharmacopoeias. There are a number of crude drugs where the plant source has not yet been scientifically identified. Hence pharmacognostic study gives the scientific information regarding the purity and quality of crude drugs.

SECTION- A

MORPHOLOGICAL STUDIES

Morphological studies include aspects of the outward appearance (shape, structure, colour and pattern) as well as the form and structure of the internal parts like cells etc. Some of these gross morphological characters of drugs such as shape, size, margin, apex and venation are identification features of drugs. These features give valuable information about the drugs.

MATERIALS AND METHODS

Collection of plant material^[35-40]

The plant specimens were collected from Yannaimalai in Madurai which is 10km from Madurai Medical College. The specimen was authenticated by the Director, The Rapinat Herbarium and Centre for Molecular Systematic, St. Joseph's College Campus, Tiruchirappalli-620002. A voucher specimen is kept in the Department of Pharmacognosy, College of Pharmacy, Madurai Medical College,

Madurai (**Fig.1**). The various aerial parts of *Justicia glauca* collected for macroscopical evaluation were leaves, flowers etc. The photographs of the macroscopic features of the plant are shown in **Fig. 2.1 to 2.3**

OBSERVATIONS AND DISCUSSION

Leaves (Fig.2.1) ^[33]

The leaves are elliptic-ovate, 3-5cm long and 2-3cm wide.

Flowers (Fig.2.3)

The flower spikes are erect, about 10cm long. Bracts are broadly ovate, and bracteoles are linear. The flowers are pink, with 5 lance-shaped sepals which are 3-nerved and have minute hair on them. The flowers are 8 x 3mm across. The flowering season is between July and December.

Fruits

The capsule is 1cm long and 0.5cm wide, pointed at the tip, tapering into a solid beak below.

The above morphological studies such as shape, size, margin, apex of the leaves and flowers and venation pattern of the leaves are identification features of drugs. These features give valuable information about the drugs.

SECTION - B

MICROSCOPICAL STUDIES ^[37,38, 39]

The microscopical study of organized crude drugs is an important parameter for evaluation. The microscopical evaluations allow more detailed examination of the plant material to identify the organized drug by its histological character. It provides detailed information about the crude drugs by virtue of its property to magnify the fine structures of minute objects to be visualized and there by confirm the structural details of the plant drugs under evaluations. It can also be used in the determination of the optical as well as micro chemical properties of the crude drug confirmation study.

MATERIALS AND METHODS

Collection of specimens

The root, stem, leaves and flowers of the plant were subjected to microscopical evaluation. The samples were cut and removed from the plant and fixed in FAA (formalin, 5mL; acetic acid, 5mL; ethyl alcohol, 90mL). After 24h of fixing, the specimens were dehydrated with graded series of t-butyl alcohol [37]. Infiltration of the specimens was carried by gradual addition of paraffin wax (M.P-58-60°C) until TBA solution attained super saturation. The specimens were cast in to paraffin blocks.

Sectioning

The rotary microtome was used to section the paraffin embedded specimens into sections of 10-12µm thickness. They were then placed on a slide and de-waxing of the sections was carried out by customary procedure [38]. The sections were stained with toluidine blue [39] since it is a polychromatic stain. The dye rendered pink color

to the cellulose walls, blue to the lignified cells, violet to the mucilage, and blue to the protein bodies and also stained with safranin.

Leaf clearing

Two methods were used for studying the stomatal morphology, venation pattern and trichome distribution. The paraffin embedded leaf was used for paradermal sections. From these sections, the epidermal layers as well as vein islets were studied. Another method employed was clearing leaf fragments by immersing the material in alcohol (to remove chlorophyll) followed by treating with 5% sodium hydroxide. The material was rendered transparent due to loss of cell contents. Epidermal peeling by partial maceration employing Jeffrey's maceration was also done. Glycerin mounted temporary preparations were made for cleared materials.

For study of elements of xylem, small fragments of stem and root were macerated with Jeffrey's maceration fluid.

Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell component were studied and measured.

Photomicrographs

The photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observations bright field was used and for the study of starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized they appear bright against dark background. The magnifications of the anatomical features are indicated by the scale-bars in the photograophs. The microscopic features are presented in **Fig. 3 to 15**.

OBSERVATIONS AND DISCUSSION

Midrib of the leaf (Fig. 3.1, 2)

The midrib consists of adaxial projection of thick broad hump and abaxial wide part. It is 400 μ m thick; the abaxial hump is 100 \times 200 μ m and the abaxial part is 300 μ m wide. The midrib consists of thick and prominent epidermal layer of square shaped cells with prominent cuticle. Some of the cells are modified into lithocysts. The adaxial cone includes a cluster of collenchyma and a layer of palisade cells. Remaining ground tissue is parenchymatous; the cells are wide thin walled and compact. The vascular strand is broadly triangular with semicircular base. The xylem elements are arranged straight, short parallel lines. Phloem occurs in small clusters beneath the xylem strands.

Lamina (Fig. 4.1)

The lamina is distinctly dorsiventral and the surfaces are even and smooth. It is 160 μ m thick. The adaxial epidermis consists of thick epidermal layer of wide rectangular cells with prominent cuticle. The adaxial epidermis bears stomata. The cells are 20 μ m thick. The abaxial epidermal cells are thin, narrowly rectangular or square shaped. These are shallow depressions in which are located glandular trichomes. The gland is capitate type. It consists of short stalk that is embedded in the epidermal layer. The body is sub spherical, two or four celled and possess prominent nuclei (**Fig. 4.1**). The gland is 15 \times 25 μ m in size. The mesophyll tissue is differentiated into adaxial single vertical narrow columnar palisade cells which have wide gaps in between the palisade. Spongy parenchyma cells include three or four layers of thin lobed cells with large air chambers.

Leaf – margin (Fig. 4.2)

The marginal portion of the leaf is blunt and the end portion is semicircular, it is 120 μ m thick. The epidermis of the lamina continues in the margins; the epidermal cells become smaller and thick walled. The palisade and spongy mesophyll tissues remain distinct; but the cells are more compact and rigid.

Some of the epidermal cells become highly dilated into long canal like cavities. Within these elongated cavities occur long, thin cylindrical cystoliths. The cavities possessing the cystoliths are called lithocysts (**Fig. 5.1**). In sectional view the leaf the litho cysts appear dilated circular cells with circular dense cystoliths. (**Fig. 4.2**)

Stomata and epidermal cells (Fig. 5.2)

When viewed in paradermal sections, the epidermal cells of both upper and lower layers are highly wavy and the cells appear amoeboid in outline because the anticlinal walls are thick and undulate. The stomata are exclusively diacytic type, in which there are two subsidiary cells with their common wall lying at right angles to the long axis of the guard cells (**Fig. 5.2**)

Venation pattern (Fig. 6.1, 2)

The veins are thin and slender. They form less dense reticulate venation system and the vein- islets are less distinct. When the islets are distinct they are variable in size and shape. The islets are wide with thin vein boundaries. The vein terminations are long thin and undulate. They are unbranched or branched once; branching is irregular. (**Fig. 6.2**)

Petiole (Fig. 7.1, 2)

The petiole is semicircular with flat adaxial side. It consists of a thick epidermal layer of squarish cells. The ground tissue is homogeneous and parenchymatous the cells are angular and compact. The vascular strand is broad slightly concave, measuring $50 \times 350 \mu\text{m}$ in size. The vascular strand is collateral; it includes parallel files of thick walled circular, fairly wide xylem elements the sieve elements of the phloem occurs in thin row beneath the xylem strand.

Stem (Fig. 8 & 9)

The stem is circular with shallow ridges and furrows. It is 1.9mm thick. The stem consists of a distinct epidermal layer of squarish cells. Some of the epidermal cells are dilated

and possess circular and chinate cystoliths. The cortex is wide and includes about five layers of collenchymas cells followed by five and six layers of wide thin walled compact parenchyma cells (**Fig. 8.2**).

The vascular system consists of a circle of six prominent collateral vascular bundles, the number of bundles corresponding to the number of ridges of the stem. The vascular segments are semicircular; the medullary rays in between the vascular segments have initial stage of secondary growth and origin of interfascicular cambium (**Fig. 8.2**): The vascular bundle have short parallel rows of wide angular thick walled xylem elements. On the outer portion of the xylem strands occur phloem elements. The phloem elements are small, diffuse and random in distribution. The pith is wide, homogeneous and parenchymatous.

Root (Fig. 10.1, 2)

The roots of two different thicknesses are studied. In thin root measuring 1.4mm in diameter consists of thin periderm and thick solid vascular cylinder (**Fig. 10.1, 2**). The periderm is very thin comprising of two or three layers of compressed cells. The cortical zone is narrow and there are the three layers of parenchyma cells. Some of the cortical parenchyma cells are dilated into lithocysts possessing cystoliths.

Thick root measuring 3.2mm in diameter exhibits thick secondary growth (**Fig. 11 & 12**). It consists of three or four layers of periderm, about six layers of tangentially elongated rectangular cortical cells with cystoliths and thick solid vascular cylinder (**Fig. 10.1, 2**).

The phloem zone is uneven in thickness; the phloem elements are fairly wide diffuse in distribution and have thick cell walls. (**Fig. 12.1**); the secondary xylem is thick, dense and solid; it exhibits two or three growth rings in the peripheral zone. The central zone has no growth rings. The growth rings are characterized by a single circle of wide vessels followed by very narrow vessels at the end of the growth rings (**Fig. 11.1, 2; 12.2**). The vessels are circular and thin walled; they are up to 70µm wide. Xylem fibers thick walled and lignified.

Powder microscopy

The powder consists of following elements.

Epidermal trichomes (Fig. 11.1; 15.1)

Long curved, multicellular, uniseriate unbranched nonglandular trichomes are common, the cells narrow and noded at the cross walls; the trichomes are upto 800 μm long.

Parenchyma cells (Fig. 13.2)

Wide plates of parenchyma cells are frequently seen. The cells are rectangular or squarish. Their walls are thick. Some cell inclusions are seen in the cells.

Fibres (Fig. 13.1,2; 15.2)

Narrow long liberiform fibres are abundant in the powder. They have thick walled and wide lumen. No pits are seen in the powder. The fibres are 300-380 μm long and 10 μm wide.

Vessel elements (Fig.14 & 15)

The vessel elements are unique. They are as long as the fibres and are very narrow resembling fibres. But the vessel elements have dense multiseriate pits and oblique perforations at the ends. Long or short tails are also present in some of the vessel elements. The vessel elements are 200-350 μm long.

SECTION – C

QUANTITATIVE MICROSCOPICAL STUDIES ^[38,39, 40]

DETERMINATION OF LEAF CONSTANTS

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

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.

Procedure

The leaves were cut into small pieces on the lamina between midrib and the margin, cleared in chloral hydrate and mounted on a slide. The 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 sample slides and the veins were traced over the square. The vein islets and vein terminations were counted in the square. Six such readings were taken and the average was calculated and the results were presented in **Table 1**

Stomatal Number and Stomatal Index

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

of stomata to the total number of epidermal cells, each stoma beings counted as one cell. The stomatal index was calculated using the formula

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

Where S = number of stomata per unit area; E = number of epidermal cells in the same unit area

Procedure

The upper and lower epidermal peelings of the leaves were mounted on a slide. A camera lucida and stage micrometer were used to draw 1mm square was on a paper. The stage micrometer was replaced by the preparation slide. Then the preparations were observed under microscope and the stomata were marked in that unit area. The number of stomata present in unit area was calculated. Six such readings were taken and the average of stomatal number was calculated and presented in **Table 1**.

The procedure adopted in the determination of stomatal number was followed for the determination of stomatal index but the preparation was observed under high power. The epidermal cells and the stomata were counted. From these values the stomatal index was calculated and the results obtained are tabulated in **Table 1**.

RESULTS AND DISCUSSION

From **Table 1**, it can be seen that the stomatal number in upper epidermis was 28.84 ± 0.61 while it was 58.34 ± 0.92 for the lower epidermis. The vein islet number was 10.84 ± 0.41 while the vein termination number was 21.17 ± 0.31 . The stomatal number in the lower epidermis was nearly twice the value seen in the upper epidermis. The determination of the leaf constants helps in the identity of this plant from the other species of the genus *Justicia*.

Table 1: Quantitative microscopical parameters of the leaf of *Justicia glauca*

| S. No. | Parameters* | Values obtained* |
|---------------|------------------------------------|-------------------------|
| 1 | Vein islet number | 10.84±0.41 |
| 2 | Vein termination number | 21.17±0.31 |
| 3 | Stomatal number in upper epidermis | 28.84±0.61 |
| 4. | Stomatal number in lower epidermis | 58.34±0.92 |
| 5. | Stomatal index in upper epidermis | 8.17±0.31 |
| 6. | Stomatal index in lower epidermis | 13.17±0.61 |

***Mean of 6 readings ± SEM**

SECTION - D

PHYSICAL PARAMETERS ^[40-42]

ASH VALUES

The ash values were determined by using air dried powdered leaves as per the official method. The total ash, acid insoluble ash and water soluble ash were determined.

(A) Total ash

Procedure

An accurate quantity of 2g of the whole plant powder was weighed and transferred into a tarred nickel crucible and 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 from carbon. Then it was cooled and weighed for constant weight. The percentage of ash with reference to the air dried drug was calculated. The values were determined in triplicate. The results are presented in **Table 2**.

(B) Acid insoluble ash

Crude drugs containing larger quantity of calcium oxalate can give variable results depending upon the conditions of ignition. Treatment of ash with HCl leaves virtually only silica. It is necessary to use ash less filter paper during filtration and subsequent incineration, to limit the error.

Procedure

The ash obtained in (A) was boiled for 5min with 25mL of 2M hydrochloric acid. The insoluble matter was collected in a tared sintered glass crucible. The residue

was washed with hot water, ignited to constant weight, cooled in desiccators and weighed. The percentage of acid insoluble ash with reference to the air dried drug was calculated. The values were determined in triplicate. The results are presented in **Table 2**

(C) Water soluble ash:

It is a measurement of detection of water soluble impurities in dry or raw material of plant.

The 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 and 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 values were determined in triplicate. The results were represented in **Table 2**.

LOSS ON DRYING

Loss on drying is defined as the amount of water and volatile matters in a sample when the sample is dried under specified conditions.

Procedure

The powdered crude drug (2g) was accurately weighed in a tarred dish and dried in an oven at 100°-105°C. It was cooled in a desiccator and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken and the experiment was repeated thrice. The results obtained are presented in **Table.2**.

DETERMINATION OF EXTRACTIVE VALUES

Procedure

(1) Petroleum ether soluble extractive value

A quantity of 5g of the air dried drug coarsely powdered was macerated in 100ml of petroleum ether in a closed flask for 24h, shaking frequently during 6h and allowed to stand for 18h, filtered rapidly taking precautions against loss of solvent. 25mL of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish and dried at 105°C, to constant weight. The percentage of the petroleum ether soluble extractive with reference to the air dried drug was calculated. The results are tabulated in **Table 2**.

(2) Ethanol and 70% ethanol soluble extractive:

A quantity of 5g of the air dried drug coarsely powdered was macerated in 100mL of ethanol in a closed flask for 24h, shaking frequently during 6h and allowed to stand for 18h, filtered rapidly taking precautions against loss of solvent. 25mL of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish and dried at 105°C, to constant weight. The percentage of alcohol soluble extractive with reference to the air dried drug was calculated and presented in **Table 2**.

(3) Water-Soluble Extractive:

A quantity of 5g of the air dried drug coarsely powdered was macerated in 100mL of chloroform water in a closed flask for 24h, shaking frequently during 6h and allowed to stand for 18h, filtered rapidly taking precautions against loss of solvent. 25mL of the filtrate was evaporated to dryness in a tared 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 2**.

(4) 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. The percentage of ether soluble extractive with reference to the air dried drug was calculated and presented in **Table 2**.

(5) Chloroform, acetone, methanol and benzene 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 benzene soluble extractive. Instead of alcohol, respective solvents were used for the determination of their extractive values. The percentage of chloroform, acetone, methanol and benzene soluble extractives were calculated and presented in **Table 2**.

RESULTS AND DISCUSSION

The results obtained for the various physical parameters like total ash, acid insoluble ash, water soluble ash, loss on drying and the extractive values are presented in **Table 2**. It can be seen that the acid insoluble ash was found to be 0.70 ± 0.36 which indicated that very little sand and other insoluble material were present. The water soluble ash was found to be 5.27 ± 0.21 which indicated that inorganic mineral like sodium; calcium salts etc. were present in the plant material. The loss on drying was found to be 9.44 ± 0.27 . This indicates the amount of the moisture that is generally present in the raw material.

Table 2: Analytical parameters of *Justicia glauca*

| S. No | Parameters* | Values* expressed as % |
|----------|--------------------------|------------------------------|
| 1 | Ash values | |
| | Total ash | 7.40±0.36 |
| | Water soluble ash | 5.27±0.21 |
| | Acid insoluble ash | 0.70±0.36 |
| 2 | Loss on drying | 9.44±0.27 |
| 3 | Extractive values | |
| | Petroleum ether extract | 12.06 |
| | Ethyl acetate | 15.95 |
| | Benzene | 10.87 |
| | Chloroform | 11.70 |
| | Acetone | 13.89 |
| | Methanol | 16.72 |
| | Ethanol | 17.26 |
| | 75% Ethanol | 18.28 |
| | Aqueous | 16.30 |

* mean of three readings ± SEM

The extractive value was found to be maximum in 70% ethanol followed by ethanol and then water. The determination of extractive values helps in determining a suitable menstrum for extraction of secondary metabolites and helps in determining if the material has been exhausted at a future date since these values can be used as a standard.

SECTION - E

POWDER ANALYSIS

Procedure ^[36, 43, 44]

The whole plant powder of *J. glauca* was treated with various chemical reagents like picric acid, iodine solution, Mayer's reagent etc. on a watch glass for the identification of the secondary metabolites. The color/precipitate obtained with various reagents are presented in **Table 3**.

The powder was taken in watch glass and was treated with various acids and alkalis and their color in daylight and under UV light was observed. The results are presented in **Table 4**. The fluorescence analysis of the various extracts was also carried out to find out whether any fluorescent compound was present in the sample. The results obtained are tabulated in **Table 5**.

RESULTS AND DISCUSSION

The behavior of the *J. glauca* powder with various chemical reagents is tabulated in **Table 3**. The powder showed the presence of phytosterols, tannins, proteins, flavonoids, phenolic compounds. The powder showed the absence of alkaloids.

The fluorescence analysis of the powder and the extracts of *J. glauca* are presented in **Tables 4 & 5**. The powder when viewed under UV light at 365nm appeared black and dark green at 254nm when treated with sodium hydroxide. The ethanolic extract when seen in day light was green

Table 3: Behavior of the *J. glauca* 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 | No blue color | Absence of starch |
| Mayer's reagent | Cream color | Alkaloids present |
| 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 4: Fluorescence analysis of powder of *J. glauca*

| Powder +reagent | Day light | UV light (254 nm) | UV light (365 nm) |
|---|------------------|--------------------------|--------------------------|
| Drug powder | Pale green | Green | Black |
| Drug powder +aqueous 1M sodium hydroxide | Yellowish green | Dark green | Black |
| Drug powder + alcoholic 1M sodium hydroxide | Yellowish green | Dark green | Black |
| Drug powder + iodine | Brown | Brownish green | Black |
| Drug powder + 10% potassium hydroxide | Yellowish green | Dark green | Brown |
| Drug powder + 1M hydrochloric acid | Gray | Green | Brown |
| Drug powder + glacial acetic acid | Pale green | Green | Brown |
| Drug powder + 50% sulphuric acid | Green | Green | Brown |
| Drug powder + 50% nitric acid | Brown | Brownish green | Brown |
| Drug powder + 50% hydrochloric acid | Gray | Green | Brown |

Table 5: Fluorescence Analysis of extracts of *J.glauca*

| Extracts | Consistency | Colour in Day Light | Colour under UV Lamp | |
|--------------------|-------------|---------------------|----------------------|----------------|
| | | | 365nm | 254nm |
| Petroleum extract | Semisolid | Green | Yellowish green | green |
| Ether extract | Semisolid | Greenish brown | green | Green |
| Chloroform extract | Semisolid | Greenish brown | Orange | Green |
| Hexane extract | Semisolid | Green | Reddish Orange | Green |
| Ethanol extract | Semisolid | Green | Reddish Orange | Green |
| Methanol extract | Semisolid | Green | Reddish Orange | Green |
| Aqueous extract | Semisolid | Greenish brown | Green | Greenish brown |

The powder analysis of the crude drug material and the extract throws light on the type of secondary metabolites present in the plant. They also help in identifying the crude material and in determining the purity of the sample.

CHAPTER-6

SECTION-A

PHYTOCHEMICAL STUDIES

COLLECTION AND PREPARATION ^[45-49]

The leaves of *Justicia glauca* were collected in and around Madurai and authenticated by taxonomist. The whole plant were washed thoroughly and dried in shade. The shade dried whole plant were powdered and used for further studies.

PRELIMINARY PHYTOCHEMICAL SCREENING

MATERIALS AND METHODS

1. Test for sterols

The powdered whole plant 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 appears red in color indicates the presence of sterols.

b. Liebermann – Burchard's Test

To the chloroform solution, a few drops of acetic anhydride and 1mL of conc. 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 will be formed and the upper layer turns green indicating the presence of sterols.

2. Test for terpenoids

A little of the powdered whole plant was extracted with chloroform and filtered. The filtrate was warmed gently with tin and thionylchloride. Pink color solution appears indicates the presence of terpenoids.

3. Test for carbohydrates

a. Molisch's Test:

The aqueous extract of the whole plant powdered when treated with alcoholic solution of α -naphthol in the presence of sulphuric acid. A purple color indicates the presence of carbohydrates.

b. Fehling's Test:

The aqueous extract of the whole plant powdered was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. A red precipitate indicates the presence of free reducing sugars.

4. Test for Flavonoids

a. Magnesium turning – conc. 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 and boiled for five minutes. A red colour indicates the presence of flavonoids.

b. Alkali Test

To a small quantity of test solution 10% aqueous sodium hydroxide solution was added. A yellow orange color indicates the presence of flavonols.

c. Acid Test

To a small quantity of test solution, few drops of concentrated sulphuric acid was added. A yellow orange color indicates the presence of flavonols.

7. Test for Proteins

a. Millon's Test

A small quantity of aciduous – alcoholic extract of the powdered drug was heated with Millon's reagent. A white precipitate turning red on heating indicates 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. A violet color indicates the presence of proteins.

8. 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 5min. It was then evaporated to dryness in a porcelain dish on a water bath. To the residue 20mL 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 the residue 5ml of dilute hydrochloric acid was added followed by 2mL of each of the following reagents.

- | | | |
|--------------------------|---|---------------------------|
| a) Mayer's Reagent | _ | cream precipitate |
| b) Dragendorff's Reagent | _ | orange brown precipitate |
| c) Hager's Reagent | _ | Yellow precipitate |
| d) Wagner's Reagent | _ | Reddish brown precipitate |

9. Test for Glycosides

a. Borntrager's Test

The whole plant powder 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. A pink color in the ammoniacal layer indicates the presence of anthraquinone glycosides.

b. Modified Borntrager's Test

About 0.1g of the powdered drug was boiled for 2min 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. A pink color indicates the presence of anthraquinone glycosides.

Test for Cardiac Glycosides (for deoxysugar)

Keller Kiliani Test

About 1g of the powdered leaf was boiled with 10ml of 70% alcohol for 2min, cooled and filtered. To the filtrate 10mL of water and 5 drops of solution of lead subacetate were added and filtered, evaporated to dryness. The residue was dissolved in 3mL of glacial acetic acid. To this, 2 drops of ferric chloride solution was added. Then 3mL of conc. sulphuric acid was added to the sides of the test tube carefully and observed. A reddish brown layer indicates the presence of deoxysugars of cardiac glycoside

Test for Cyanogenetic Glycosides

A 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 2h in a warm place. A brick red color was produced on the paper indicating the absence of cyanogenetic glycosides.

10. Test for saponins

About 0.5g of the powdered drug was boiled gently for 2min with 20mL of water and filtered while hot and allowed to cool. 5mL of the filtrate was then diluted with water and shaken vigorously. A frothing indicates the presence of saponins.

11. 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. A bluish black color indicates the presence of tannins.

12. Test for the presence of Volatile oil

A weighed quantity of 250gm of fresh leaves was subjected to hydrodistillation using volatile oil estimation apparatus (BP 1980). A flavoured oil collected indicates the presence of volatile oil.

The above preliminary phytochemical reactions were carried out the powdered material of *Justicia glauca* and on the various extracts of the crude powder. The results obtained are tabulated in **Tables 6 & 7**.

RESULTS AND DISCUSSION

The results obtained for the preliminary phytochemical screening of the powder indicated the presence of phytosterols, proteins, carbohydrates, phenolic compounds, alkaloids, flavonoids and cardiac glycosides. It showed absence for anthraquinone glycosides, terpenoids and volatile oil.

The aqueous extract of the *J. glauca* showed the presence of flavonoids, phenolic compounds, alkaloids, proteins and carbohydrates. The ethanolic extract of *J. glauca* shows the presence of flavonoids, phenolic compounds, alkaloids, protein and carbohydrates. The methanolic extract of *J. glauca* shows the presence of flavonoids, phenolic compounds, alkaloids, proteins and carbohydrates.

Table 6: Preliminary phytochemical screening for the powder of *Justicia glauca*

| 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 | + |
| 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 | - |
| 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 | - |

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

The petroleum ether extract of *J. glauca* shows the presence of sterols and cardiac glycosides. The chloroform extract of *J. glauca* shows the presence of sterols and cardiac glycosides. The acetone extract of *J. glauca* shows the presence of cardiac glycosides, tannins and flavonoids.

Table 7: Preliminary phytochemical screening of various extracts of *Justicia Glauca*

| Tests | Pet. ether Extract | Ether Extract | Benzene Extract | Chloroform Extract | Acetone Extract | Methanol Extract | Ethanol Extract | Ethanol extract (70%) | Aqueous extract |
|-----------------------------------|--------------------|---------------|-----------------|--------------------|-----------------|------------------|-----------------|-----------------------|-----------------|
| I Test for sterols | | | | | | | | | |
| Salkowski's test | + | - | - | + | - | - | - | - | - |
| Liebermann-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 | - | - | - | - | - | + | + | + | + |
| V. Test for Glycosides | | | | | | | | | |
| Anthraquinone glycosides | - | - | - | + | - | - | - | - | - |
| i) Borntrager's test | - | - | - | + | - | - | - | - | - |
| ii) Modified Borntrager's test | - | - | - | + | - | - | - | - | - |
| Cardiac glycosides | | | | | | | | | |
| i) Keller Killiani test | + | + | + | + | + | + | + | + | + |
| Cyanogenetic 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 Terpenoid | | | | | | | | | |
| | - | - | - | - | - | - | - | - | - |

(+) indicates positive reaction

(-) indicates negative reaction

SECTION-B

TOTAL PHENOL, TOTAL FLAVANOID AND VITAMIN C DETERMINATION ^[50-54]

PREPARATION OF EXTRACT

About 1kg of the dried powdered leaf of *Justicia glauca* was defatted with 2.5L of petroleum ether (60-80°C) by maceration. The solvent was then removed by filtration and the marc is dried. To the dried marc 2.5L of 70% ethanol was added and the extraction was performed by triple maceration. It was then filtered and the combined filtrate was evaporated to a cohesive mass using rota vapour.

TOTAL PHENOL DETERMINATION

Principle

The total phenolic content of the various extracts of *J. glauca* were determined by Folin Ciocalteu reagent method. All the phenolic compounds are oxidised by the Folin-Ciocalteu 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.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

- 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°C.

b) Sodium carbonate solution (10%)

c) Standard gallic acid solution.

Procedure

Gallic acid was accurately weighed and diluted in water to concentration of 1mg/mL. This solution was suitably diluted to get concentrations ranging from 2, 4, 6, 8 and 10µg/mL. 0.5mL of Folin Ciocalteu reagent was added and allowed to stand for 15min. Then 1mL of 10% sodium carbonate solution was added. Finally the mixtures were mixed with distilled water and made upto10mL, allowed to stand for 30min at room temperature and total phenols were determined spectrophotometrically at 760nm using the reagent as blank.

The ethanolic extract of *J. glauca* was weighed and diluted to get a solution of 1mg/mL. Different concentrations of the solution were taken in separate test tubes. 0.5mL of Folin Ciocalteu reagent was added and allowed to stand for 15min. Then 1mL of 10% sodium carbonate solution was added. Finally the mixtures were mixed with distilled water and made upto 10mL, allowed to stand for 30min at room temperature and total phenols were determined spectrophotometrically at 760nm using the reagent as blank.

A calibration curve was generated by plotting concentration of gallic acid versus absorbance (**Fig. 16**). A linear regression equation was determined. The total phenol content was calculated using the linear regression equation and expressed in terms mg of gallic acid equivalent per gm of extract (mg GAE/g). The results obtained are presented in **Table 8**.

TOTAL FLAVANOIDS CONTENT

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 C3 or C5 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 flavonoids. These complexes showed a strong absorption at 415nm which is used for the estimation of flavonoids.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

10S% aluminum chloride

1M potassium acetate

Procedure

An aliquot quantity of quercetin was dissolved in ethanol to get a stock solution of 1mg/mL. Further dilutions were made to get concentrations ranging from 20-100 μ g/mL. 1ml of the above standard solutions were taken in different volumetric flasks, 0.1mL 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 sample without aluminium chloride was used as a blank. From the absorbance obtained, a calibration curve was constructed by plotting concentration versus absorbance of quercetin (**Fig.17**). 1mL of methanolic extract at concentrations 40 μ g/mL and 80 μ g/mL were taken and the reaction was carried out as above and the absorbance was measured at 415nm after 20min and the readings were tabulated in **Table 9**. The amount of

flavonoids present can be determined by linear regression analysis. The total flavonoid content was expressed as mg quercetin equivalents /g of extract.

ESTIMATION OF VITAMIN C ^[55]

Principle

Ascorbic acid was estimated according to the method *Sarajini et al.* The keto group of ascorbic acid undergoes a condensation reaction with 2,4 dinitro phenyl hydrazine to form a hydrazone which is orange yellow and has a maximum absorbance of about 520nm.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagent

Dinitro phenyl hydrazine

85% sulphuric acid

Preparation of test solution

1gm cut pieces of fresh plant material was soaked in 70% ethanol for 24h. The plant extract was filtered and used.

Procedure

An aliquot quantity of ascorbic acid was weighed and dissolved in water to get stock solution of 1mg/mL. Further dilutions were made to get the concentrations ranging from 5-25µg/mL. To 1mL of sample, 0.5mL of dinitro phenyl hydrazine solution was added and incubated for 3h at 37°C. After 3h, 2.5mL of 85% sulphuric acid was added and the absorbance was measured after 30min at 520nm. A calibration curve was constructed by plotting concentration versus absorbance of ascorbic acid (**Fig. 18**). The procedure was repeated for the plant extract as above and the absorbance was measured at 520nm after 3h and the reading were tabulated in table

10. The amount of the vitamin C present can be determined by linear regression analysis. The vitamin C content was expressed as mg/g of extract. The results obtained are presented in **Table 10**.

RESULTS AND DISCUSSION

Total phenol estimation

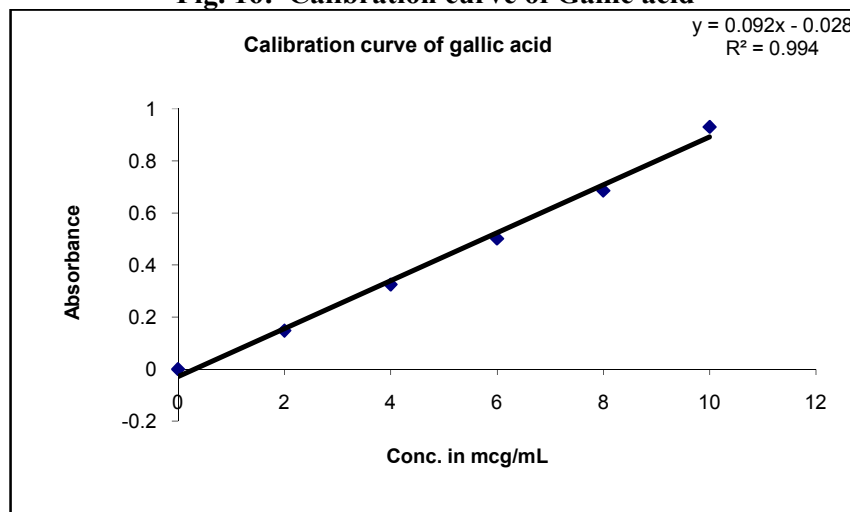
The results for the total phenol estimation of ethanolic extract of *Justicia glauca* are tabulated in **Table 8**.

Table 8: Total phenolic content in ethanolic extract of *Justicia glauca* in terms of gallic acid equivalents

| S. No. | Conc. of gallic acid in µg/mL | Absorbance at 760nm | Conc. of ethanolic extract in µg/mL | Absorbance at 760nm* | Amount of total phenolic content in terms mgGAE/g of extract* |
|--------|-------------------------------|---------------------|-------------------------------------|----------------------|---|
| 1 | 2 | 0.229 ± 0.010 | 50 | 0.306±0.02 | 53.4±0.02 |
| 2 | 4 | 0.452 ± 0.006 | 100 | 0.724±0.03 | 62.8±0.06 |
| 3 | 6 | 0.695 ± 0.005 | | | |
| 4 | 8 | 0.918 ± 0.031 | | | |
| 5 | 10 | 1.162 ± 0.028 | | Average | 58.6±0.03 |

* mean of three readings ±SEM

Fig. 16: Calibration curve of Gallic acid



The linear regression equation was found to be $y = 0.092x - 0.028$ while the correlation was found to be 0.994. The amount of phenol content present in the extract

in terms mg GAE/g of extract was found to be 58.6 ± 0.03 by using the above linear regression equation.

Total flavonoid estimation

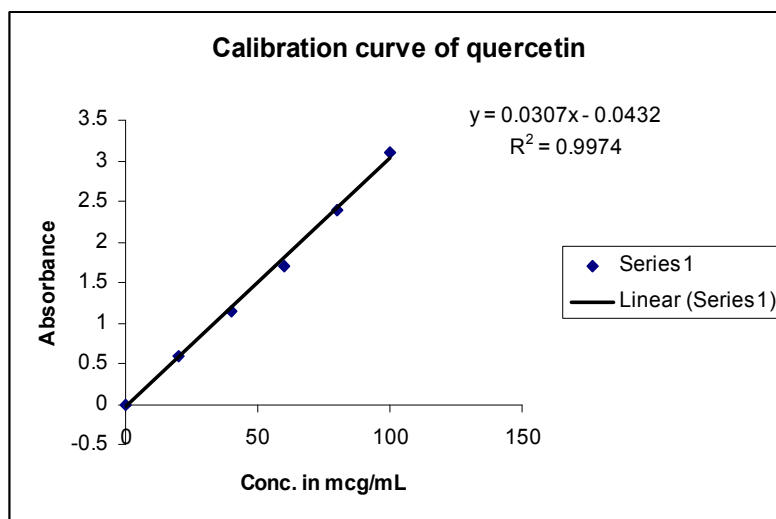
The results for the total flavonoid estimation of ethanolic extract of *Justicia glauca* are tabulated in Table 9.

Table 9: Total flavonoid content per gram of extract in terms of quercetin by aluminium chloride method

| S. No. | Conc. of quercetin in $\mu\text{g/mL}$ | Absorbance at 415nm | Conc. of methanolic extract in $\mu\text{g/mL}$ | Absorbance at 415nm | Amt of total flavonoid content in terms mg quercetin equivalent/ g of extract |
|--------|--|---------------------|---|---------------------|---|
| 1 | 20 | 0.589 ± 0.01 | 100 | 0.048 ± 0.03 | 29.07 ± 0.06 |
| 2 | 40 | 1.151 ± 0.04 | 200 | 0.128 ± 0.01 | 27.02 ± 0.04 |
| 3 | 60 | 1.710 ± 0.09 | | | |
| 4 | 80 | 2.390 ± 0.03 | | | |
| 5 | 100 | 3.112 ± 0.03 | | Average | 29.35 ± 0.03 |

*mean of three readings \pm SEM

Fig. 17: Calibration curve of quercetin



The linear regression equation was found to be $y = 0.0307x - 0.0432$ while the correlation was found to be 0.9974. The amount of flavonoid content present in the

extract in terms mg quercetin equivalent/g of extract was found to be 29.35 ± 0.03 by using the above linear regression equation.

Estimation of vitamin C

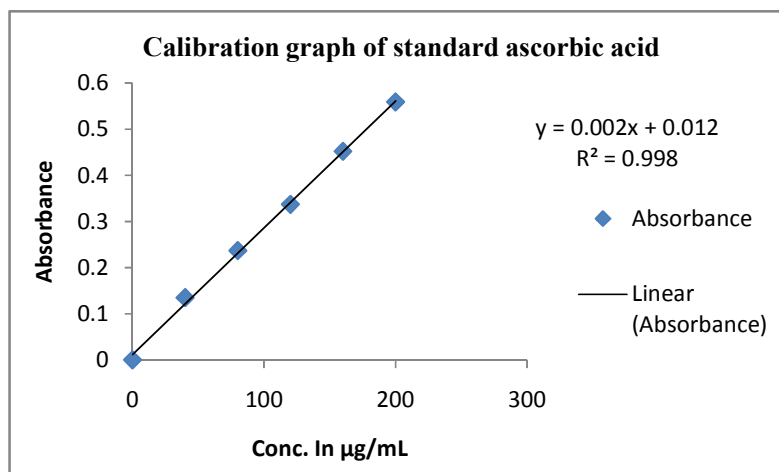
The results for vitamin C content of ethanolic extract of *Justicia glauca* are presented in Table 10.

Table 10: Estimation of Vitamin C in *Justicia glauca*

| S. No. | Conc. of ascorbic acid in $\mu\text{g/mL}$ | Absorbance at 520nm | Conc. of methanolic extract in $\mu\text{g/mL}$ | Absorbance at 520nm | Amt of vitamin C present / g of extract |
|--------|--|---------------------|---|---------------------|---|
| 1 | 40 | 0.135 ± 0.000 | 40 | 0.028 ± 0.02 | 8.02 ± 0.02 |
| 2 | 80 | 0.265 ± 0.015 | 80 | 0.032 ± 0.01 | 10.08 ± 0.01 |
| 3 | 120 | 0.346 ± 0.010 | 120 | 0.045 ± 0.03 | 14.52 ± 0.07 |
| 4 | 160 | 0.468 ± 0.011 | 160 | 0.055 ± 0.07 | 20.78 ± 0.02 |
| 5 | 200 | 0.525 ± 0.010 | 200 | 0.069 ± 0.02 | 26.32 ± 0.03 |
| | | | | Average | 15.94 ± 0.01 |

*mean of three readings \pm SEM

Fig. 18: Calibration curve of ascorbic acid



The linear regression equation was found to be $y = 0.002x + 0.012$ and a correlation coefficient of 0.998. The amount of vitamin C content present in the

ethanolic extract of *Justicia glauca* was found to be 15.94 ± 0.01 mg/gm of fresh material by using the above linear regression equation.

The estimation of secondary metabolites in terms standard marker compounds helps in understanding the type of secondary metabolites present. The results obtained show that the ethanolic extract of *Justicia glauca* was found to possess 58.6 ± 0.03 mg/g GAE of phenolic compounds and 29.35 ± 0.03 mg/g quercetin equivalent of flavonoids and 15.94 ± 0.01 mg/gm of ascorbic acid. The high amount of phenolic and flavonoids may be attributed to the pharmacological activity of the plant

SECTION-C

CHROMATOGRAPHY - TLC AND HPTLC STUDIES ^[56-59]

Plant extracts contains complex mixture of different components and it is necessary to separate them before quantification. Chromatography comprises a group of techniques for isolation of molecular mixtures that depends on the differential affinities of the solutes between two immiscible phases. One of the phases is a fixed bed of large surface area known as stationary phase, while the other is fluid, which moves through or over the surface of the fixed phase known as mobile phase. The principle involved in chromatographic techniques is adsorption and partition.

The different methods of chromatography include paper chromatography (PC), thin layer chromatography (TLC), column chromatography (CC), gas chromatography (GC), high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC).

The phytochemical evaluation of ethanolic extract of *Justicia glauca* was carried out using TLC and HPTLC studies.

DEVELOPMENT OF CHROMATOGRAMS

Materials and method

The extracts were dissolved in ethanol and the spot was applied on the TLC plates using capillary tube.

The plates were developed in the chromatographic tank containing the solvent systems. Various solvent systems were tried for better results. The TLC plates were allowed to develop upto 2/3rd of the plate length and dried. The TLC plates were examined visually or under UV light.

Stationary phase - Silicagel G

- Mobile phase - Toluene: Ethylacetate: Acetic aci (7:3:0.1)
 Detecting agent - Visual & UV light

The R_f value of the spots obtained were calculated using the formula,

$$R_f \text{ value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

The results obtained for the TLC analysis is presented in **Table 11**.

HPTLC EVALUATAION OF ETHANOLIC EXTRACT OF *Justicia glauca*

- Instrument used : CAMAG make HPTLC.
 Software : winCATS 1.4.3
 Sample Applicator : Linomat 5.
 Detection : @254nm in Densitometry TLC Scanner 3
 STD Preparation : 10 mg of STD is dissolved in 1ml Ethanol
 Sample preparation : The sample was prepared in Ethanol 50mg / 1 ml
 Stationary Phase : HPTLC plates silica gel 60 F 254.
 Mobile Phase : Toluene: Ethyl acetate: Acetic Acid (7:3:0.1)
 Sample Applied : 2 μ l sample is applied as 8mm band.

The results obtained are presented in **Table 12** and the TLC visualization and 3D peak display are presented in **Figs. 19 to 21**.

RESULTS AND DISCUSSION

The results obtained for the TLC analysis with the mobile phase is presented in **Table 11**. The TLC plates when examined under UV light at 365nm showed the presence of 5 spots which may be attributed to the presence of different active principles in ethanolic extract of *Justicia glauca*. The spots showed fluorescence when

viewed under UV light at 365nm. The active principle at R_f value 0.7 may be responsible for the antioxidant activity due to its phenolic nature.

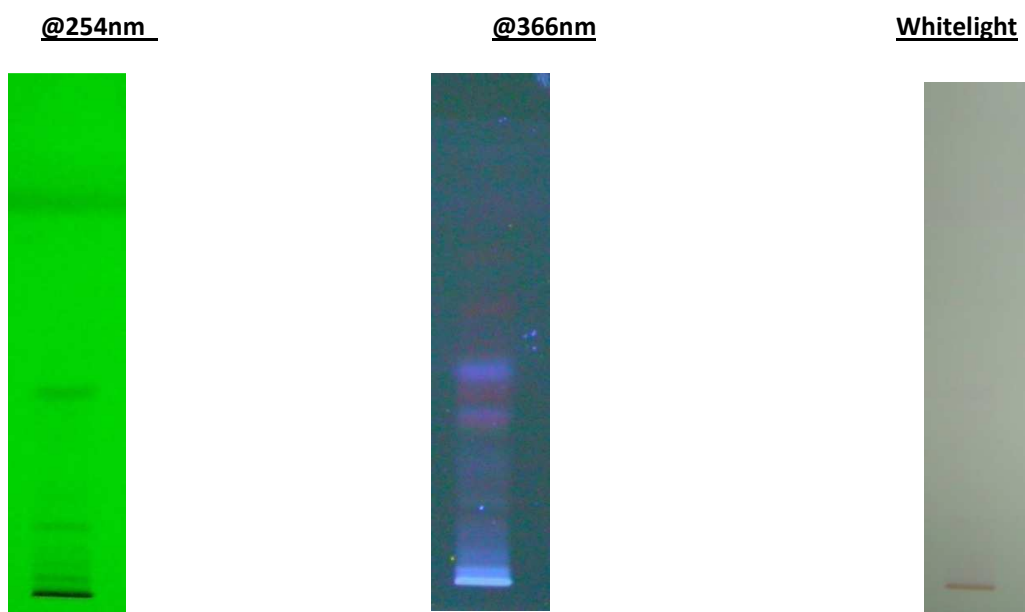
Table 11: Phytochemical evaluation of ethanolic extract by TLC studies.

| S. NO. | SOLVENT SYSTEM | EXTRACT | NO. OF SPOTS | RF VALUE | DETECTING AGENT |
|--------|--|---|--------------|---------------------------------|-----------------|
| 1. | TOLUENE : ETHYLACETATE: ACETIAC ACID (7 : 3 : 0.1) | Ethanolic extract of <i>Justicia glauca</i> | 5 | 0.3 0.4 0.6 0.7 0.8 | UV - 366 |

High Performance Thin Layer Chromatography

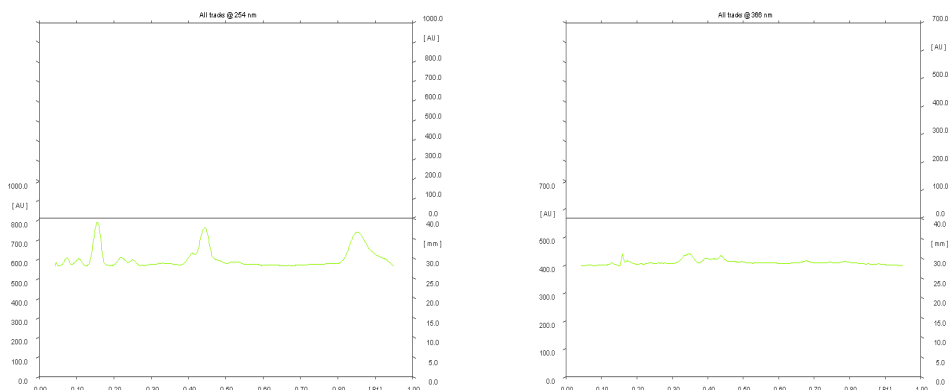
The visualization of the TLC plate of ethanolic extract of *J. glauca* at 254nm, 366nm and white light is presented in **Fig. 19**. The photo of plate at 254nm showed the presence of 13 spots while at 366nm showed the presence 9 spots and one spot was seen when viewed under white light.

Fig. 19: Visualization at 254nm, 366nm & white light



The 3D display of the fingerprint profile and the peak display of ethanolic extract of *J. glauca* at 254nm, 366nm and white light is presented in **Figs. 20 & 21**. The display at 254nm showed the presence of 13 peaks while at 366nm showed the presence 9 peaks and 1 peak was under white light. The R_f values of the peaks along with the area under the curve for each peak at 254,366 nm and white light are tabulated in **Table 12**.

Fig. 20: 3D Display of the fingerprint profile at 254nm and 366nm
@ 254nm **@ 366nm**



White light

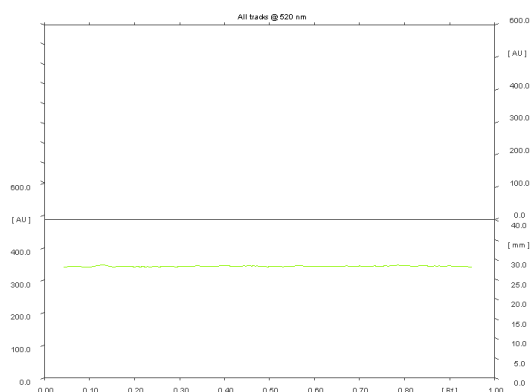
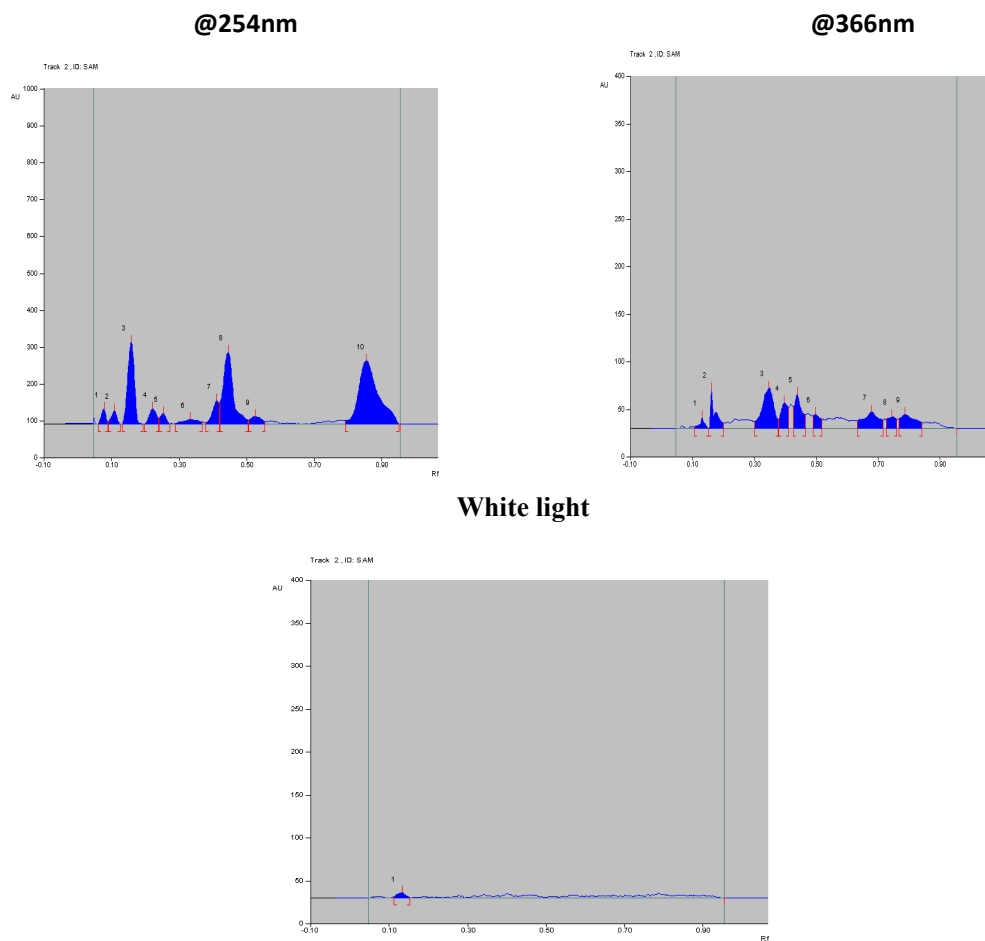


Fig. 21: Peak display of ethanolic extract of *J. glauca* at 254nm and 366nmTable 12: R_f values and area under the curve for each peak at 254, 366nm and white light

| S. No | @254nm | | @366nm | | White light | |
|-------|-------------|-----------|-------------|-----------|-------------|-----------|
| | R_f Value | AREA (AU) | R_f Value | AREA (AU) | R_f Value | AREA (AU) |
| 1 | 0.08 | 481.4 | 0.13 | 149.3 | 0.13 | 121.0 |
| 2 | 0.11 | 512.8 | 0.16 | 517.9 | | |
| 3 | 0.16 | 3869.2 | 0.35 | 1409.6 | | |
| 4 | 0.22 | 783.2 | 0.39 | 550.7 | | |
| 5 | 0.25 | 426.2 | 0.44 | 742.8 | | |
| 6 | 0.33 | 502.9 | 0.49 | 296.5 | | |
| 7 | 0.41 | 1081.8 | 0.68 | 759.4 | | |
| 8 | 0.44 | 5234.0 | 0.74 | 294.5 | | |
| 9 | 0.52 | 574.4 | 0.78 | 625.6 | | |
| 10 | 0.57 | 178.5 | | | | |
| 11 | 0.74 | 215.1 | | | | |
| 12 | 0.78 | 224.8 | | | | |
| 13 | 0.85 | 8812.2 | | | | |

The HPTLC finger print profile of the ethanolic crude plant extract of *J. glauca* showed spots with different Rf values indicating the plant contains many medicinally active compounds which may be responsible for its therapeutic activity. The peak display and finger print profile can be utilized for the quality control for different test samples of *J. glauca* by comparing different chromatograms with the standard obtained in the present study. No two finger prints are same, similarly no two finger print profiles of the extract of different plants are same and hence useful in the identity of *J. glauca* in future.

CHAPTER - 7

PHARMACOLOGICAL SCREENING

SECTION A

INVITRO ANTIOXIDANT ACTIVITY

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell.

Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols.

The antioxidants scavenge the free radical generated, due to oxidative stress and exhibit a protective effect against many diseases like cancer, cardiovascular disease, diabetes and ageing. Generation of ROS overtakes the antioxidants defence of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates and this leads to development of degenerative diseases .Hence the rationale for the use of antioxidant is well established in prevention and treatment of diseases where oxidative stress plays a major aetiopathological role.

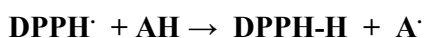
Antioxidant may protect the body against ROS toxicity either by preventing the formation of ROS by bringing interruption in ROS attack by scavenging the reactive metabolites or by converting them to less reactive molecules . The antioxidant capacity gives information about the duration while the activity describes the starting dynamics of antioxidant action. Therefore the uses of antioxidants, both natural and

synthetic are gaining wide importance in prevention of diseases. Some of the in-vitro models for the evaluation of antioxidant activity are listed below.

- ✓ DPPH method
- ✓ Superoxide radical scavenging activity
- ✓ Hydroxyl radical scavenging activity
- ✓ Nitric oxide radical inhibition assay
- ✓ Reducing power method
- ✓ Phosphomolybdenum method
- ✓ Peroxy nitrile radical scavenging activity
- ✓ Xanthine oxidase method
- ✓ Ferric reducing ability of Plasma
- ✓ Thiobarbituric acid assay etc.

Method 1 : Diphenyl picryl hydrazyl (DPPH) method^[60-64]

DPPH is a well-known radical and a trap ("scavenger") for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. Because of a strong absorption band centered at about 520 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520 nm or in the EPR signal of the DPPH.(ref.2)



Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

0.1mM diphenyl picryl hydrazyl in ethanol

Procedure

To the 1mL of test samples of different concentrations, 4mL of DPPH was added. Control without test compound was prepared in an identical manner. Blank was prepared in the similar way, where 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 read 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

$$\% \text{inhibition} = [(\text{Control}-\text{Test})/\text{Control}] \times 100$$

A graph was constructed using concentration versus percentage inhibition (**Fig. 22**). 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**.

Method 2: Nitric oxide scavenging activity assay ^[65]

Principle

Nitric oxide scavenging activity was determined according to the method reported by Green *et al.*, 1982 [78]. Sodium nitropruside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interact with oxygen to produce nitrite ions. These nitrite ions can be determined by Griess Illosvoy reaction.

The nitrite ions produced diazotizes sulphanilamide and the diazonium salt thus obtained reacts with NN naphthyl ethylene diamine dihydrochloride to give a pink colour chromophore which has a maximum absorption at 546nm.

Nitric oxide (NO), being a potent pleiotropic mediator in physiological process and a diffusible free radical in pathological conditions, reacts with superoxide anion

and form a potentially cytotoxic molecule, the peroxynitrite (ONOO⁻). Its protonated form, peroxynitrous acid (ONOOH) is a very strong oxidant (ref.6)

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

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 sodium nitroprusside, 2.5mL phosphate buffered saline pH 7.4 was added. 1mL of extracts at various concentrations were added to the above solution and the mixture was incubated at 25°C for 30min. To 1.5mL of the incubated mixture add 1mL of sulphanilamide in phosphoric acid and 0.5mL of naphthyl ethylene diamine dihydrochloride. The absorbance was measured at 546nm. Ascorbic acid was used as a standard. The percentage inhibition of nitric oxide radical generated was calculated using the following formula:

$$\% \text{ inhibition} = [(\text{Control}-\text{Test})/\text{Control}] \times 100.$$

The IC₅₀ was calculated using linear regression analysis. The results were presented in **Table 14** and **Fig. 23**.

Method 3: Scavenging of hydrogen peroxide activity ^[61, 62, 66]

Principle

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol(-SH) groups. Hydrogen peroxide can probably react with Fe⁺² and possibly Cu⁺² to form hydroxyl radical and this may be

the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

Hydrogen peroxide

Procedure

A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230nm using a spectrophotometer. Extracts (0.1-1mg/mL) in distilled water were added to hydrogen peroxide solution. (0.6 ml 4mM). The absorbance of hydrogen peroxide at 230nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The standard vitamin C was treated in a similar manner. The results are tabulated in **Table 15** and **Fig. 24**.

RESULTS AND DISCUSSION

Method 1: Diphenyl picryl hydrazyl (DPPH) method

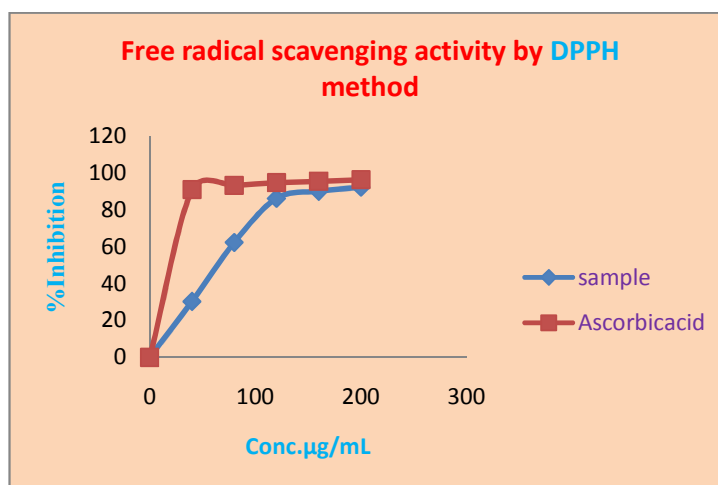
The results obtained for radical scavenging activity against DPPH is presented in **Table 13**. From the table, it can be seen that the ethanolic extract of *J. glauca* showed a percentage inhibition of 92.36 ± 0.42 while ascorbic acid showed a percentage inhibition of 96.38 ± 0.36 at a concentration of $200 \mu\text{g/mL}$. The IC_{50} value calculated using the linear regression analysis was found to be **19.69** and **78.46 $\mu\text{g/mL}$** for ethanolic extract and ascorbic acid respectively (**Fig.22**). The extract possessed a good radical scavenging capacity.

Table 13: Percentage inhibition of ethanolic extract of *J. glauca* and standard ascorbic acid against DPPH at 517nm

| S. No. | Conc. in $\mu\text{g/mL}$ | Percentage inhibition by ascorbic acid | Percentage inhibition by <i>J. glauca</i> |
|--------|---------------------------|---|---|
| 1 | 40 | 91.06 \pm 0.15 | 30.33 \pm 0.32 |
| 2 | 80 | 93.27 \pm 0.26 | 62.36 \pm 0.28 |
| 3 | 120 | 94.77 \pm 0.23 | 86.28 \pm 0.29 |
| 4 | 160 | 95.58 \pm 0.46 | 90.17 \pm 0.38 |
| 5 | 200 | 96.38 \pm 0.36 | 92.36 \pm 0.42 |
| | IC₅₀ | 19.69$\mu\text{g/mL}$ | 78.46$\mu\text{g/mL}$ |

*mean of three readings \pm SEM

Fig. 22: Free radical scavenging of 70% ethanolic extract of *J. glauca* and ascorbic acid against DPPH



Method 2: Nitric oxide scavenging activity

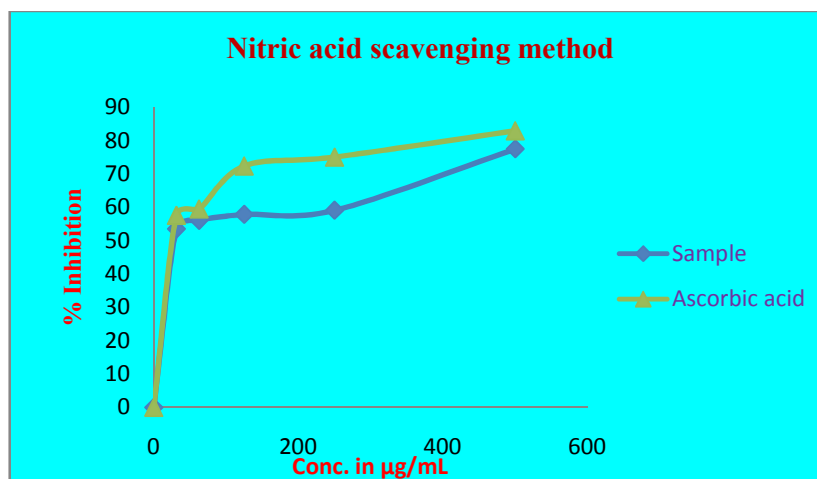
The results obtained for the free radical scavenging activity against nitric oxide radical are presented in **Table 14**. From the table, it can be seen that the ethanolic extract of *J. glauca* showed a percentage inhibition of 77.45 ± 0.05 while ascorbic acid showed a percentage inhibition of 82.94 ± 0.27 at a concentration of $500 \mu\text{g/mL}$. The IC_{50} value calculated using the linear regression analysis was found to be **155.26** and **87.61 $\mu\text{g/mL}$** (**Fig. 23**) for ethanolic extract and ascorbic acid respectively

Table 14: Percentage inhibition of ethanolic extract of *Justicia glauca* against nitric oxide at 546nm

| S. No. | Conc. in $\mu\text{g/mL}$ | Percentage inhibition by ascorbic acid | Percentage inhibition by extract |
|--------|---------------------------|---|--|
| 1 | 31.25 | 57.58 \pm 0.56 | 53.44 \pm 0.46 |
| 2 | 62.5 | 59.47 \pm 0.24 | 56.06 \pm 0.22 |
| 4 | 125 | 72.38 \pm 0.37 | 57.83 \pm 0.32 |
| 5 | 250 | 75.04 \pm 0.45 | 59.08 \pm 0.38 |
| 6 | 500 | 82.94 \pm 0.27 | 77.45 \pm 0.05 |
| | IC ₅₀ | 87.61$\mu\text{g/mL}$ | 155.26$\mu\text{g/mL}$ |

*mean of three readings \pm SEM

Fig. 23: Nitric oxide radical scavenging by 70% ethanolic extract of *J. glauca* and ascorbic acid



Method 3: Determination of scavenging activity against hydrogen peroxide

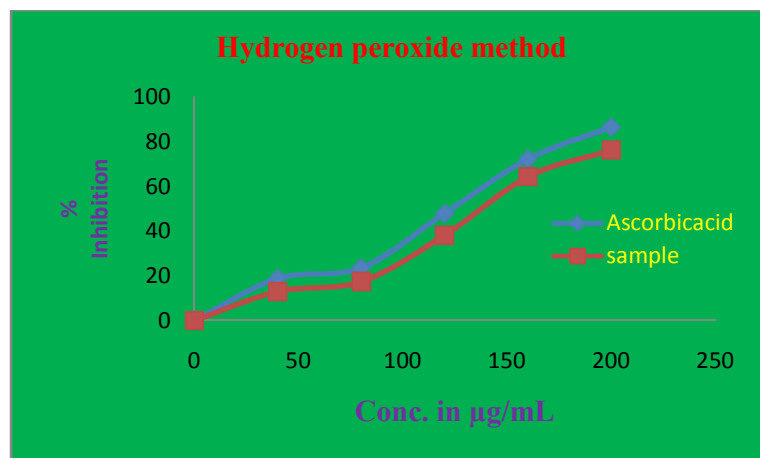
The results obtained for the scavenging activity against hydrogen peroxide are presented in **Table 15**. From the table, it can be seen that the ethanolic extract of *J. glauca* showed a percentage inhibition of 78.12 \pm 0.36 while ascorbic acid showed a percentage inhibition of 76.29 \pm 0.85 at a concentration of 200 $\mu\text{g/mL}$. The IC₅₀ value calculated using the linear regression analysis was found to be **192.16** and **119.55 $\mu\text{g/mL}$** (**Fig. 24**) for ethanolic extract and ascorbic acid respectively.

Table 15: Percentage inhibition of hydrogen peroxide by ethanolic extract of *Justicia glauca*

| S. No. | Conc. in $\mu\text{g/mL}$ | Percentage inhibition | |
|--------|---------------------------|--|---|
| | | Ethanolic extract of <i>J. glauca</i> | Ascorbic acid |
| 1 | 40 | 38.56 \pm 0.12 | 12.92 \pm 0.30 |
| 2 | 80 | 47.21 \pm 0.25 | 17.40 \pm 0.53 |
| 3 | 120 | 59.76 \pm 0.32 | 38.12 \pm 0.96 |
| 4 | 160 | 65.42 \pm 0.23 | 64.37 \pm 0.40 |
| 5 | 200 | 78.12 \pm 0.36 | 76.29 \pm 0.85 |
| | IC ₅₀ | 192.16$\mu\text{g/mL}$ | 119.55 $\mu\text{g/mL}$ |

*mean of three readings \pm SEM

Fig. 24: Scavenging activity by hydrogen peroxide method



SECTION - B

CARDIOPROTECTIVE EFFECT OF LEAF EXTRACT OF *JUSTICIA GLAUCA* ON DOXORUBICIN INDUCED CARDIOTOXICITY IN RATS ^[67-76]

Doxorubicin is an effective and broad spectrum antineoplastic agent used in the treatment of a variety of haematologic and solid malignancies, such as leukaemias, bladder, lung and breast cancers, Hodgkin's and non-hodgkin's lymphomas. Its clinical uses are often limited by its adverse effect namely cardiotoxicity. An initial acute effect includes hypotension and transient ECG abnormalities reported upto 41% of patients. The chronic effect may occur several weeks or months after cumulative doxorubicin administration and occurrence is dose dependent cardiomyopathy which accounts for as high as 50% mortality within two years after diagnosis. The high level of doxorubicin could damage membranes, proteins eg. enzymes, structural and receptors and DNA that may leads to cardiac dysfunction and apoptosis . Administration of the doxorubicin at 15mg/kg body weight of albino rats leads to cardiomyopathy and heart failure.

MATERIALS AND METHODS

Animals

Male albino rats (150-200g) were procured from the Central Animal House, Institute of Pharmacology, Madurai Medical College. The animals were housed under standard conditions of temperature ($25^{\circ} \pm 2^{\circ}\text{C}$) and photoperiod of 12h dark/light cycle with food and water *ad libitum*. The Institutional Animal Ethical Committee clearance was obtained and the experimental study was conducted according to the protocol approved by them.

Chemicals and reagents

- ✓ CK-MB Diagnostic Kit
- ✓ LDH Diagnostic Kit
- ✓ Tris HCl Buffer
- ✓ Thiobarbituric acid (TBARS)
- ✓ DTNB Reagent
- ✓ Doxorubicin - reconstituted with sterile water for injection administered intraperitoneally to rats at a dose of 15mg/kg to induce cardiotoxicity.

Experimental Protocol

The animals were divided into 4 groups and each group consists of six animals (n=6). Group I and II animals were administered 1% CMC alone while group III and IV animals were administered with ethanolic extract of *Justicia glauca* - (suspension in 1% CMC) at 100mg/kg and 200mg/kg doses respectively. On the fifth day, group III, IV animals were administered extracts an hour prior to the administration of doxorubicin

| GROUP | TREATMENT |
|-------|---|
| I | 1% CMC in distilled water for five consecutive days orally |
| II | Single dose for doxorubicin at 15mg/kg IP was administered on fifth day alone. |
| III | EJG at 100mg/kg po was administered for 5 consecutive days and doxorubicin 15mg/kg IP on the fifth day alone. |
| IV | EJG at 200mg/kg po was administered for 5 consecutive days and doxorubicin 15mg/kg IP on the fifth day alone. |

After 24 hours of doxorubicin administration blood samples were collected. All the animals were sacrificed as per the CPCSEA guidelines. The heart was isolated and used for histopathological and *in vivo* antioxidant studies.

Parameters evaluated

- **Serum parameters**

CK-MB and LDH

- **Estimation of *in vivo* antioxidants in cardiac tissue homogenate**

TBARS, GSH and Total protein.

- **Histopathological studies**

Estimation of serum parameters

The marker enzymes CK-MB and LDH were assayed in serum using Standard Kits. The results obtained are presented in the **Table 16** and the graphical representation is presented in **Figs. 25 & 26**.

Estimation of *in vivo* antioxidants

Preparation of tissue homogenate

The isolated heart was washed and blotted in a filter paper. A10% tissue homogenate was prepared by using chilled 0.15M TRIS HCl (pH 7.4) buffer and centrifuged at 5000rpm for 5min. The clear supernatant liquid was used for the estimation TBARS, GSH and total protein.

Estimation of lipid peroxidation (TBARS)

The lipid peroxidation was estimated by the method described by Rajkumar D.V. *et al.* To 1mL of the tissue homogenate, 2.5mL of trichloroacetic acid (20%) were added and centrifuged. 2.5mL of 0.05M sulphuric acid and 3.5mL thiobarbituric acid were added to the supernatant liquid and incubated at 37°C for 30min. It was then extracted with n-butanol. The intensity of the chromogen in the layer was measured at

530nm using UV spectrophotometer using n-butanol as blank. The results obtained are presented in the **Table 17** and the graphical representation is presented in **Fig. 27**.

Estimation of reduced glutathione (GSH)

The reduced glutathione was estimated by the method described by Morn *et al.*, (1979). 1mL of tissue homogenate was precipitated with 1mL of 10% TCA and was centrifuged to remove the precipitate. To 0.5mL of the supernatant added 2mL of 0.6 mM 5,5'-dithiobis-2-nitrobenzoic acid(DTNB) in 0.2M sodium phosphate and the total volume was made upto 3mL with 0.2M phosphate buffer (pH 8). The absorbance was read out at 412nm. The results obtained are presented in **Table 17** and the graphical representation of the same are presented in **Fig 28**.

Histopathological studies

The hearts isolated from each group were preserved in 10% formalin, processed and embedded in paraffin blocks. A four μ m thick paraffin section were cut and stained with eosin and hematoxylin over a glass slide. They were observed under light microscope and the pathological changes were recorded and presented in **Fig. 29**.

Statistical Analysis

All the data were expressed as standard error of mean (SEM). Data of biochemical parameters were analyzed using one way ANOVA. Tukeys's multiple range test was applied for post-hoc analysis. A value of $p < 0.001$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The results obtained for the serum biochemical parameters are presented in **Table 16**. The creatine kinase (CK MB) and lactate dehydrogenase (LDB) values were determined. The ethanolic extract treated animals at a dose of 200mg/kg body weight

showed significant reduction of the elevated levels of CK-MB and LDH in doxorubicin treated animals. The value obtained was close the normal animals.

Table 16: Effect of ethanolic extract of *J. glauca* on serum biochemical parameters

| Groups | CK-MB IU | LDH IU |
|-----------------|----------------|----------------|
| Control | 129.25 ± 1.38 | 105.07 ± 1.28 |
| Doxorubicin(15) | 280.56± 0.57 | 222.65± 0.92 |
| EJG(100) | 198.84±1.53* | 178.25 ± 1.33* |
| EJG(200) | 139.57 ± 0.71* | 134.6 ± 1.37* |

- Note :** 1. Values expressed as mean SEM *p<0.001 when compared with doxorubicin treated group
 2. Digits in parentheses indicate dose in mg/kg
 3. CK-MB- Creatine kinase - MB,
 4. LDH - Lactate dehydrogenase
 5. EJG - Ethanolic extract(70%) of *Justicia glauca*

Fig 25: Effect of ethanolic extract of *J. glauca* on serum biochemical parameter - CK-MB in normal & doxorubicin induced cardiotoxic animals

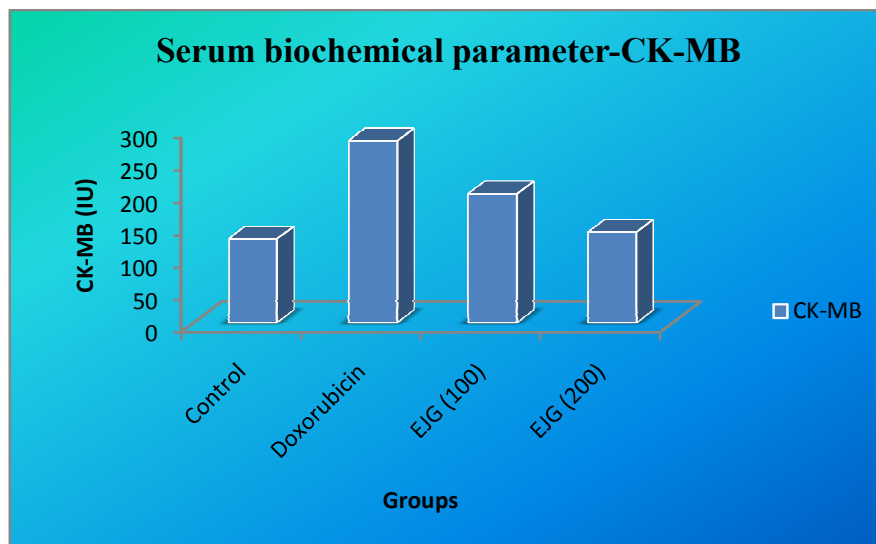
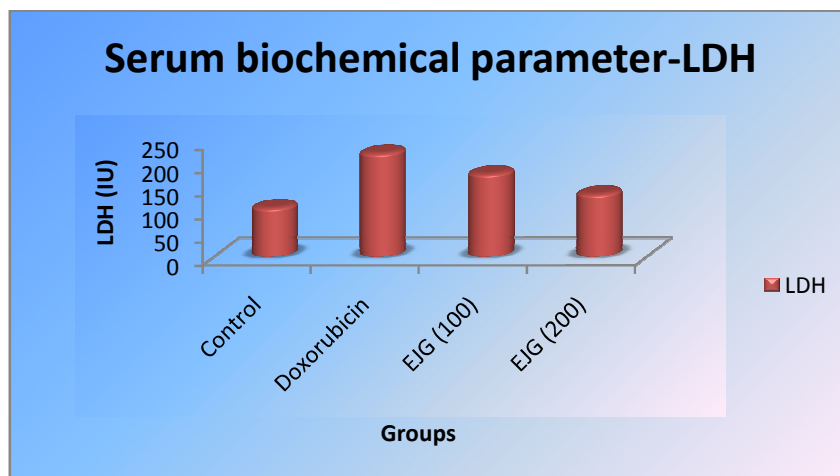


Fig 26: Effect of ethanolic extract of *J. glauca* on serum biochemical parameter - LDH in normal & doxorubicin induced cardiotoxic animals



The results obtained for the tissue biochemical parameters are presented in **Table 17**. The TBARS and GSH values were determined. The ethanolic extract treated animals at a dose of 200mg/kg body weight showed significant reduction of the elevated levels of TBARS and GSH in doxorubicin treated animals. The value obtained was close the normal animals. The values obtained were statistically significant ($p < 0.001$) [77, 78]

Table 17: Effect of ethanolic extract of *J. glauca* on tissue biochemical parameters

| Groups | TBARS nmoles/g protein | GSH nmoles/g protein |
|-------------|------------------------|----------------------|
| Control | 69.51 ± 0.67 | 2.86 ± 0.3 |
| Doxorubicin | 125.11 ± 0.67 | 5.01 ± 0.2 |
| EJG(100) | 104.96 ± 1.1* | 2.68 ± 0.2* |
| EJG(200) | 81.98 ± 0.91* | 2.38 ± 0.1* |

Note: 1. Values expressed as mean SEM * $p < 0.001$ when compared with Doxorubicin treated group
 2. Digits in parentheses indicate dose in mg/kg
 3. TBARS – Thiobarbituric acid
 4. GSH - Reduced glutathione

Fig 27: Effect of ethanolic extract of *J. glauca* on tissue biochemical parameter – TBARS in normal & doxorubicin induced cardiotoxic animals

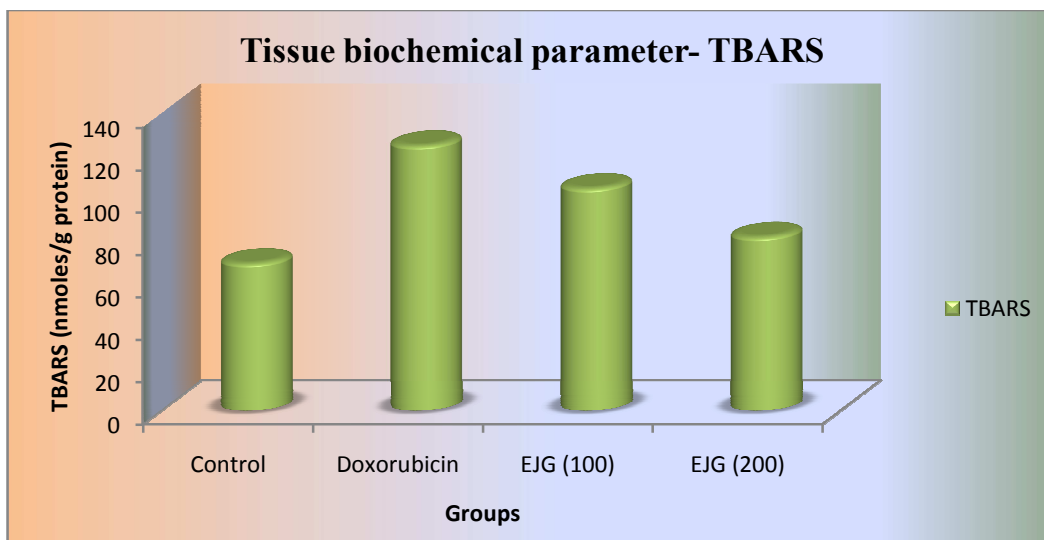
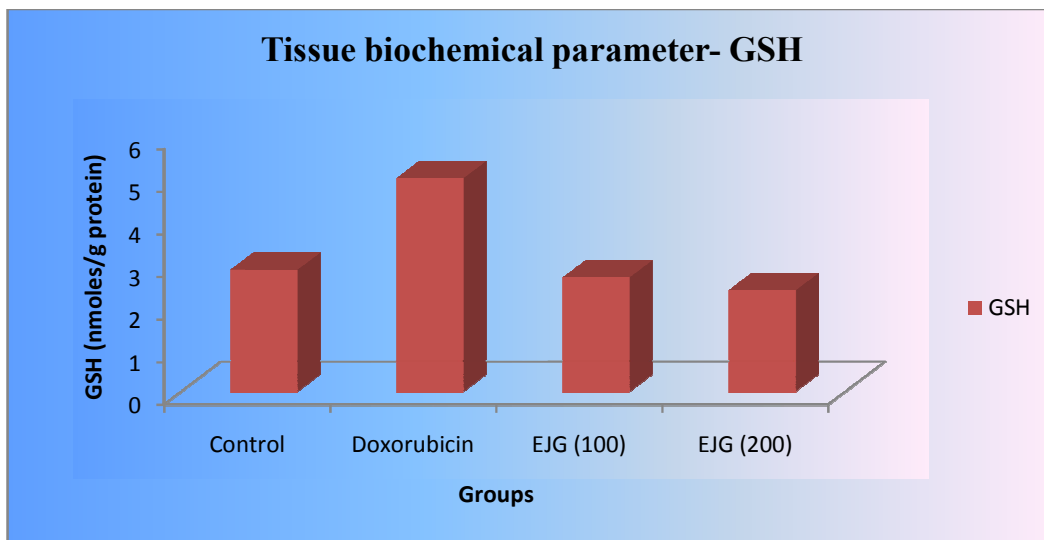


Fig 28: Effect of ethanolic extract of *J. glauca* on tissue biochemical parameter – GSH in normal & doxorubicin induced cardiotoxic animals



Histopathology (Fig. 29)

1. **Control group** – the section of the heart of the normal animals showed normal myocardial structure.
2. **Doxorubicin treated group** – the section of the heart of the doxorubicin treated animals showed destruction of cardiac muscle

3. **Extract treated group (100mg/kg bw)** - the section of the heart of the extract treated animals showed moderate pathology of the myocardial structure.
4. **Extract treated group (200mg/kg bw)** - the section of the heart of the extract treated animals showed normal myocardial structure.

Doxorubicin used to treat a variety of malignancies such as leukemia, breast and ovarian cancer, Hodgkin and non Hodgkin lymphomas. The major limitation is the development of cardiotoxicity. The adverse effects are dose dependent and recent studies have suggested that the doxorubicin induced toxicity involves the generation of ROS especially superoxide anion and hydrogen peroxide (1-3) which causes cellular damage. The cellular damage includes lipid peroxidation, protein cross linking, DNA fragmentation leading to cardiac dysfunction, apoptosis and cardiomyopathy. Oxidative stress, lipid peroxidation and generation of free radical induced by doxorubicin leads to increased CK-MB, LDH, TBARS level. (4-7).

Alleviation of toxicity can be achieved by administration of antioxidants like vitamins E and C but their effects remain controversial (8-10). Ascorbic acid protects against lipid damage but not against protein damage (11). Hence a search for a good cardioprotective agent against doxorubicin induced cardiotoxicity.

The extract treated animals resulted in decrease of elevated endogenous CK-MB, LDH and TBARS. The extract has been reported for the presence of tannins phenolics and flavonoids which may be attributed to the cardioprotective effect

The extract also showed potential *in vitro* antioxidant activity by DPPH assay, nitric oxide scavenging activity and hydrogen peroxide scavenging activity. The mechanism behind the cardiotoxicity is free radical generation and oxidative stress. Since the plant extract also exhibited *in vivo* antioxidant activity.

Hence the result suggested that the cardioprotective effect against doxorubicin induced cardiotoxicity at 100mg/kg & 200mg/kg of EGJ.

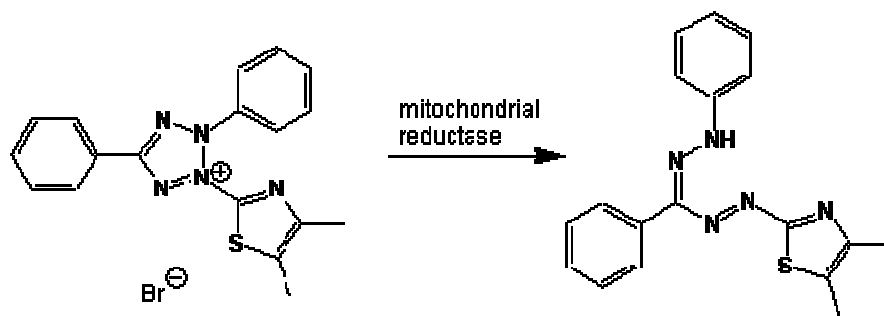
SECTION - C

IN VITRO ANTI CANCER ACTIVITY [79-82]

Cancer is an abnormal growth of cells which tend to proliferate in an uncontrolled way and in some cases spread to other parts of the body.

MTT ASSAY**Principle**

MTT assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethyl thiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured(dark purple) formazan product. The cells are then solubilised with an organic solvent eg.isopropanol and the released,solubilized formazan reagent is measured spectrophotometrically^[81,82] Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

**Methodology**

The human stomach adenocarcinoma cell line (AGS) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Hams F12 K medium containing 10% fetal bovine serum (FBS). All cells were maintained at 37⁰C, 5%

CO₂, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediamine tetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1×10^5 cells/ml. one hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37⁰C, 5% CO₂, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five drug concentrations. Aliquots of 100 µl of these different drug dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final drug concentrations. Following drug addition the plates were incubated for 48 h at 37⁰C, 5% CO₂, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37⁰C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

$$\% \text{ cell Inhibition} = 100 - \text{Abs (sample)}/\text{Abs (control)} \times 100.$$

Nonlinear regression graph was plotted between % Cell inhibition and Log₁₀ concentration and IC₅₀ was determined using Graph Pad Prism software.

The results obtained for the MTT assay is presented in **Table 18 and Figs. 30 & 31.**

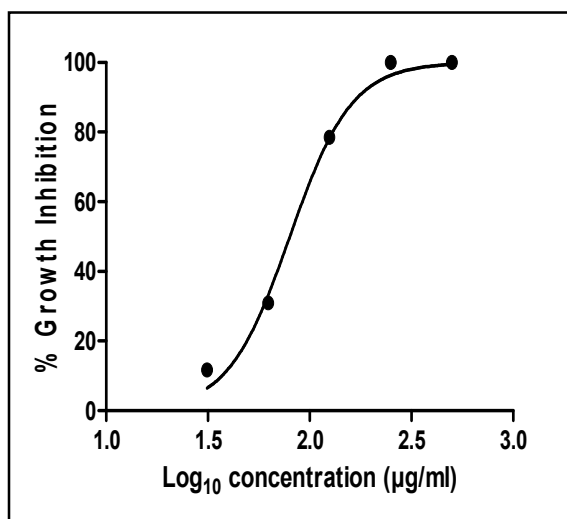
RESULTS AND DISCUSSION

The results obtained for anticancer activity of 70% ethanolic extract of *Justicia glauca* on stomach adenocarcinoma cell lines (AGS) by MTT assay was presented in **Table 18 and Figs. 30 & 31.**

Table.18: Anticancer activity using stomach adenocarcinoma cell lines by ethanolic extract of *Justicia glauca*

| S. No | Conc.in µg/mL | Percentage cell inhibition | IC 50 |
|-------|---------------|----------------------------|-------------|
| 1 | 31.25 | 11.717 | 79.82 µg/mL |
| 2 | 62.5 | 30.95 | |
| 3 | 125 | 78.542 | |
| 4 | 250 | 100 | |
| 5 | 500 | 100 | |

Fig. 30: Anticancer activity using stomach adenocarcinoma cell lines by ethanolic extract of *Justicia glauca*



The amount of formazan crystals produced by MTT is directly proportional to the number of viable cells. A decrease in the cell count was observed as the concentration of the extract increases. There was a dose depended increase in the cytotoxic activity. The extract at low concentration (**31.25 µg/mL**) showed **11.717 %** cell inhibition and at high concentration (**500 µg/mL**) **100%** cell inhibition. The inhibitory concentration (**IC₅₀**) value was found to be **79.82µg**. So the ethanolic extract of *Justicia glauca* possess significant anti cancer activity.

SECTION - D

ANTIBACTERIAL ACTIVITY [83-87]

Many higher plants accumulate extractable organic substances in quantities sufficient to be economically useful as pharmaceutical or antibiotics. Species of higher plants are much less surveyed for antibacterial activity. Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defence against predation or infection.

Alternative to available antibiotics for disease management are increasingly felt due to the increase in the resistance of bacterial isolates. This has necessitated the requirement of second and third line drug. Antibacterial active principles isolated from higher plants appear to be one of the important alternative approaches to contain antibiotic resistance and the management of disease. It is believed that plant based drugs cause less or no side effect when compared with synthetic antibiotics.

Bacterial infection is any type of infection that is caused by bacterial (rather than a virus). Bacteria are very common in our bodies and in the world around us. Many of them are helpful. Less than 1% of bacteria will actually makes us sick. when they do make sick it is called as “bacterial infection”.

Bacteria

The various micro-organisms used in the present study include *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus albus* and *Streptocococcus pyogens*. The purity of the cultures prior to their use was checked and confirmed by conventional cultures, morphological and biochemical methods.

Preparation of media

Mull Hinton Agar media

Muller Hinton Agar (MH, Hi media) was used. The formula (gm/litre): Beef -2g, Caesin acid hydrolysate 17.5g, starch 1.5g and agar 17g. MH agar (38g) was weighed and dissolved in 1000ml of distilled water and adjusted to Ph 7.3 ± 0.2 . The medium is sterilized by autoclaving at 121°C for 15 minutes at 15psi pressure and was used for sensitivity tests.

Blood agar media

The formula for the medium is nutrient substrate (heart extract and peptones) - 20 g/liter; sodium chloride - 5g/L; agar-agar - 15g/L. 40g of the above powder was suspended in 1L of purified water and autoclaved for 15min at 121°C and cooled to $45\text{-}50^{\circ}\text{C}$. The medium obtained was clear and yellowish-brown. To the above suspension 5-8% defibrinated blood was added and mixed. The medium was poured into petridishes. This medium was used for studying antibacterial activity against *Streptococcus pyogenes*.

Preparation of bacterial cultures

Few colonies of the bacterial strains selected for study were picked from the agar slopes and inoculated into 4ml peptone water in a test tube and incubated for 2-4 hours to produce suspensions. The suspension were then diluted with saline, if necessary 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.

Drugs

Amikacin (30 μg /disc) was used as standard

Ethanollic extract of *Justicia glauca* (EJG).

Preparation of extract

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 and the petridishes were swirled until the agar begins to set.

Disc Diffusion technique

The pathogenic strains were then seeded evenly all over the plate on the prepared MH agar medium by streaking the plate with the help of a sterile swab and 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 methanolic extract of *J. glauca* dissolved in DMSO. 30µg of amikacin discs was used as a standard. The standard, extract and DMSO discs were then placed on the seeded medium plates. The plates were then incubated at 37°C for 24h. The results were read by the presence or absence of zone of inhibition. The zone of inhibition was then measured and the activity of different concentration of extracts was compared with the standard. The results are tabulated in **Table. 19**. The zone of inhibition obtained in the antibiotic disc diffusion against various microorganisms are presented in **Table 20**. The photographic representations of the antibacterial activity against various organisms are presented in **Figs. 32 to 35**.

Experimental procedure for *Streptococcus pyogens*

The procedure adopted above was followed except the medium used was blood agar medium

RESULTS AND DISCUSSION

Minimum inhibitory concentration (MIC)

MIC is the concentration of antimicrobial required to inhibit the growth of a particular bacterial isolate *in vitro*. Clinically the MIC is used to assign an organism to a susceptibility category. The results obtained for MIC for various organisms are presented in Table 19.

Table 19: MIC of 70%ethanolic extract of *J. glauca* against various microorganisms

| S. No | Name of the organism | Minimum inhibitory concentration (in mg/disc) |
|-------|-------------------------------|---|
| 1. | <i>Escherichia coli</i> | 1.5 |
| 2. | <i>Klebsiella pneumoniae</i> | 1.5 |
| 3. | <i>Proteus mirabilis</i> | 1.5 |
| 4. | <i>Staphylococcus aureus</i> | 1.5 |
| 5 | <i>Staphylococcus albus</i> | 1.5 |
| 6 | <i>Pseudomonas aeruginosa</i> | 1.5 |
| 7 | <i>Streptococcus pyogens</i> | 1.5 |

Table 20: Antibiotic disc diffusion assay against various microorganism

| S. No | Name of the organism | Zone of inhibition (in mm)* | |
|-------|-------------------------------|-----------------------------|----------------------|
| | | Standard | 70%ethanolic extract |
| 1. | <i>Escherichia coli</i> | 26.0 ± 0.0 | 18.0 ± 0.5 |
| 2. | <i>Klebsiella pneumonia</i> | 26.0 ± 1.0 | 22.0 ± 0.5 |
| 3. | <i>Proteus mirabilis</i> | 25.0 ± 0.0 | 21.0± 0.5 |
| 4. | <i>Staphylococcus aureus</i> | 27.0 ± 0.5 | 22.0 ± 0.5 |
| 5. | <i>Staphylococcus albus</i> | 26.0 ± 0.0 | 22.0 ± 0.5 |
| 6. | <i>Pseudomonas aeruginosa</i> | 25.0 ± 0.0 | 20.0 ± 0.5 |
| 7. | <i>Streptococcus pyogens</i> | 28.0 ± 0.0 | 14.0 ± 0.5 |

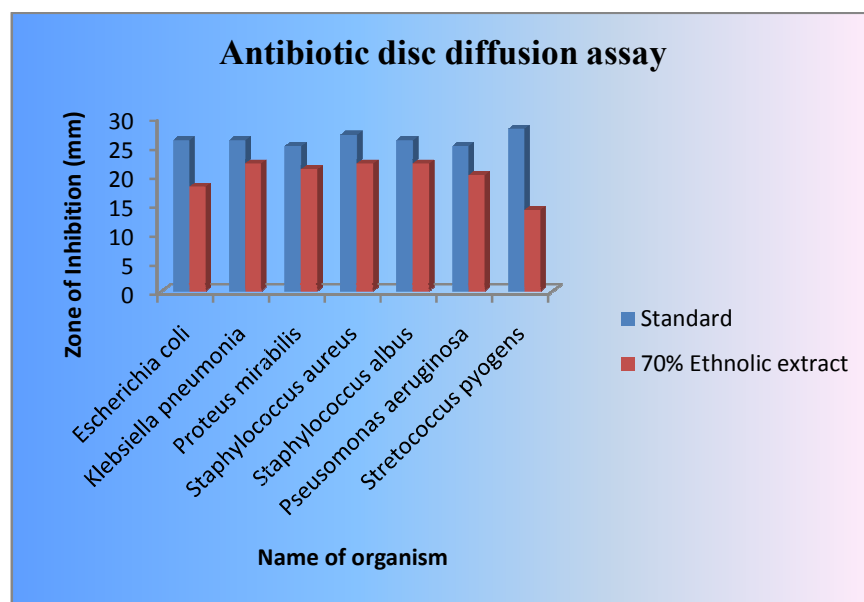
* mean of 2 readings \pm SEM

From the **Table 19**, it can be observed that the MIC for all the seven tested organisms *E. coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Staphylococcus aureus*, *Staphylococcus albus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* were found to be 1.5mg/disc.

The results obtained for the antibiotic disc diffusion technique are presented in **Table 20** and From the **table 19**, it can be observed that the zones of inhibition of the 70% ethanolic extract of *Justicia glauca* for the tested organisms were comparable as that of standard amikacin.

The pictorial representation of zones of inhibition obtained by the ethanolic extract against various micro organisms is presented in **Fig. 36**.

Fig. 36: Zone of inhibition obtained against various organisms



SECTION - E

ANTI FUNGAL ACTIVITY ^[88-91]

Disc diffusion method

Inoculum preparation

The fungal colony to be tested was grown in potato dextrose agar slant at 35° C to induce the conidium and sporangiospore formation. After 7-10 days of incubation with well grown spores, the culture was taken for testing. 5ml of 0.85% sterile saline was added to the culture tube and the suspension were made by gently probing the colonies with the tip of Pasteur pipette. With the help of sterile pipettes, the saline with conidia was transferred in to a sterile screw cap tube. The tube was then vortexed for 30sec to 1min and allowed to stand at room temperature for 5 to 10min for the heavier particles to settle down. The upper homogenous suspensions were collected and the densities of the conidial suspensions were read and adjusted the optical density (OD) to be between 0.09 and 0.11 for *Aspergillus* species, 0.15 to 0.17 for *Fusarium* species by using UV Visible spectrophotometer at 530nm. The suspensions were diluted 1:50 in RPMI 1640 medium. The final concentration of the conidia was 0.2 – 1 X 10⁴ cfu/mL.

Medium

Disc diffusion test was performed on Muller-Hinder agar plates supplemented with 2% glucose and 0.5µg / L Methylene blue.

Preparation of extract

The plant extract of *Justicia glauca* was dissolved in DMSO solution to produced final concentration 10mg, 15mg, 20mg.

Procedure

The entire dried agar surface was evenly streaked in three different directions with a sterile cotton swap dipped into the inoculum suspension. The plate was allowed to dry for 20min. Using a pair of flame sterilized forceps the antifungal discs were applied on to the surface of the inoculated plates. The plates were incubated at 35°C for 48h. The plates were read at 24h and 48h.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was defined as the lowest concentration of extract that allows no more than 20% growth of microbes after incubation on SDA media at 37°C for 24h. The results obtained for the antifungal activity is tabulated in **Table 21** and the pictorial representations are presented in **Figs. 36 to 39**.

RESULTS AND DISCUSSION

The results obtained for antifungal activity is presented in **Table 21**. From the table it can be seen that the Minimum inhibitory concentration for *Aspergillus fumigatus* and *Candida albicans* was 8mg/disc while it was 10mg/disc for *Aspergillus flavus* and for *Epidermophyton floccosum* it was found to be 12mg/disc.

Table 21: Minimum Inhibitory concentration of the ethanolic extract of *J. glauca* against various fungi

| S. No | Name of the fungal isolate | MICin (in mg/disc) |
|-------|---------------------------------|--------------------|
| 1. | <i>Epidermophyton floccosum</i> | 12 |
| 2. | <i>Aspergillus flavus</i> | 10 |
| 3. | <i>Aspergillus fumigates</i> | 08 |
| 4. | <i>Candida albicans</i> | 08 |

CHAPTER-8

SUMMARY AND CONCLUSION

There are many unknown plants with high medicinal value still not been recognised for their importance. They have not been brought to the light of scientific world. This dissertation covers pharmacognostical, phytochemical and pharmacological studies on the commonly available medicinal plant *Justicia glauca* belonging to the family Acanthaceae.

Pharmacognostical parameters have been determined on the leaves in order to substantiate and identify the plant for future work.

Preliminary phytochemical screening carried out on the plant powder and extracts confirmed the presence of flavonoids, phenols, tannins, sterols, alkaloids and carbohydrates. The determination of total phenolics (58.06 mg/g), total flavanoid content (29.35mg/g) and total vitamin C content (15.97mg/g) confirms the significant concentration of these phytoconstituents in the ethanolic extract of *Justicia glauca*. The TLC and HPTLC studies on ethanolic extract of *Justicia glauca* will help us in the fingerprinting the profile of the plant for future reference.

The pharmacological screening confirms

- The ethanolic extract of *Justicia glauca* have exhibited radical scavenging activity by DPPH assay and hydrogen peroxide method shown potent antioxidant activity.
- Significant cardioprotective activity at 100mg/kg of S (p<0.001), 200 mg/kg of EJS (p<0.001) against Doxorubicin induced cardiotoxicity in rats and the *in vivo* antioxidant activity of these extracts may be ascribed for this cardioprotective activity which is confirmed by elevation of GSH

($p < 0.001$) and reduction of TBARS ($p < 0.001$) in the tissue parameters of the treated animals.

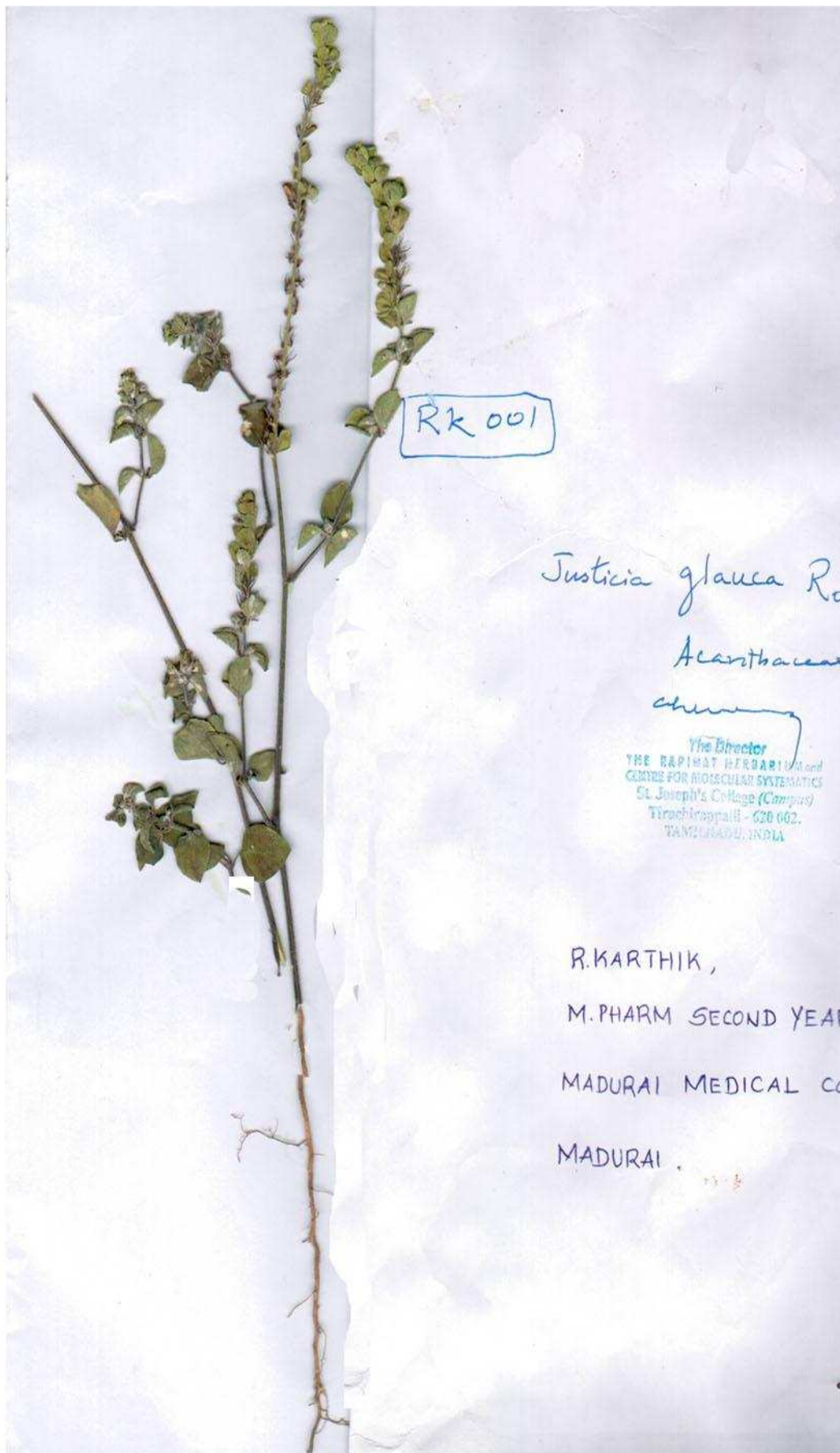
- Significant anti-bacterial activity exhibited by ethanolic extract (1.5mg/ disc) against *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Staphylococcus aureus*, *Staphylococcus albus* and *Pseudomonas aeruginosa*.
- Significant anti-fungal activity exhibited by ethanolic extract (8/ disc) against *Aspergillus flavus* and *Candida albicans*.

It was evident from the phytochemical studies of this plant, that substantial amount of phenols and flavanoids were present in these extracts which exhibited significant invitro antioxidant activity.

Cardiomyopathy may be due to viruses, amyloidosis, diabetes, thyroid diseases, medications such as chemotherapy drugs (Doxorubicin, Donorubicin, etc). Though many synthetic cardioprotective agents are available, herbal drugs are preferred for their safety and efficacy. Cardiotoxicity was induced in the animal model using Doxorubicin (antineoplastic agent). Doxorubicin cause oxidative stress leads to cardiac tissue damage at 15mg/kg i.p in rats. The extract has shown significant cardioprotective action and anti-bacterial activity against the pathogenic organisms.

The ethanolic extract of *Justicia glauca* may serve as a lead medicinal plant to synthesise various semi-synthetic drugs to treat various life threatening diseases like congestive heart failure, cancer, bacterial. fungal infections.

Fig. 1: Herbarium of the Plant



RK 001

Justicia glauca Ro.
Acanthaceae

chinn

The Director
THE RAJAMAT HERBARIUM and
CENTRE FOR MOLECULAR SYSTEMATICS
St. Joseph's College (Campus)
Tiruchirappalli - 620 002.
TAMILNADU, INDIA

R.KARTHIK,
M.PHARM SECOND YEAR
MADURAI MEDICAL COL
MADURAI

Fig . 2.1: Habitat



Fig. 2.2: Leaf



Fig. 2.3 : Flower



Fig. 2.4: Leaf dorsal view



Fig. 2.5: Leaf ventral view



Fig. 3.1: T.S of leaf through midrib

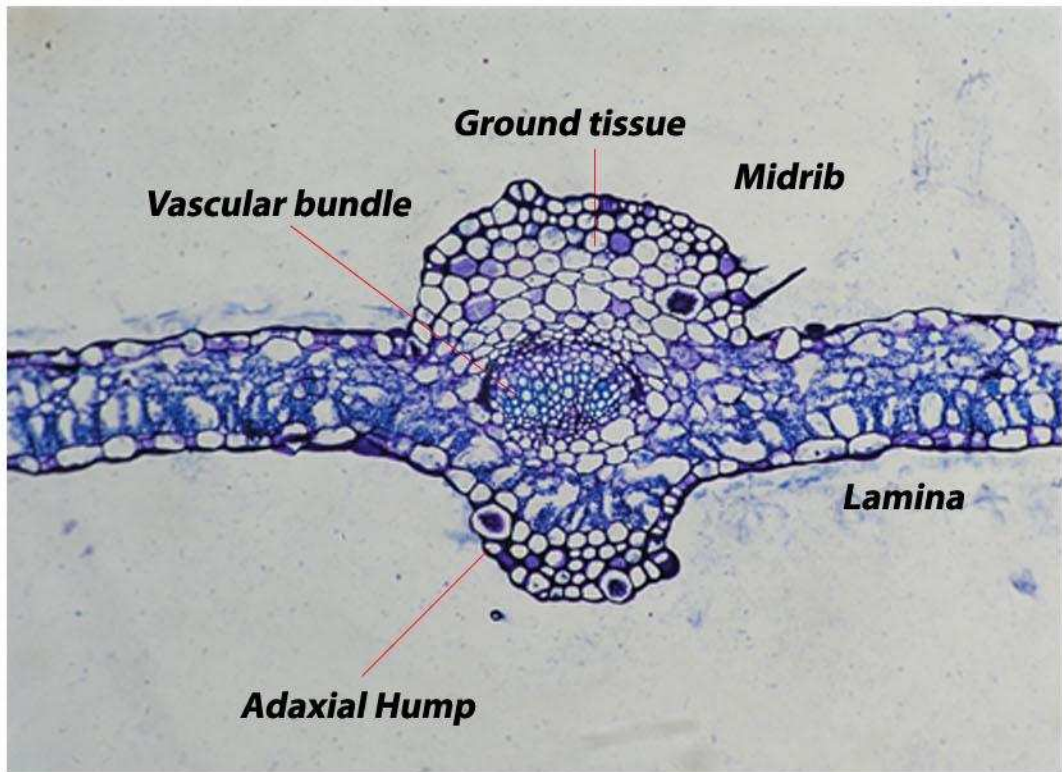


Fig. 3.2: T.S of midrib enlarged

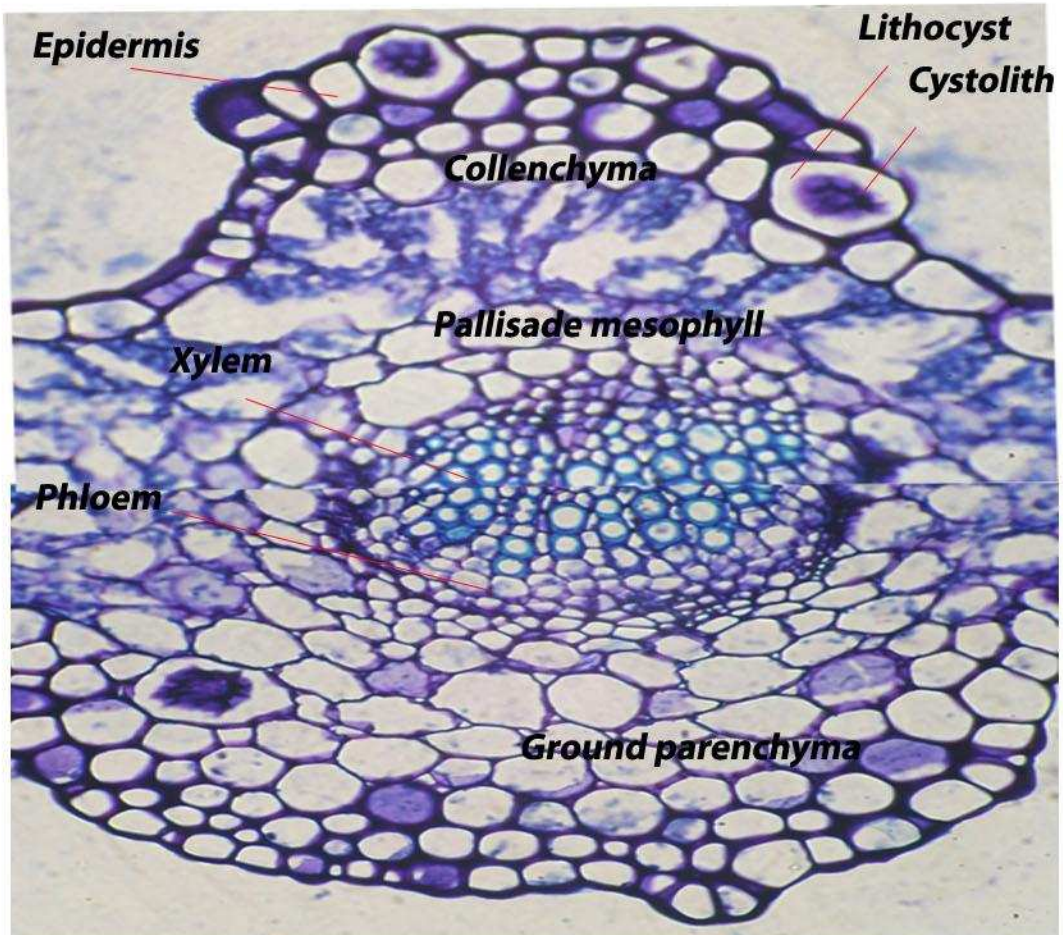


Fig. 4.1: T.S of lamina

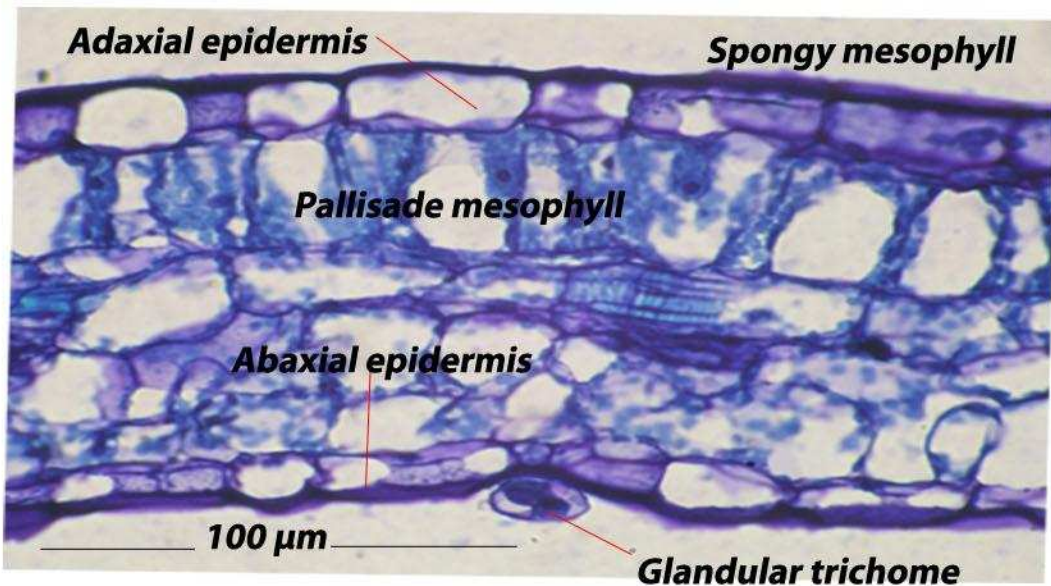


Fig. 4.2: T.S of leaf margin

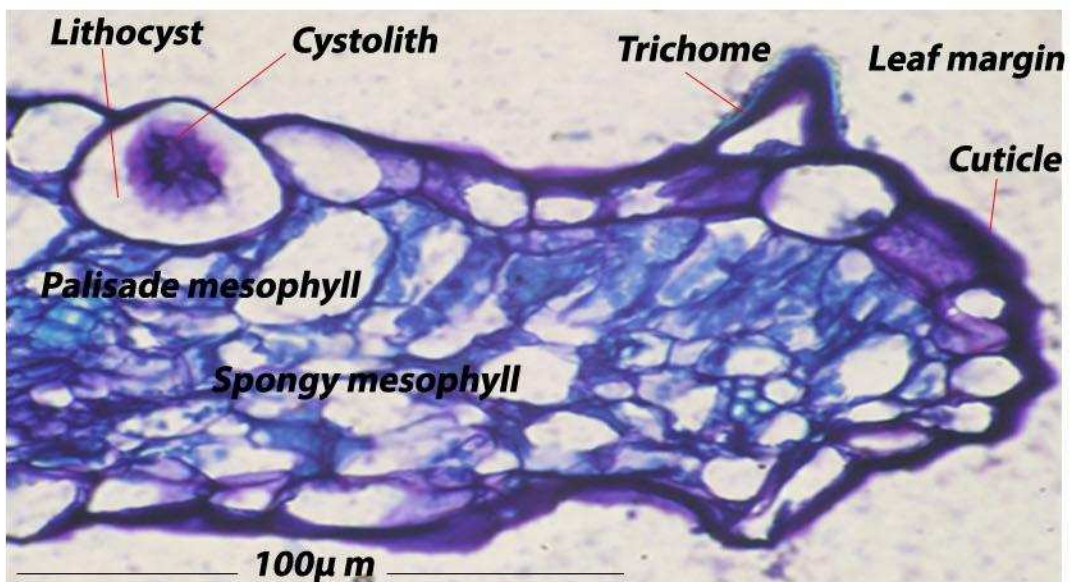


Fig. 4.3: T.S of leaf showing adaxial stomata

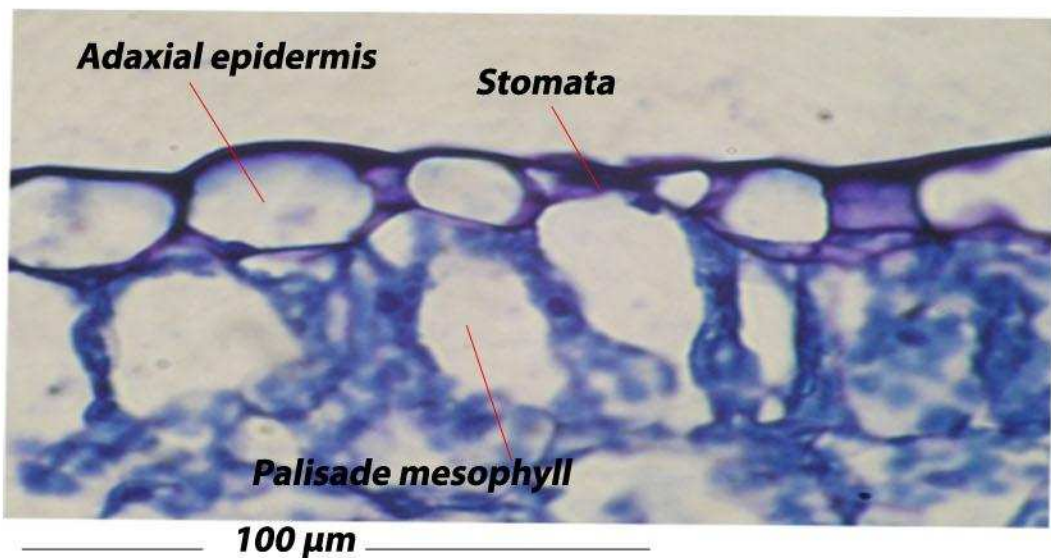


Fig. 7.1: T.S of Petiole entire view

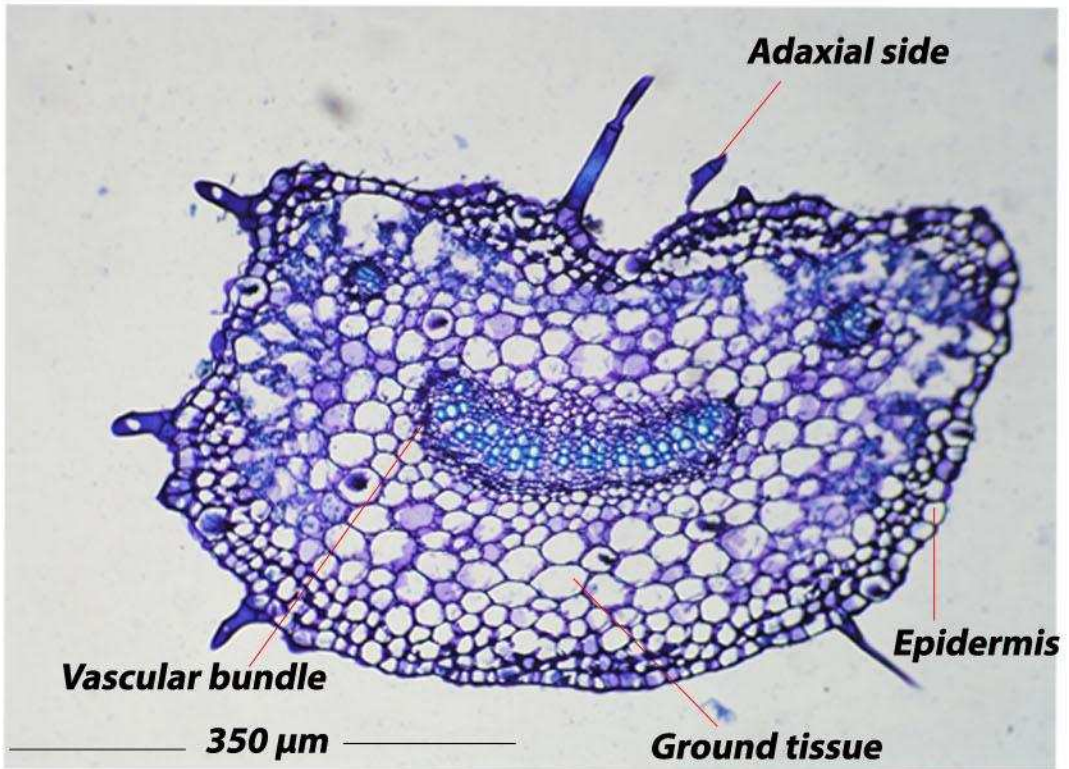


Fig. 7.2: T.S of Petiole a sector large

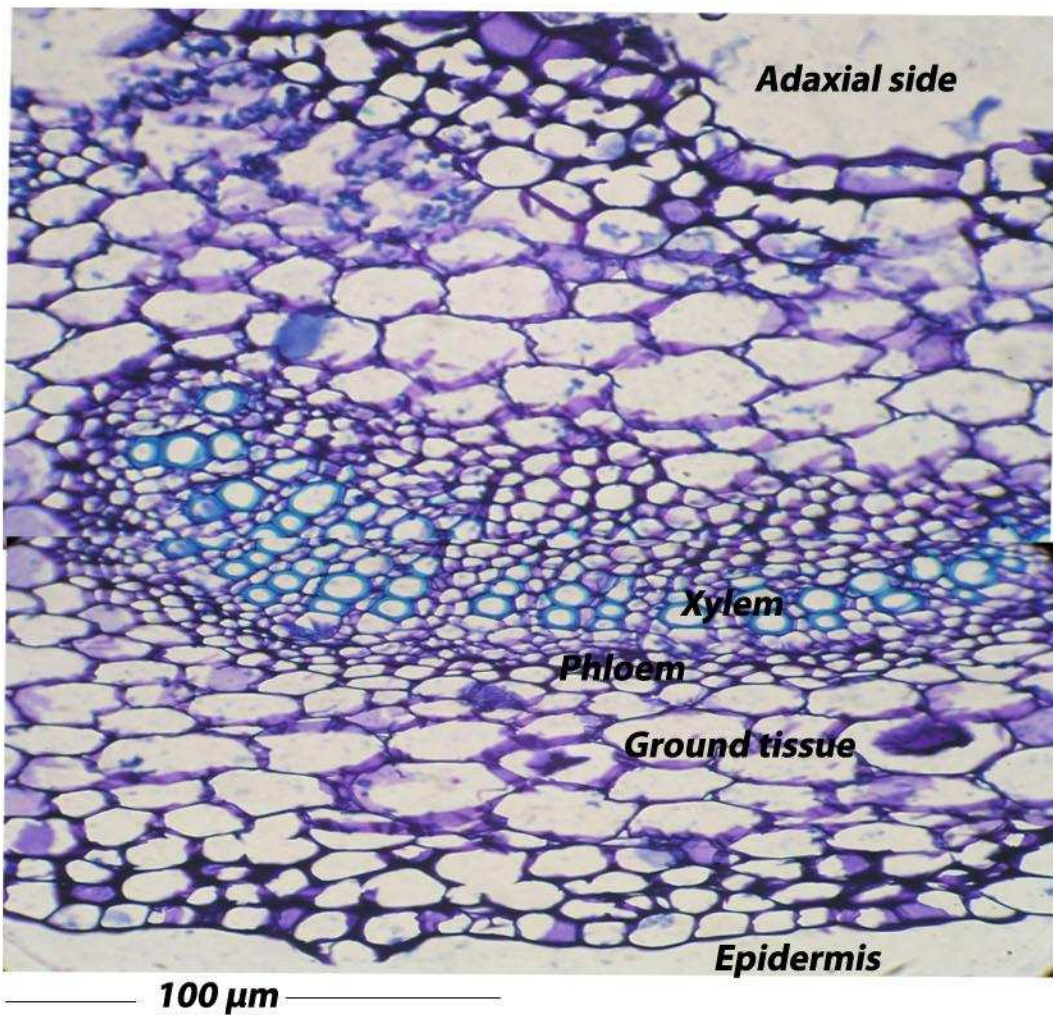


Fig. 8.1: T.S of stem entire view

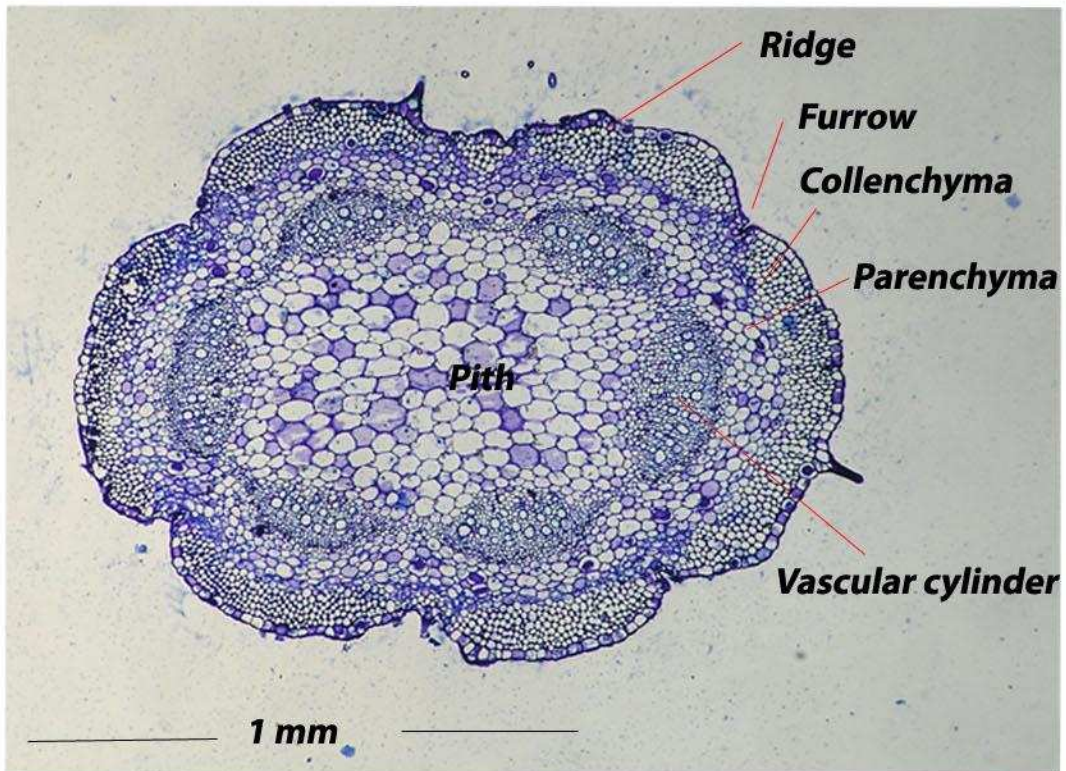


Fig. 8.2: T.S of stem a sector enlarged

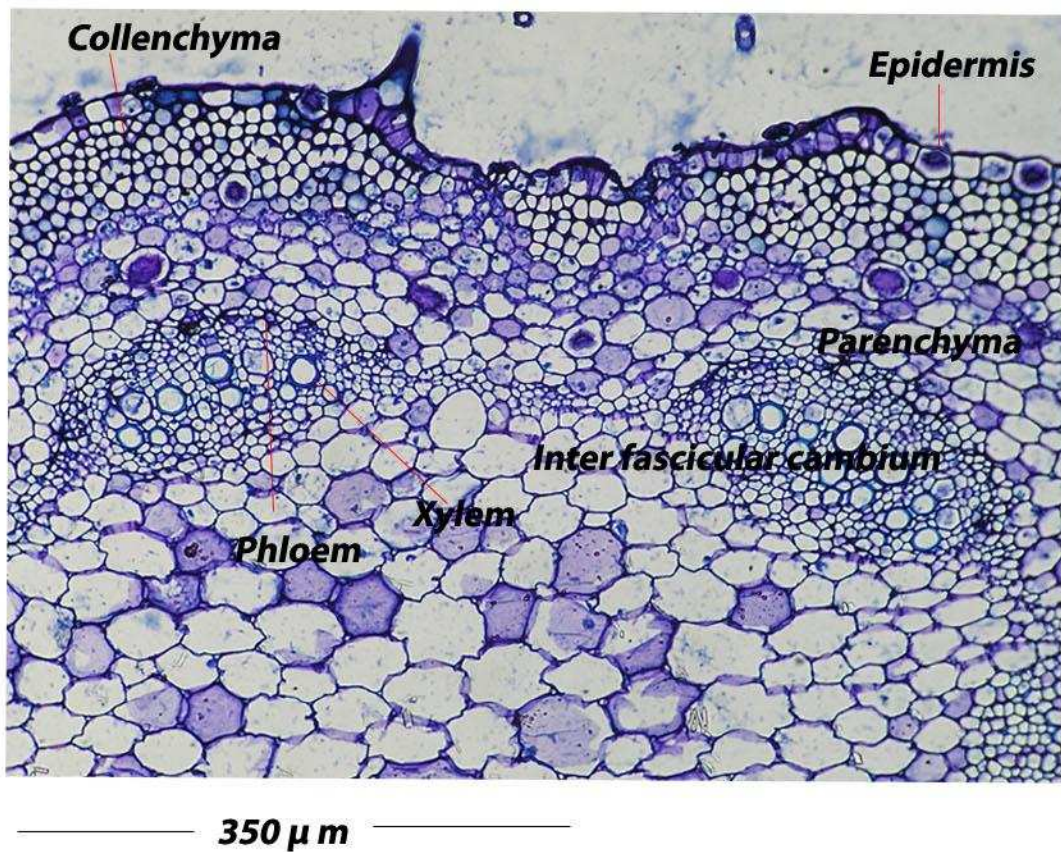


Fig. 9 : T.S of stem showing cortex and vascular segments

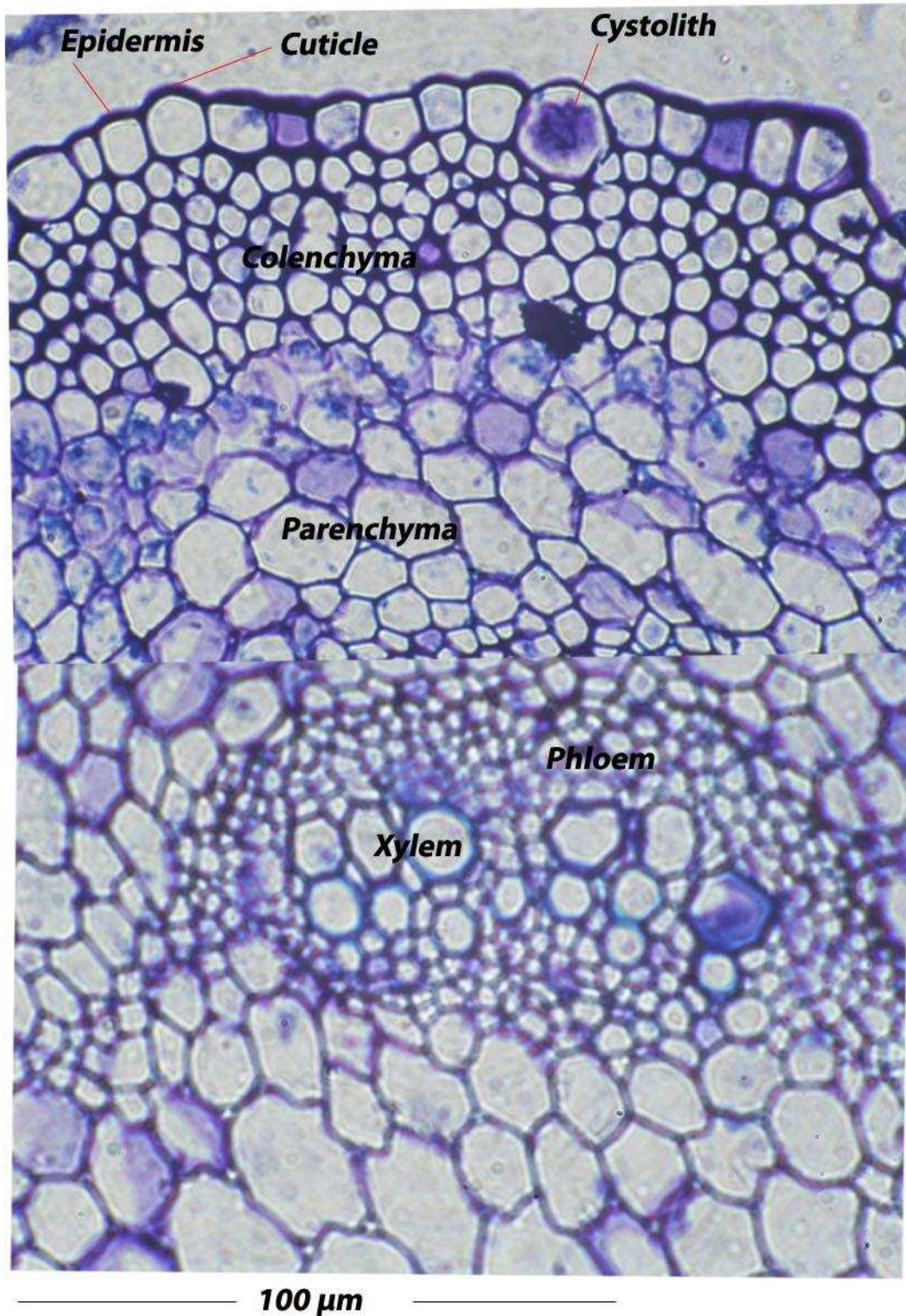


Fig. 10.1: T.S of thin Root

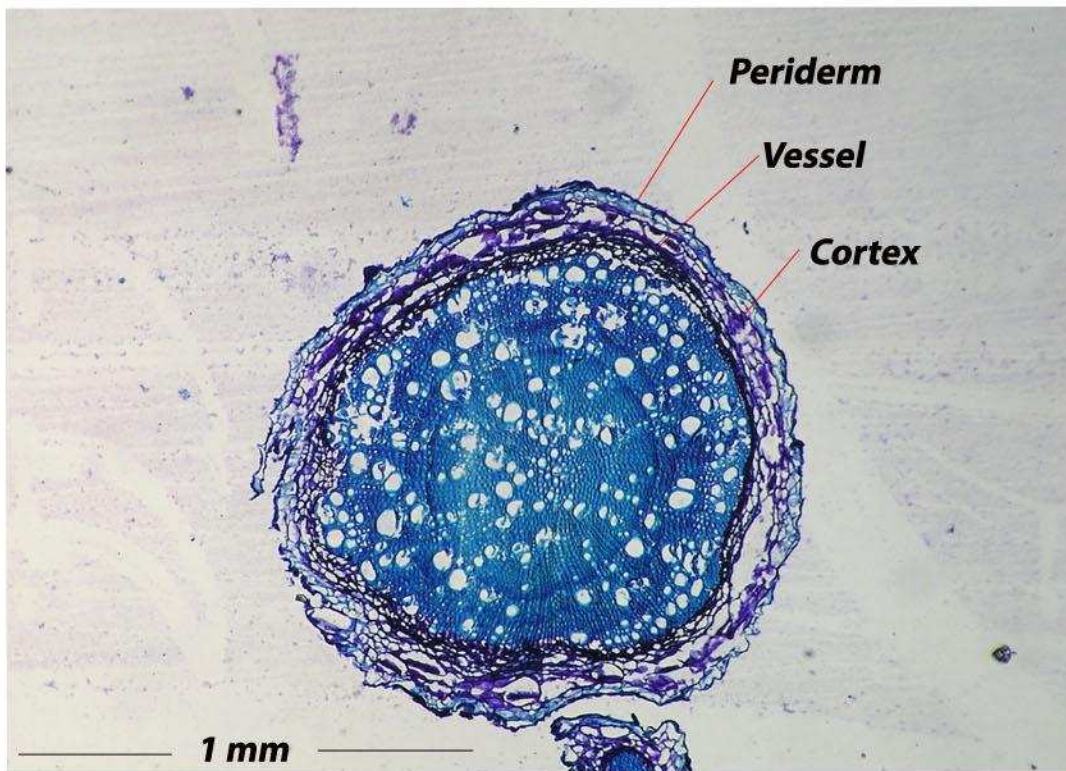


Fig. 10.2: A sector enlarged

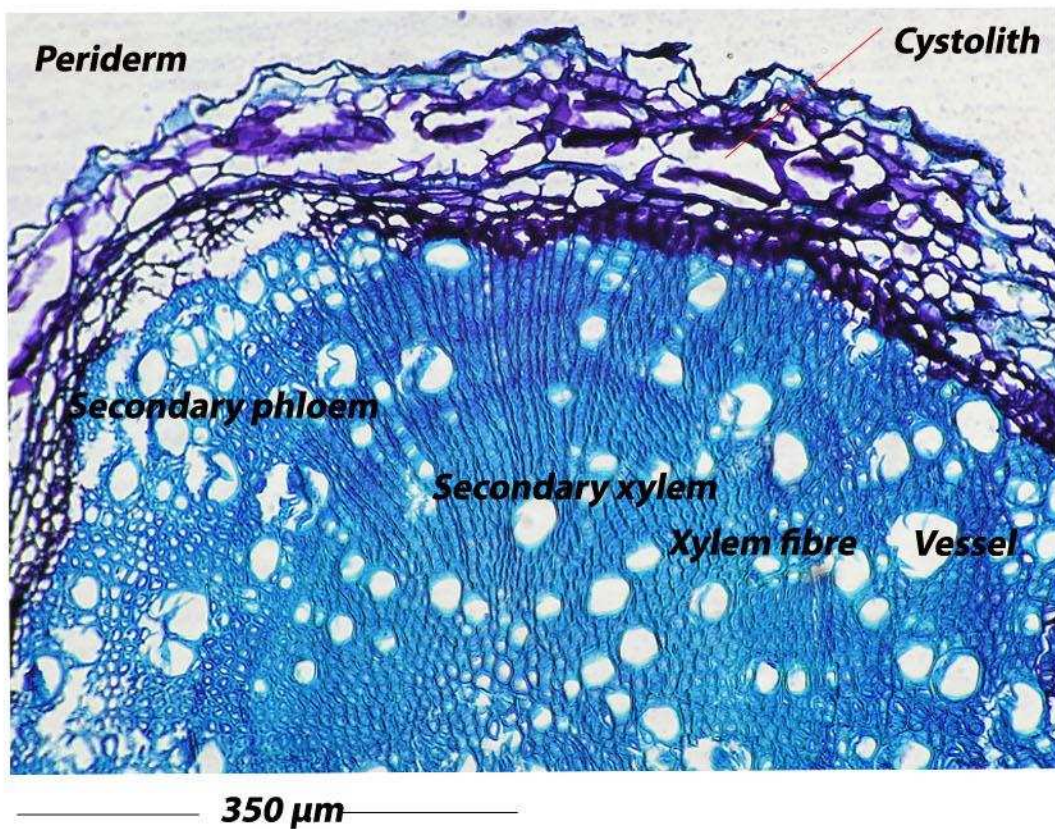


Fig. 11.1: T.S of thick Root

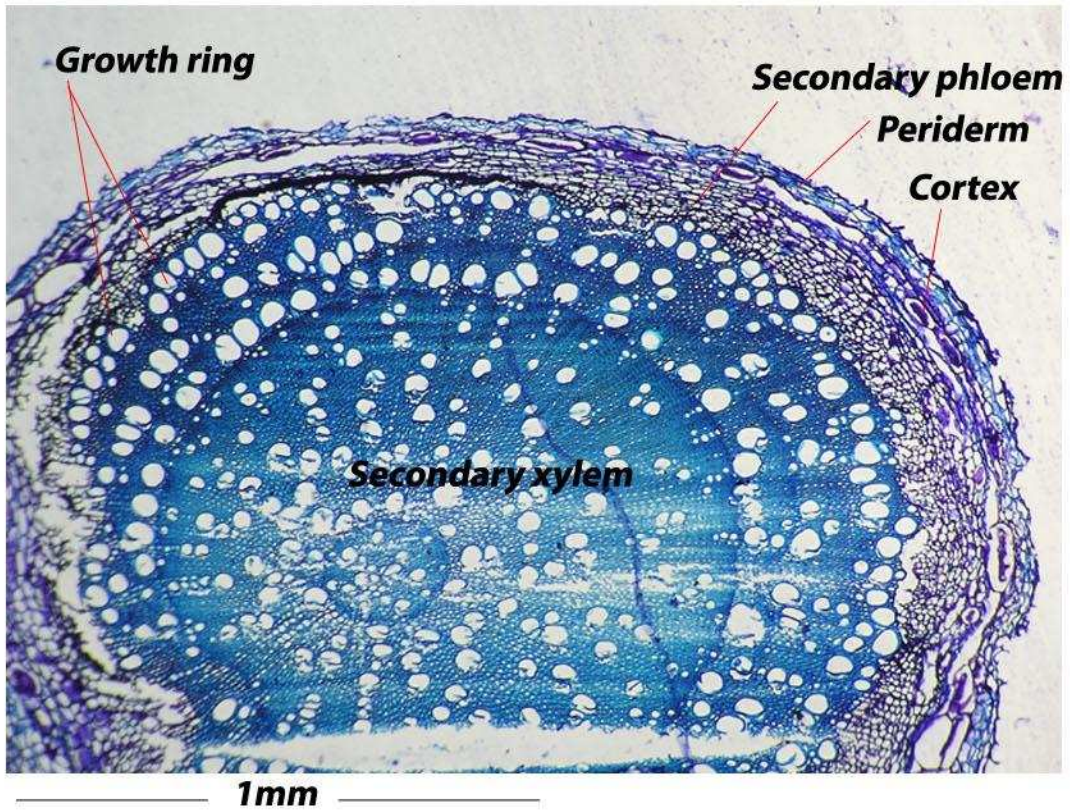


Fig. 11.2: A sector enlarged

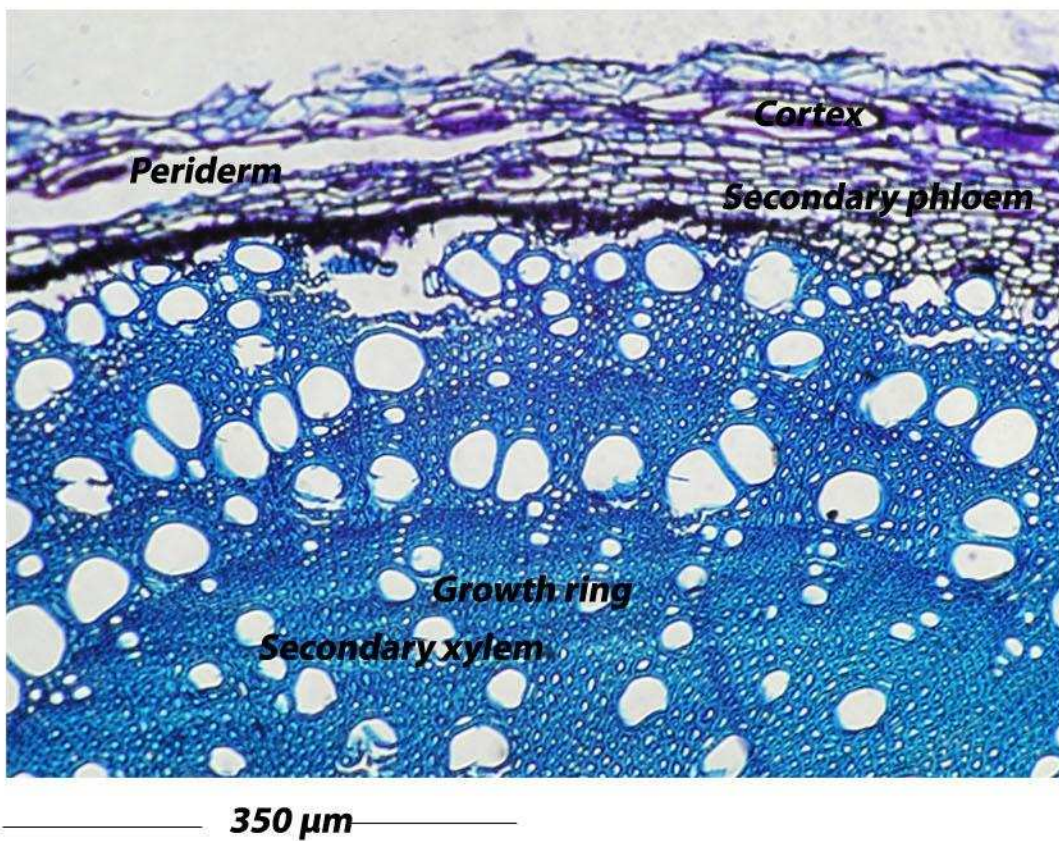


Fig. 12.1: T.S of Root showing phloem

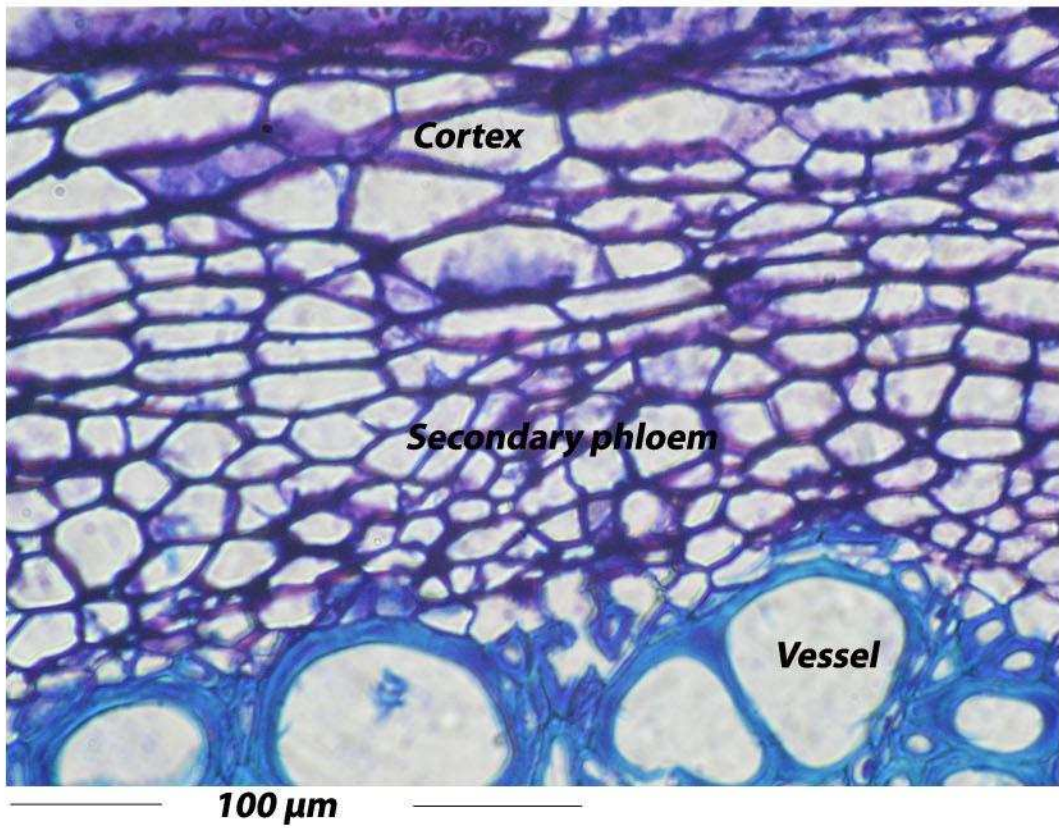


Fig. 12.2: T.S of thick Root showing secondary xylem

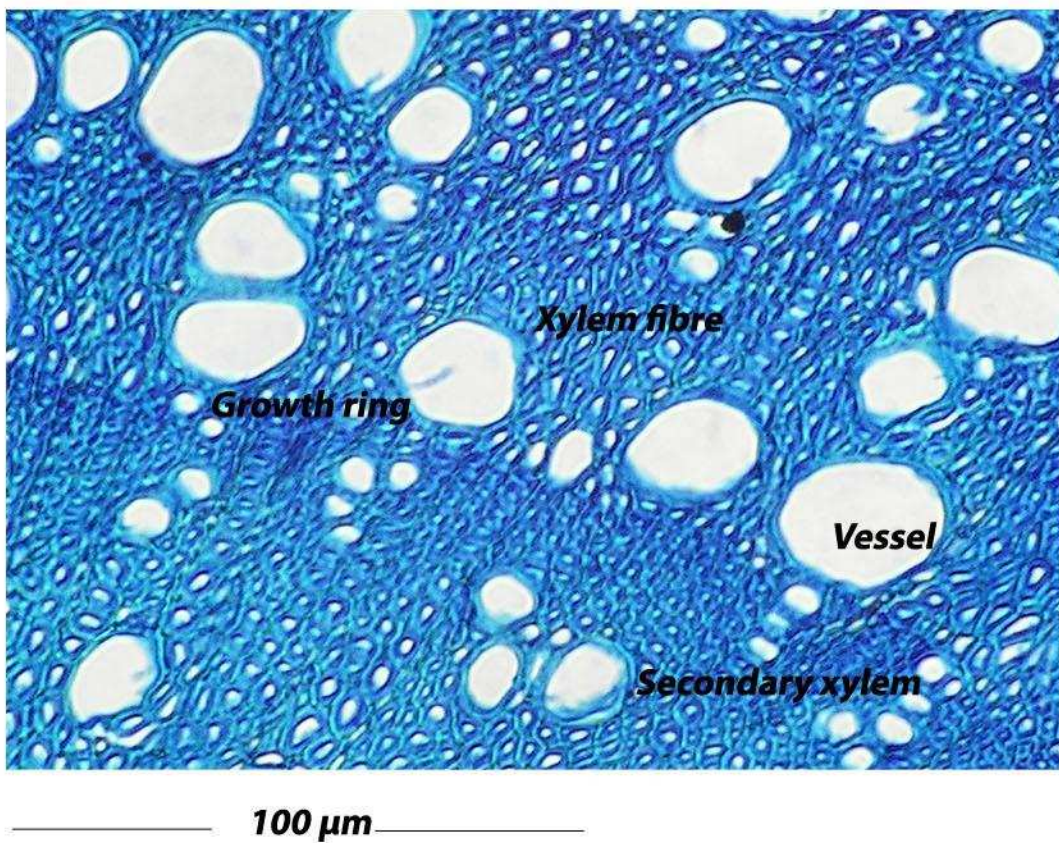


Fig. 13.1: Fibres and Non-glandular trichome

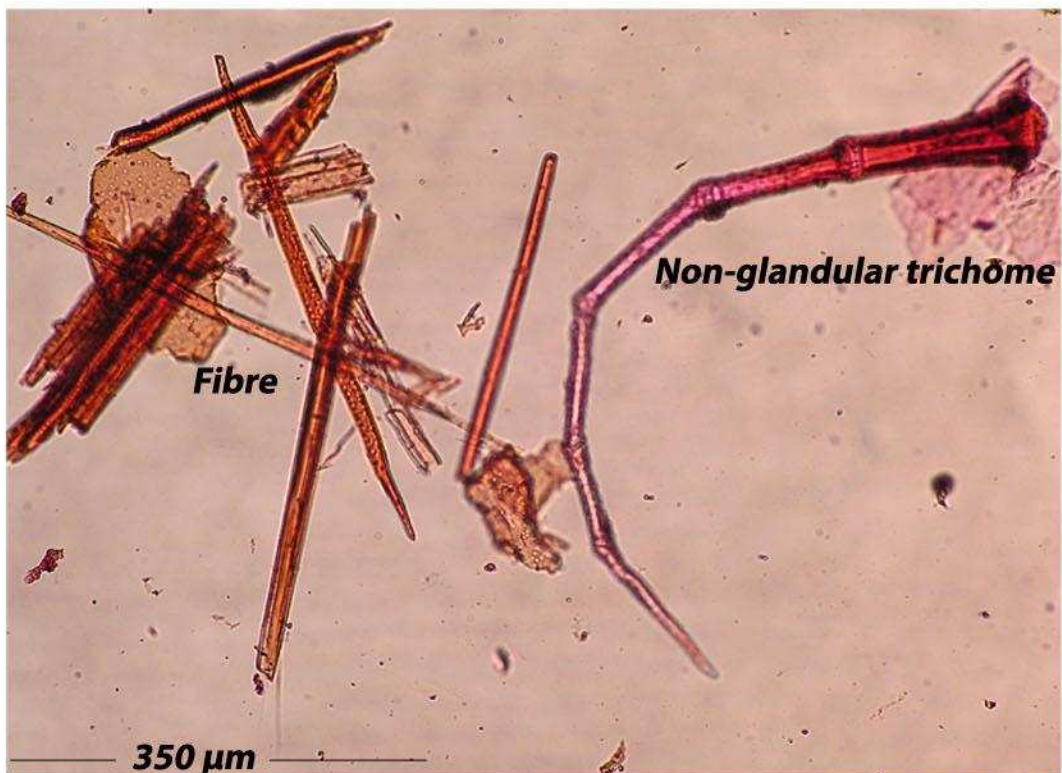


Fig. 13.2: Parenchyma , vessel elements and fibre

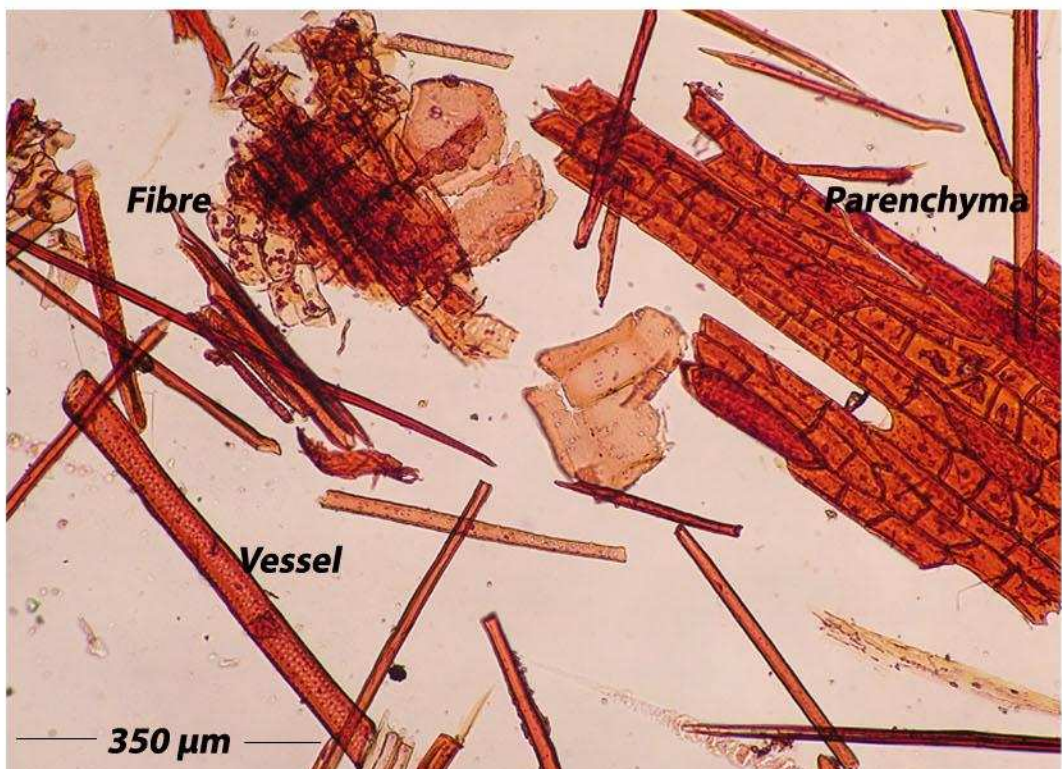


Fig. 14.1: Vessel elements enlarged

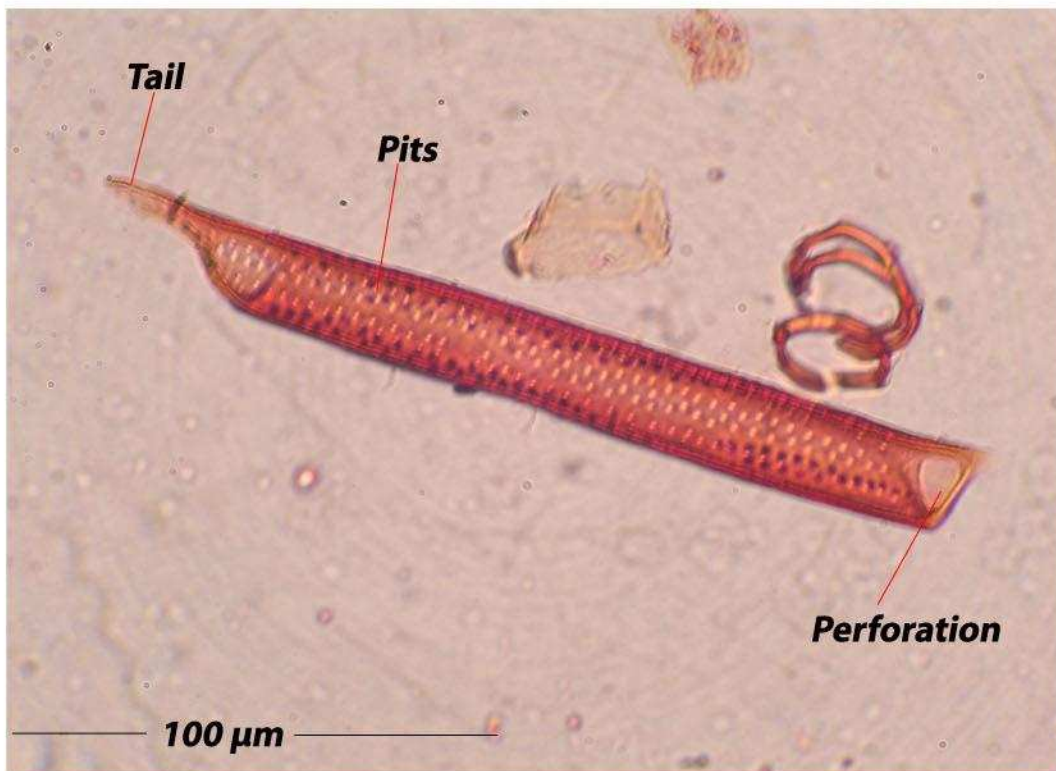


Fig. 14.2: Vessel elements enlarged

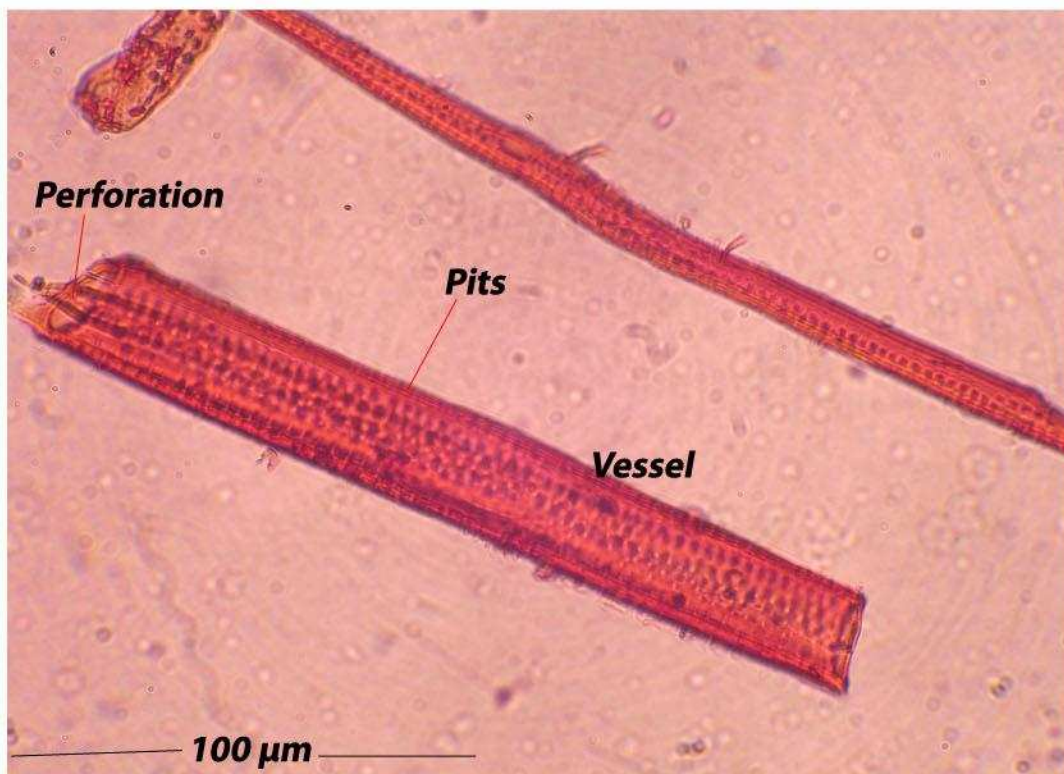


Fig. 15.1: Vessel elements and trichome

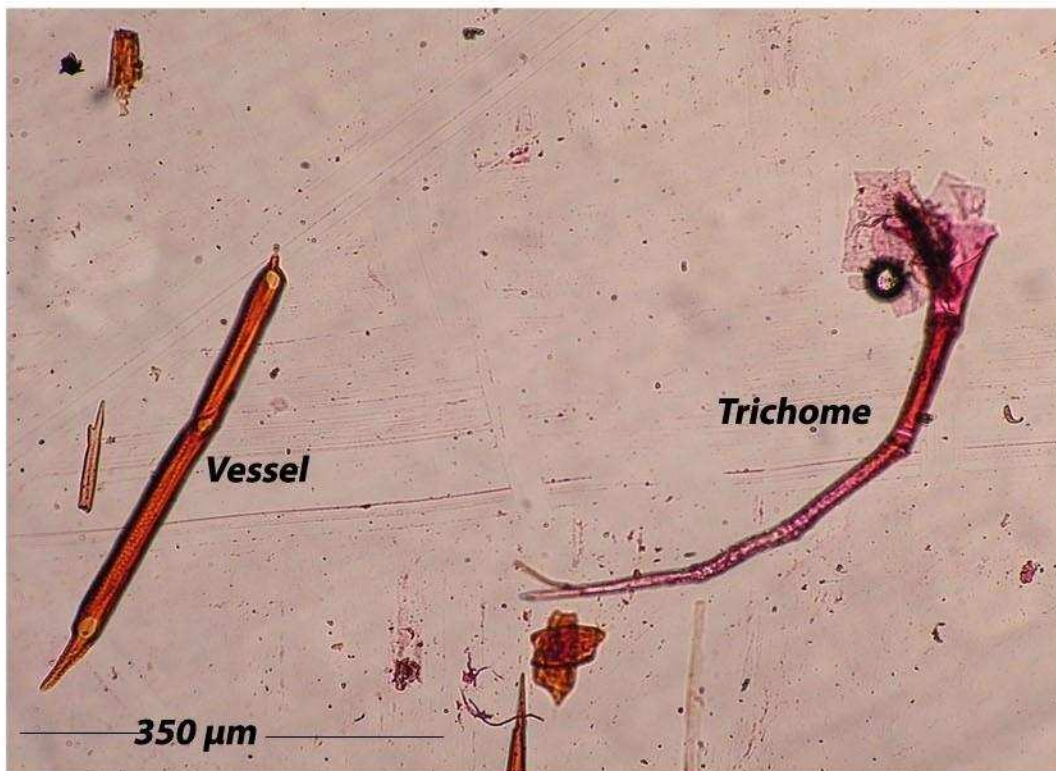


Fig. 15.2: Vessel elements and enlarged fibre

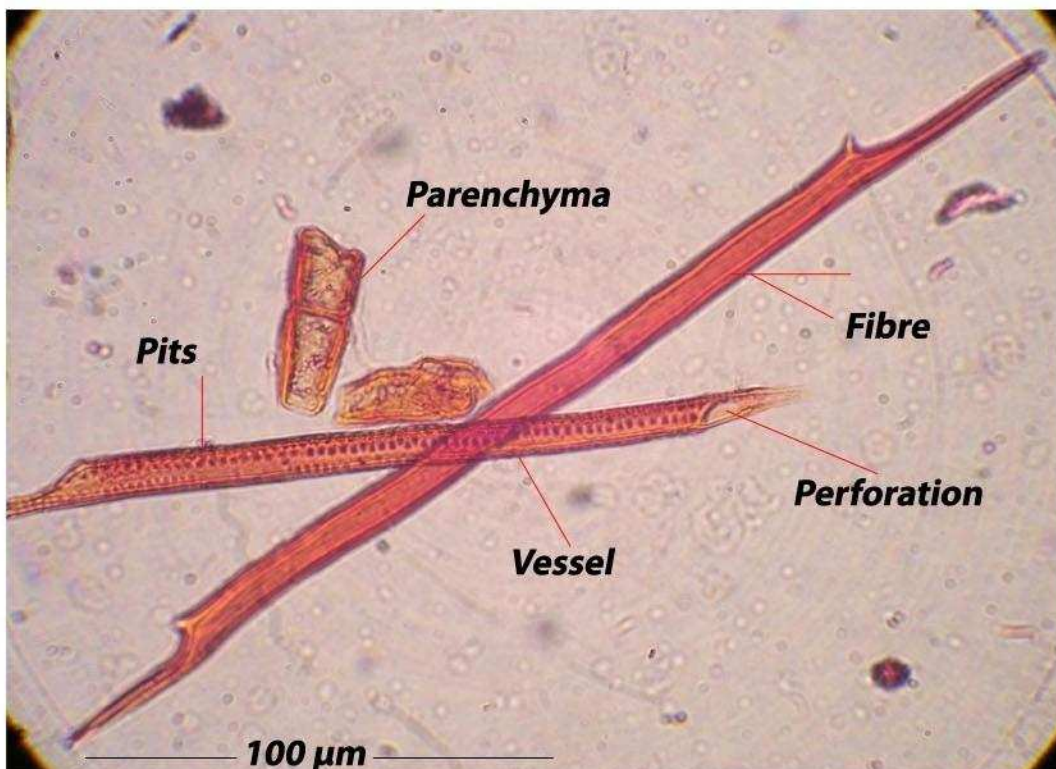


Fig. 29.1: Administration of 1% CMC in distilled water



Fig. 29.2: Administration of Doxorubicin at 15mg/kg CMC in distilled water



Fig. 29.3: Administration of ethanolic extract of Justicia glauca 10mg/kg PO



Fig. 29.3: Administration of ethanolic extract of Justicia glauca 200 mg/kg PO

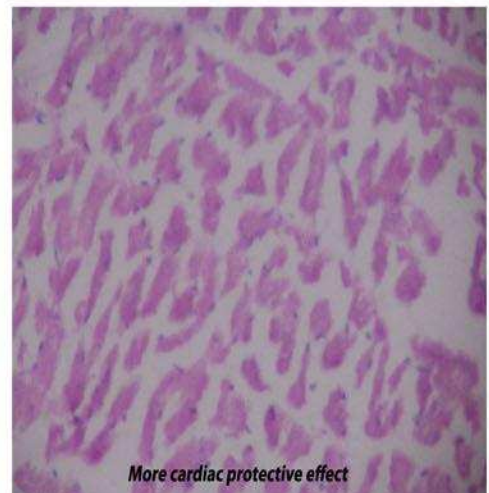
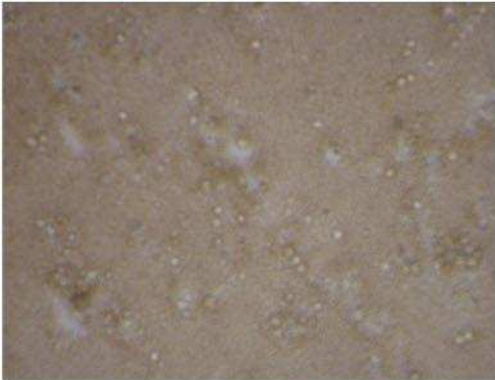
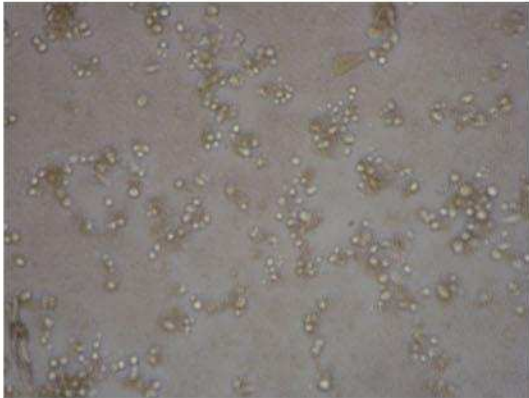


Fig. 30 : Cell inhibition (formazan crystals produced) at various concentration of 70% ethanolic extract after MTT treatment by using Stomach adeno carcinoma cell lines

32.5 μg



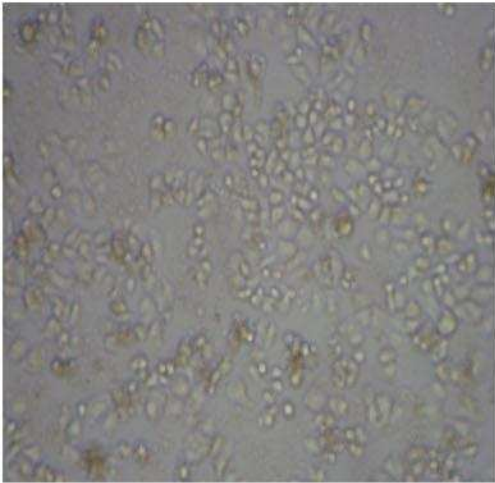
62.5 μg



125 μg



250 μg



500 μg

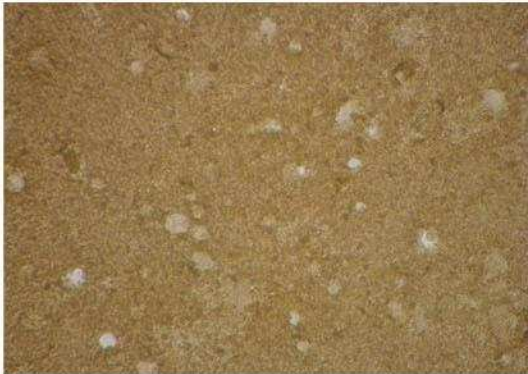


Fig. 32 : Antibacterial activity of *Justicia glauca* against *Escherichia coli* and *Klebsiella pneumonia*

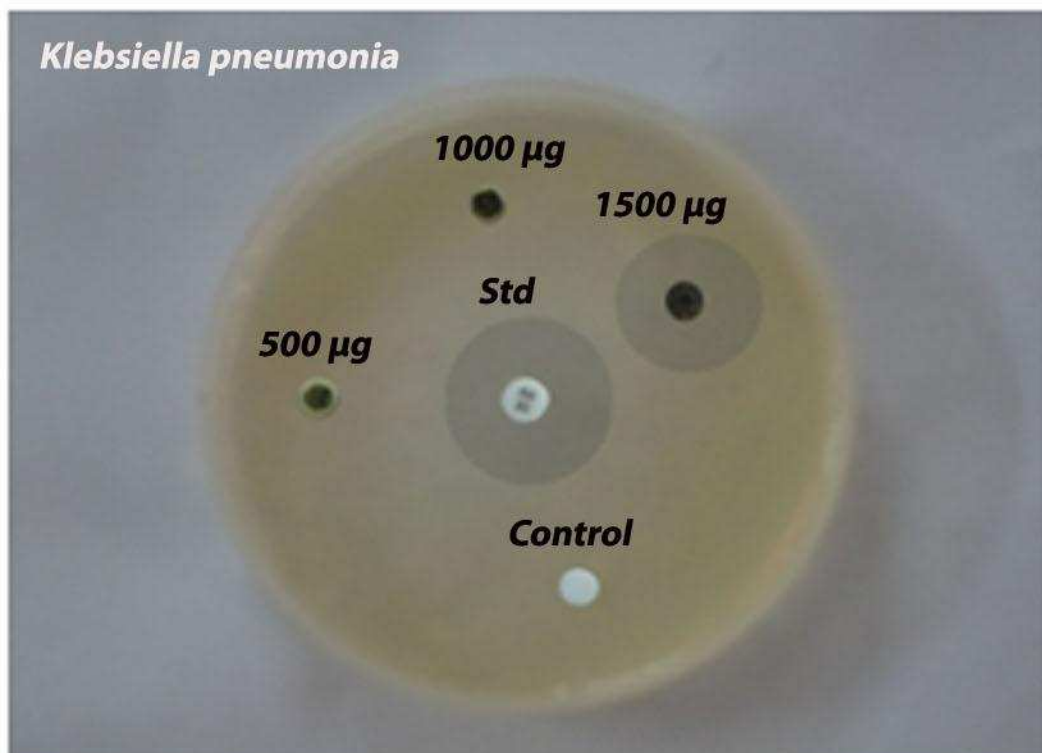
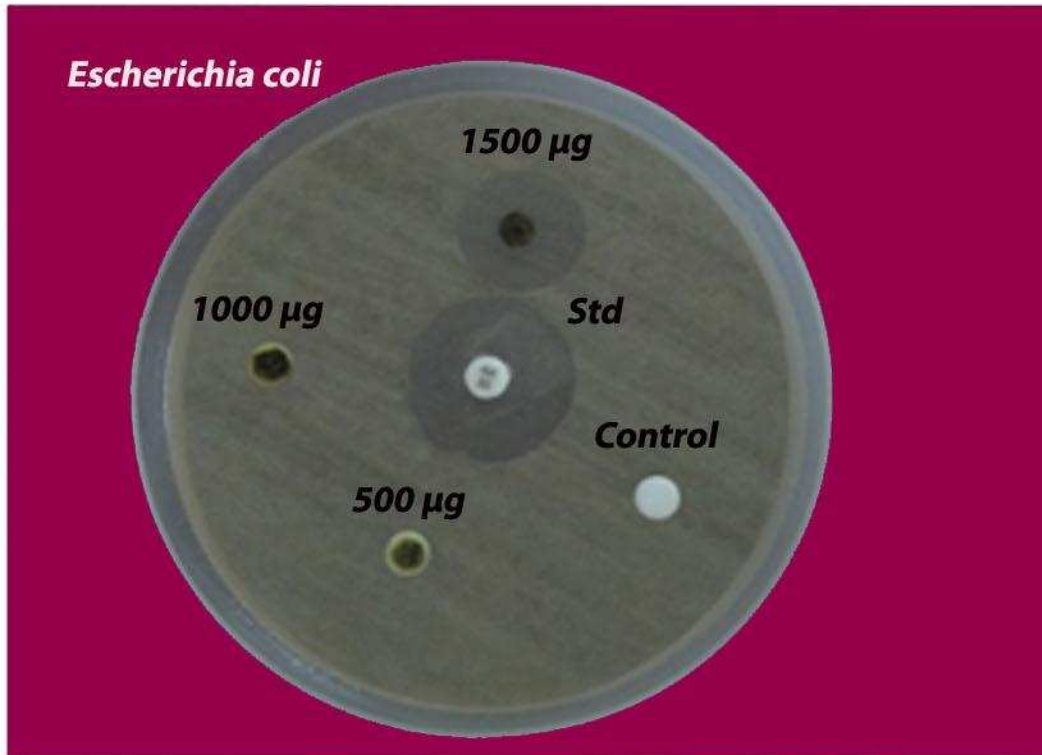


Fig. 33 : Antibacterial activity of *Justicia glauca* against *Proteus mirabilis* and *Pseudomonas aeruginosa*

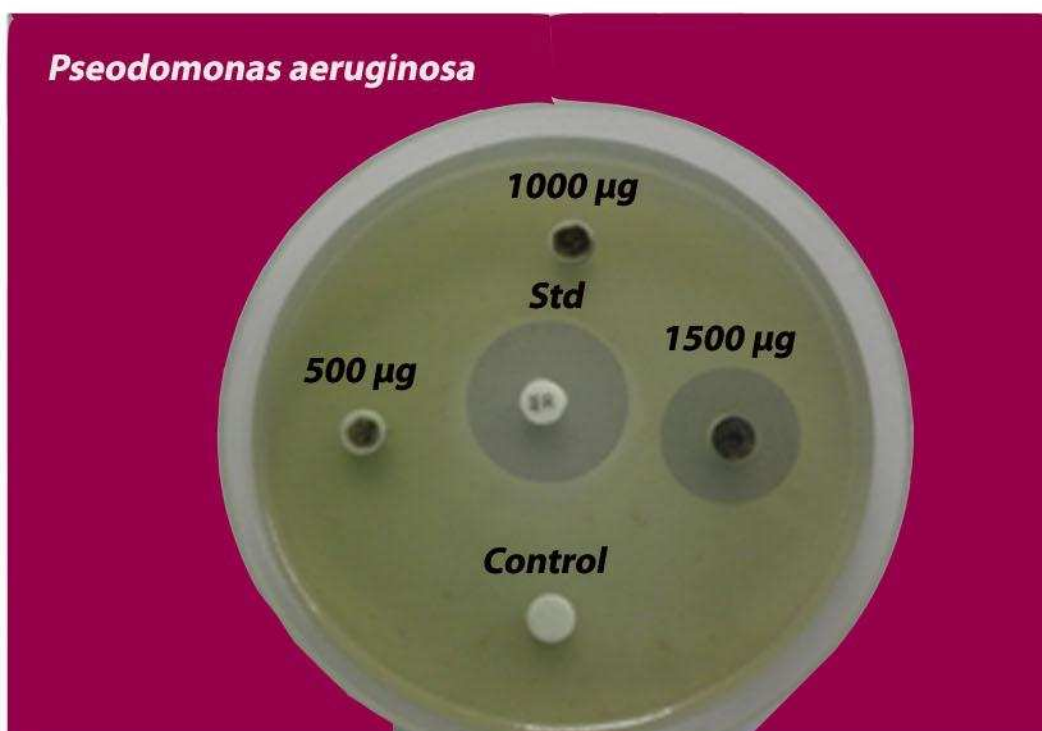
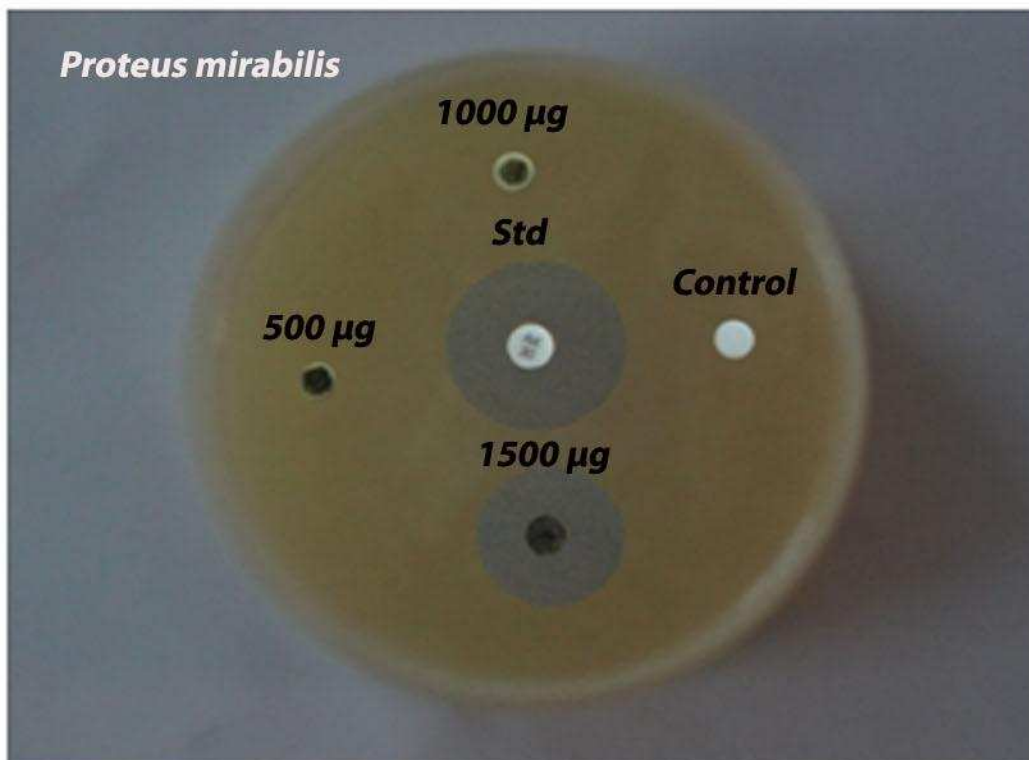


Fig. 34 : Antibacterial activity of *Justicia glauca* against *Staphylococcus albus* and *Staphylococcus aureus*

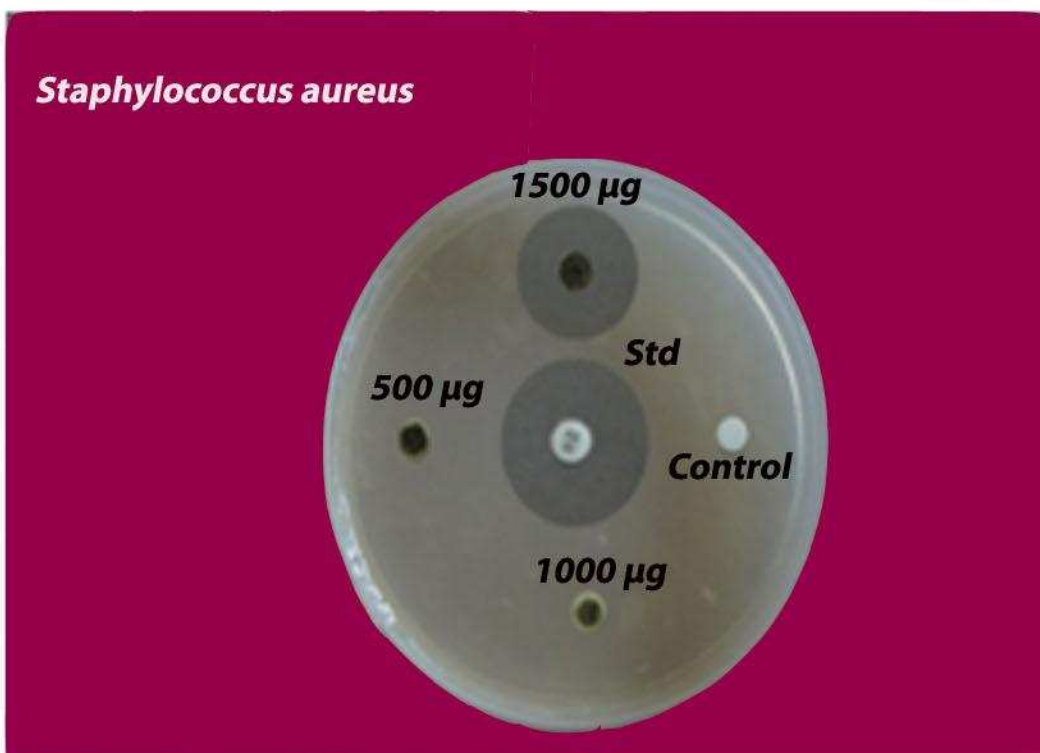


Fig. 35: Antibacterial activity of *Justicia glauca* against *Streptococci pyogenes* and *Streptococci viridans*

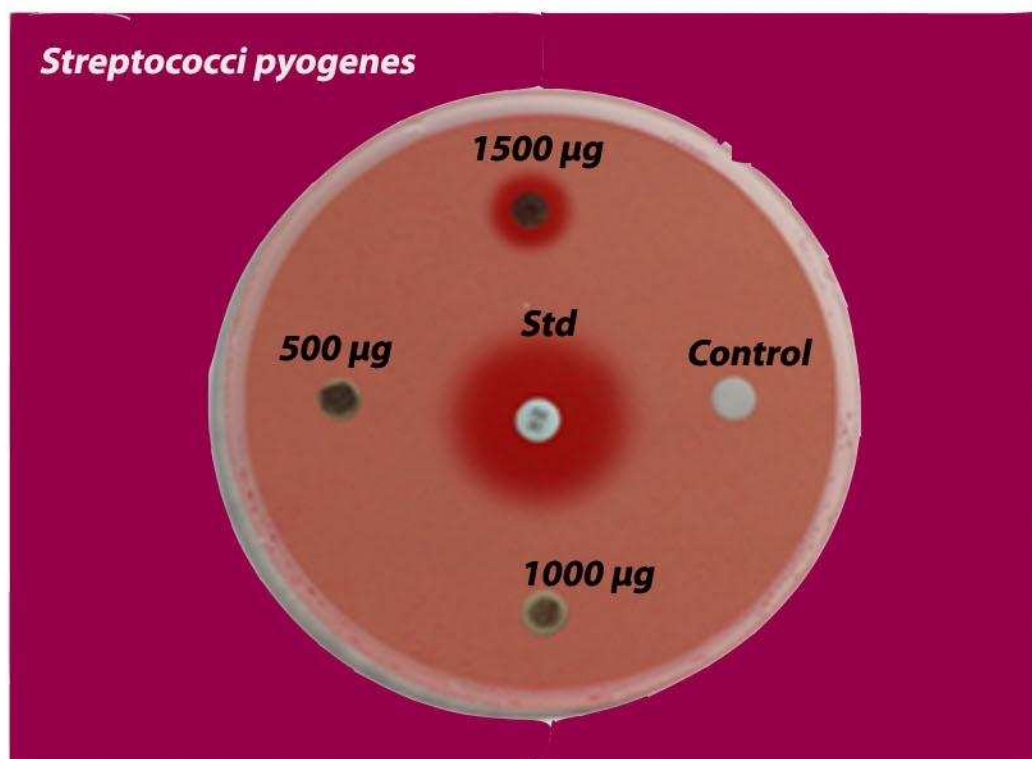


fig. 37 : Antifungal activity of Extract of *Justicia glauca* against *Aspergillus flavus* and *Candida albicans*



fig. 38 : Antifungal activity of Extract of *Justicia glauca* against *Epidermophyton floccosum* and *Aspergillus fumigatus*



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