PHARMACOGNOSTIC PERSPECTIVES OF THE LEAVES OF *Commiphora caudata* (Wt&Arn).Engl. – *Invivo* CARDIOPROTECTIVE POTENTIAL OF ITS ETHANOLIC EXTRACT ON DOXORUBICIN INDUCED CARDIOTOXICITY IN ZEBRAFISH LARVAE MODEL.

A dissertation submitted to

THE TAMIL NADU Dr.M.G.R.MEDICAL UNIVERSITY

CHENNAI - 600 032.

In partial fulfilment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

BRANCH III – PHARMACOGNOSY

Submitted by

Miss.D.SANGEETHA

(REG.NO: 261620707)

Under the Guidance of

PROF.Dr.K.PERIYANAYAGAM,M.Pharm.,Ph.D.,

Professor & HOD

DEPARTMENT OF PHARMACOGNOSY

COLLEGE OF PHARMACY

MADURAI MEDICAL COLLEGE

MADURAI - 625 020

MAY – 2018

CERTIFICATE

This is to certify that the dissertation entitled **"PHARMACOGNOSTIC PERSPECTIVES OF THE LEAVES OF *Commiphora caudata* (Wt&Arn) Engl. – *Invivo* CARDIOPROTECTIVE POTENTIAL OF ITS ETHANOLIC EXTRACT ON DOXORUBICIN INDUCED CARDIOTOXICITY IN ZEBRAFISH LARVAE MODEL"** is a bonafide work done by **Miss.D.Sangeetha (Reg. No: 261620707),** DEPARTMENT OF PHARMACOGNOSY, COLLEGE OF PHARMACY, **MADURAI MEDICAL COLLEGE, MADURAI-625020** in partial fulfilment of the Tamil Nadu Dr. M.G.R. Medical University rules and regulations for the award of **MASTER OF PHARMACY IN PHARMACOGNOSY** under my guidance and supervision during the academic year 2017-2018.

Name & Signature of the Guide

Name & Signature of the Head of Department

Name & Signature of the Dean/Principal

Dr. D.STEPHEN, M.Sc.,Ph.D.,
ASSISTANT PROFESSOR
MADURAI-625002

DEPARTMENT OF BOTANY
THE AMERICAN COLLEGE

CERTIFICATE

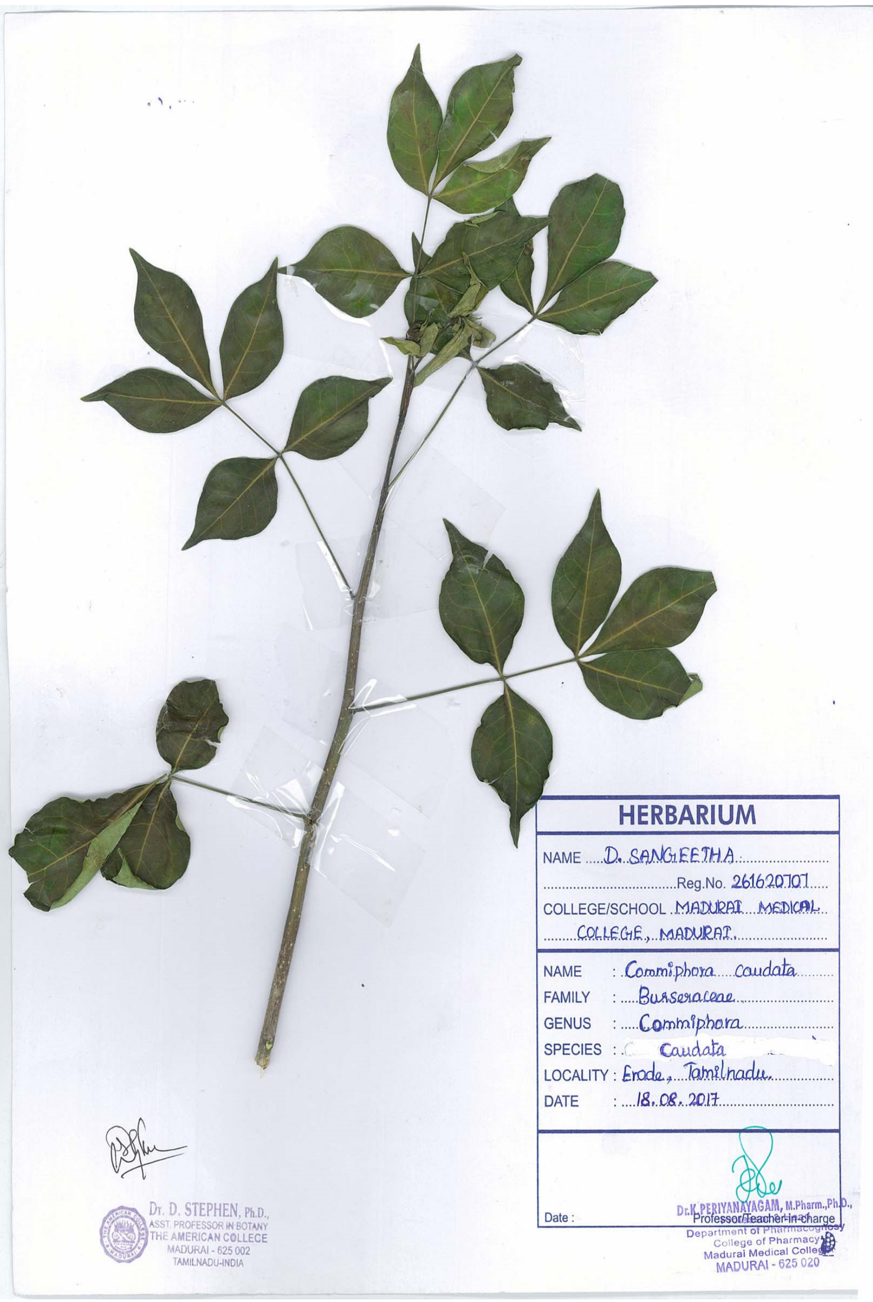
This is to certify that the specimen brought by Miss.D.Sangeetha, II M.Pharm, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai is identified as *Commiphora caudata(Wt&Arn)Engl* .belonging to the family Burseraceae.

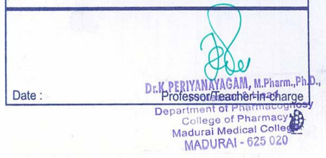
(Dr.D.STEPHEN)

Station : Madurai.

Date : 18.08.2017.

Dr. D. STEPHEN, Ph.D.,
ASST. PROFESSOR IN BOTANY
THE AMERICAN COLLEGE
MADURAI - 625 002
TAMILNADU-INDIA


HERBARIUM
NAME D. SANGEETHA
Reg.No. 261620707
COLLEGE/SCHOOL MADURAI MEDICAL COLLEGE, MADURAI.
NAME : Commiphora caudata
FAMILY : Burseraceae
GENUS : Commiphora
SPECIES : Caudata
LOCALITY : Erode, Tamilnadu.
DATE : 18.08.2017
Date :
Dr. D. STEPHEN, Ph.D.,
ASST. PROFESSOR IN BOTANY
THE AMERICAN COLLEGE
MADURAI - 625 002
TAMILNADU-INDIA


Dr.K.PERIYANAYAGAM, M.Pharm.,Ph.D.,
Professor/Teacher-in-charge
Department of Pharmacognosy
College of Pharmacy
Madurai Medical College
MADURAI - 625 020

Manonmaniam Sundarannar University
Constituent Model College,
NAGALAPURAM TK,
Thoothukudi TK. 628904

Sub: Your Ref:

Our ref: Date:19.09.2017

Prof. (Major) P.Chandra Sekaran,
Principal, MSU Constituent Model College
Nagalapuram 628 904.
Formerly Faculty of PG & Research
Department of Zoology and Biotechnology
Vivekananda College (Autonomous)
Tiruvedakam west 625217.

CERTIFICATE

This is to certify that the specimen brought by **Ms. D.SANGEETHA**, II M.Pharm. Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai has been identified as, "*Danio rerio*", family: Cyprinidae

Station: Madurai

Date:

Prof (Major) P.CHANDRASEKARAN,
PRINCIPAL,
MS University Constituent Model College,
NAGALAPURAM,
Vilathikulam Taluk. Thoothukudi Dt 628 904
majorpcs@yahoo.co.in

E-Mail id: Phone: 04638-242666 Fax No:

ACKNOWLEDGEMENT

I first and foremost express my revered regard and obeisance to the **ALIMIGHTY GOD** with whose blessings I was able to complete my project work with my beloved professor.

I am grateful to express my thanks to **DR.D.MARUDUPANDI, M.S., FICS., FAIS.,** Dean, Madurai Medical College, Madurai, for giving an opportunity to carry out my project work.

I thank to **Dr.V.DHANALAKSHMI. M.D.,** Vice Principal, Madurai Medical College, Madurai for her support and encouragement to carry out the work.

I express my thanks to Prof. **Dr.A.ABDUL HASAN SATHALI**, **M.Pharm., Ph.D.,** Principal and Head of Department of Pharmaceutics, College of Pharmacy, Madurai Medical College, Madurai for his support to do my project work.

I owe a great debt of gratitude and heartfelt thanks to my dear **Professor. Dr. K. PERIYANAYAGAM, M.Pharm., Ph.D., P.G. Diploma in Clinical Pharmacy (Australia),** Professor and Head of Department of Pharmcognosy, College of Pharmacy, Madurai Medical College, Madurai, for his enthusiastic co-operation as my project guide and all the constant encouragement, sacrificing, suggestions, contribution and support rendered during the project work. As a gesture of respect I would like to extend my thanks for his patience in listening and answering to my questions.

I thank **Mrs.Dr.A.SETHURAMANI, M.Pharm., Ph.D., Mrs.Dr.A.KRISHNAVENI, M.Pharm., Ph.D., Mr.G.SATHYABALAN,**

M.Pharm., (Ph.D) , Mr.M.R.VINIAYAKAMURTHI, M.Pharm., Assistant Professors, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai for their help.

I thank **Mrs.K.RADHA**, Lab supervisor**,** for her extraoridinary support and encouragement to complete my project work. I thank **Mrs.A.CHITRA,** Lab technician and **Mrs.VIMALA** for their support during my study of this work.

I thank to **Dr. D. STEPHEN, M.Sc., Ph.D.,** Department of Botany, The American College, Madurai for plant authentication. And also I thank **Dr.SASIKALA** (Retd.) Director of Siddha Central Research Institute, Arumbakkam, Chennai who helped me in the microscopic studies.

I express my special thanks to my dear parents **Mr. K. DEVARAJ, Mrs. D. DHANALAKSHMI** and my brother **Mr.D.DEVENDRAN** and my sisters **Miss.R.PRABA, Miss. R. UMAMAHESWARI , Miss.R.KEERTHANA** and **Miss. K. GEETHA** for their support and co-operation to complete my project work.

I express my special thanks to my dear sister **Mrs.G.HEMALATHA,** and my dear brother **Mr.T.PRABAHAR,** and my dear best friend **Mr.K.RAJASEKARAN** for their support and encouragement in full of my completion of project work in this course .

I thank my M.Pharm Pharmacognosy Classmates**, Mr. M. MOHANRAJ, Mrs. L. SRIKALA, Mr.A.IYAPPAN, Mr. S. RAJASEKAR , Mrs. R. SUGANYA, Mr.B.EZHILARASAN** and all my classmates of Pharmaceutics and Pharmaceutical chemistry for helping my project.

I also express my thanks to my M.Pharm Pharmacognosy juniors especially **Mr.K.MUTHUKRISHNAN** and **Miss.P.BRINDHA, Miss.A.DEEBIKA, Miss R.DHANALAKSHMI, Mr.B.KARTHIKEYAN, Mrs.P.KOHILA VANI, Mr.M.RAJAMANICKAM, Mrs.J.SELVARANI, Mr.M.VIJAYA GANESH** for their extraordinary support and help to complete my project work.

CHAPTER	TITLE	PAGE NO
1	**INTRODUCTION**	1
2	**REVIEW OF LITERATURE**	15
3	**AIM AND OBJECTIVE**	43
4	**MATERIALS AND METHODS**	47
	4.1 PLANT COLLECTION AND AUTHENTICATION	47
	4.2 PHARMACOGNOSTIC STUDIES	47
	4.2.1 Morphological studies of *C.caudata*	48
	4.2.2 Microscopical studies of the leaves of *C.caudata*	48
	4.2.3 Microscopical study of a leaf using Scanning Electron Microscope	50
	4.2.4 Powder Microscopy	51
	4.2.5 Microscopic schedules	52
	4.2.6 Physicochemical parameters	54
	4.3 PHYTOCHEMICAL STUDIES	56
	4.3.1 Preliminary Phytochemical Screening	56
	4.3.2 Fluorescence analysis of Powdered leaf	62
	4.3.3 Estimation of Flavonoid content	63

	4.3.4 Estimation of Total Phenolic content	64
	4.3.5 Determination of trace elements in the leaf of *C.caudata* by Energy dispersive X-ray Spectrometer [EDS]	65
	4.3.6 Preparation of the ethanolic extract of leaves of *C.caudata* (EECCL)	67
	4.3.7 Identification of compounds present in the EECCL by HPTLC analysis	67
	4.4 PHARMACOLOGICAL STUDIES	68
	4.4.1 Preliminary toxicological studies of ethanolic extract of *C.caudata* on the Embryo and larvae of Zebrafish A. Whole embryo culture toxicity study B. Larval toxicity study	68 72 73
	4.4.2 Effects of EECCL in the Doxorubicin induced cardiotoxicity zebrafish models	73
5	**RESULTS**	
	5.1 Pharmacognosy	75
	5.1.1 Morphological features of *C.caudata*	75
	5.1.2 Microscopy of the leaf	77
	5.1.3 . Microscopical study of leaf using Scanning Electron Microscope	78
	5.1.4 Powder microscopy	78
	5.1.5 Microscopic schedules	78
	5.1.6 Physicochemical parameters	80
	5.2 Phytochemical studies	81
	5.2.1 Preliminary Phytochemical screening	81

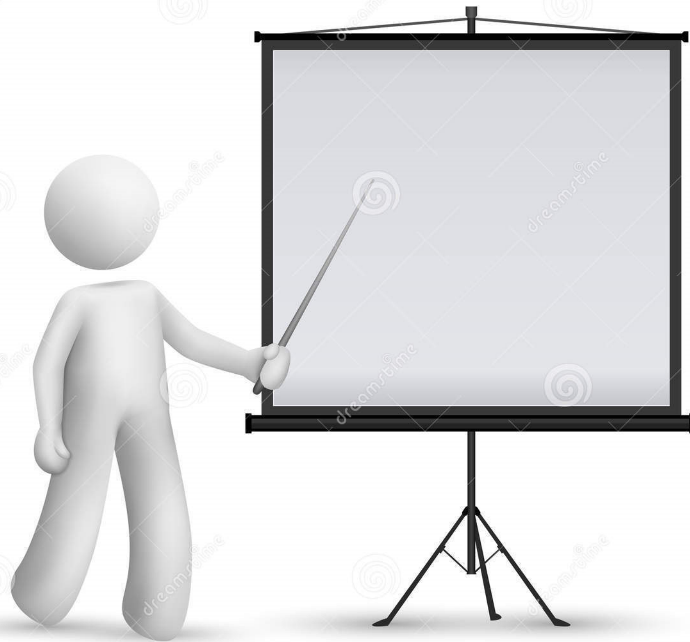
	5.2.2 Fluorescence analysis of Powdered leaf	86
	5.2.3 Estimation of Flavonoid content	86
	5.2.4 Estimation of Total Phenolic content	86
	5.2.5 Identification of compounds present in the ethanolic extract of leaves by HPTLC analysis	87
	5.2.6 Determination of trace elements in the leaf of *C.caudata* by Energy Dispersive X-ray Spectrometer [EDS]	89
	5.3 PHARMACOLOGICAL STUDIES	90
	5.3.1 Whole embryo culture toxicity study	90
	5.3.2 Zebrafish Larval toxicity study	91
	5.3.3 Effect of EECCL on Doxorubicin induced Cardiotoxicity ZF larvae	93
6	**DISCUSSION**	94
7	**CONCLUSION**	104
8	**REFERENCES**	108

LIST OF TABLES

S.NO	TABLES	PAGE NO
1	Vein islet and vein termination number of *C.caudata* leaves	79
2	Stomatal number of *C.caudata* leaves	79
3	Stomatal index of *C.caudata* leaves	80
4	Ash values of the of *C.caudata* leaves	80
5	Loss on drying (LOD) of *C.caudata* leaves	81
6	Extractive values for *C.caudata* leaves	81
7	Preliminary phytochemical screening of *C.caudata* leaves	85
8	Fluorescence analysis of *C.caudata* leaves	86
9	Elements weight percentage.	90
10	HPTLC profile of the ethanolic extract of the *C.caudata* leaves	88
11	Scores for the whole embryo toxicity	91
12	Zebrafish larval toxicity study	92
13	Effect of EECCL on Doxorubicin induced cardiotoxicity In zebrafish larvae model	93

LIST OF PLATES

S.NO	PLATES
1	Habit and habitat of *Commiphora caudata*
2	A branch *C.caudata* showing leaf arrangement
3	Leaf of *C.caudata* dorsal and ventral side
4	Flowers of *C.caudata*
5	Fruits of *C.caudata*
6	Bark of *C.caudata*
7	Diagrammatic representation of *C.caudata*
8	T.S of leaf of *C.caudata* through the midrib
9	T.S of leaf of *C.caudata* through lamina
10	Upper and Lower epidermis surface view of stomata of *C.caudata*
11	T.S of petiole of *C.caudata*
12	Venation pattern of *C.caudata* leaf
13	SEM of *C.caudata* leaf
14	Powder microscopy of *C.caudata* leaf
15	Vehicle control Zf larvae (normal)
16	Doxorubicin induced cardiotoxic Zf larvae showing pericardial edema
17	Effect of EECCL on Doxorubicin induced cardiotoxic Zf larvae



Introduction

INTRODUCTION

Ayurveda, Unani and Allopathy use several plant species to treat various ailments. The use of plant medicine is becoming trendy due to toxicity and side effects of allopathic medicines. This led to rapid increase in the number of herbal drug manufactures. Plant medicines, as the main remedy in traditional system of medicine have been used in health practices since ancient times. The practice continue today because of its biomedical benefits as well as takes place in cultural beliefs in many parts of world and have made a great contribution in maintaining human health . In India around 20,000 medicinal plant species have been recorded recently but more than 500 traditional communities use about 800 plant species for curing various diseases. Presently 80% of the world population depends on plant-derived medicine for primary health care for varied human diseases as it has no side effects. Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, nearly 121 pharmaceutical products were formulated based on the traditional awareness obtained from various sources.

HERBAL MEDICINES OBTAINED FROM PLANTS:

India has one of the richest medicinal plant traditions in the world. They are estimated to be around 25,000 effective plant-based formulations, used in folk medicine and well- known to rural communities in India. There are more than 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and remedial applications. It is estimated that there are more than 7800 medicinal drug-manufacturing units

in India, which consume about 2000 tonnes of herbs annually.

Medicinal plants play an essential role in the progress of effective therapeutic agents. During 1950 nearly 100 plants derived new drugs were introduced in the USA drug market including deserpidine , reserpine, vinblastine and vincristine which are derived from higher plants. From 1971 to 1990 recent drugs such as ectoposide, teniposide, plaunotol, artemisinin and ginkgolides appeared all over the world. Many drugs were introduced from 1991 to 1996 including toptecan, gomishin, irinotecan etc. Plant based drugs provide exceptional contribution to contemporary therapeutics; for example: serpentine isolated from the root of Indian plant *Rauwolfia serpentina* in 1950's, was a remarkable event in the management of hypertension and lowering of blood pressure. Vinblastine isolated from the *Catharanthus rosesus* is used for the treatment of choriocarcinoma, non-hodgkins lymphomas, leukemia in children, testicular and neck cancer. Vincristine is suggested for acute lymphocytic leukemia in childhood and in advanced stages of Hodgkins, lymophosarcoma, cervical and breast cancer. Podophyllotoxin is a constituent of *Phodophyllum emodi* presently used against testicular, small cell lung cancer and lymphomas. Plant based drugs are used to cure mental illness, skin diseases, tuberculosis, diabetes, Jaundice, hypertension and cancer. Medicinal plants play a significant role in the development of potent therapeutic agents. They came into use in the modern medicine through the use of plants, as native cure in folklore medicine. More than 64 plants have been found to possess antibacterial properties; and more than 24 plants have been found to possess antidiabetic properties. Venom neutralization is effected by lupeol

acetate isolated from the root extract of Indian sarsaparilla *Hemidesmus indicus*.

MARKET POTENTIAL OF MEDICINAL PLANTS:

The market for ayurvedic medicines is found to be increasing at 25% annually. Sales of medicinal plants have grown by nearly 20% in India in past ten years (1987-96), the highest rate of growth in the world. But the per capital overheads in India on medicines per annum is amongest the lowest in the world. In other developing countries too, plants are the main source of medicine. Two of the largest users of medicinal plants are China and India. Traditional Chinese Medicine accounts over 5000 plant species; India accounts about 7000. According to Export Import Bank, the international market for Medicinal plant linked trade having a growth rate of nearly 7% per annum. China’s share in world herbal market is US$ 6 billion while India’s share is only US$1 billion. The annual export of medicinal plants from India is valued at Rs. 1200 million.

All the major plant-based pharmaceutical companies are showing a constant growth of about 15 per cent. Traditional medicine has served as a tool of alternative medicine, new pharmaceuticals, and healthcare products. Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. A significant number of modern pharmaceutical drugs are derived from medicinal plants. The derivatives of medicinal plants are non-narcotic with little or no side effects.

FUTURE OF MEDICINAL PLANTS MARKET:

According to WHO about 25% of modern medicines are descended from plants first used traditionally. Many others are synthetic analogues built on prototype compounds isolated from plants. Almost, 70% modern medicines in India are derived from natural products. The basic uses of plants in medicine will continue in the future, as a source of therapeutic agents, and as raw material base for the extraction of semi-synthetic chemical compounds such as cosmetics, perfumes and food industries. Popularity of healthcare plant- based products has been traced to their increasing acceptance and use in the cosmetic industry as well as to increasing public costs in the daily maintanance of personal health and well being. In the dual role as a source of healthcare and income, medicinal plants make an important contribution to the larger development process. Though the efficacy of plants requires development of quality consciousness in respect of the evaluation related evidences, supplying the demand for botanicals and herbals is a booming business. Recently, even developed countries are using medicinal systems that involve the use of plant drugs and remedies. Undoubtedly the demand for plant derived products has increased worldwide. The demand is estimated to grow in the years to come fuelled by the growth of sales of herbal supplements and remedies. This means that scientists, doctors and pharmaceutical companies will be looking at countries like China, India, etc. for their requirements, as they have the most number of medicinal plant species and are the top exporters of medicinal plants.

CURRENT REGULATIONS FOR STANDARDIZATION OF CRUDE DRUGS:

In recent years there is a surge in the interest regarding survival of Ayurvedic forms of medication. In the global perspective, there is a shift towards the use of medicine of plant origin, as the dangers and the short coming of modern medicine have started getting more apparent, majority of Ayurvedic formulation are prepared from herbs. It is the cardinal responsibility of the regulatory authorities to ensure that the consumers get the medication, which guarantee with purity, safety, potency and efficacy. The quality control of crude drugs and herbal formulations is of vital importance in justifying their acceptability in modern system of medicine. But one of the major problems faced by the plant drug industry is non availability of rigid quality control profile for plant material and their formulations. Patent proprietary Ayurvedic medicines are sold over the counter in pharmacies. These products appear to represent a major share of branded traditional medicine in India. Nevertheless systems like Ayurveda still need to gain an empirical support of modern medical sciences to make them reliable and acceptable for all. An innovative research effort to define the advantage of traditional system of medicine with respect to their safety and efficacy could result in a better utilization of these corresponding systems of medicine. Internationally several pharmacopoeias have provided monographs stating parameter and

Standard of many herbs Several pharmacopoeias like:

- Chinese Herbal Pharmacopoeia
- United States Herbal Pharmacopoeia

- British Herbal Pharmacopoeia
- British Herbal Compendium
- Japanese Standards for Herbal Medicine
- The Ayurvedic Pharmacopoeia of India(API) and some product made out of these herbs.

These Pharmacopoeia's lay down monograph for herbs and herbal products to maintain their quality in their respective nations. Government of India too has brought out Ayurvedic Pharmacopoeia of India, which recommends basic quality parameters for eighty common Ayurvedic herbal drugs.

FUTURE RESEARCH OF TRIBAL MEDICINES:

Tribal healers in most of the countries, where ethnomedical treatment is frequently used to treat cut wounds, skin infection, swelling, aging, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal diseases, snakebite and gastric ulcer, provide instructions to local people as how to prepare medicine from herbal. They keep no records and the information is mainly passed on verbally from generation to generation. World Health Organization (WHO) has shown great interest in documenting the use of medicinal plants used by tribals from different parts of the world. Many developing countries have intensified their efforts in documenting the ethnomedical data on medicinal plants. Research to find out scientific evidence for claims by tribal healers on Indian herbs has been intensified. Once these local ethnomedical preparations are scientifically evaluated and disseminated properly, people will be better informed regarding efficacious drug treatment and improved health status (Verma. S and Singh.S.P, 2008).

HERBAL MEDICINES AND VARIOUS DISEASES:

Herbal medicine is used to treat several conditions, such as allergies, asthma, eczema, premenstrual syndrome, rheumatoid arthritis, fibromyalgia, migraine, menopausal symptoms, chronic fatigue, irritable bowel syndrome, and cancer.

Some herbs and their significance are detailed below;

- ❖ **Ginkgo** (*Ginkgo biloba*) has been used traditionally to treat circulatory disorders and enhance memory. Even though not all studies agree, ginkgo may be chiefly effective in treating dementia (including Alzheimer disease) and intermittent claudication (poor circulation in the legs). It also shows assurance for enhancing memory in older adults. Laboratory studies have revealed that ginkgo improves blood circulation by dilating blood vessels and reducing the stickiness of blood platelets.
- ❖ **Kava kava** (*Piper methysticum*) is said to elevate mood, enhance well being and contentment, and produce a feeling of relaxation. Numerous studies show that kava may help treat anxiety, insomnia, and related nervous disorders. However, there is serious concern that kava may cause liver damage.
- ❖ **Saw palmetto** (*Serenoa repens*) is used for the treatment of benign prostatic hyperplasia (BPH), a non-cancerous enlargement of the prostate gland. Several studies recommend that the herb is effective for treating symptoms, including frequent urination, having trouble starting or maintaining urination, and needing to urinate during the night.

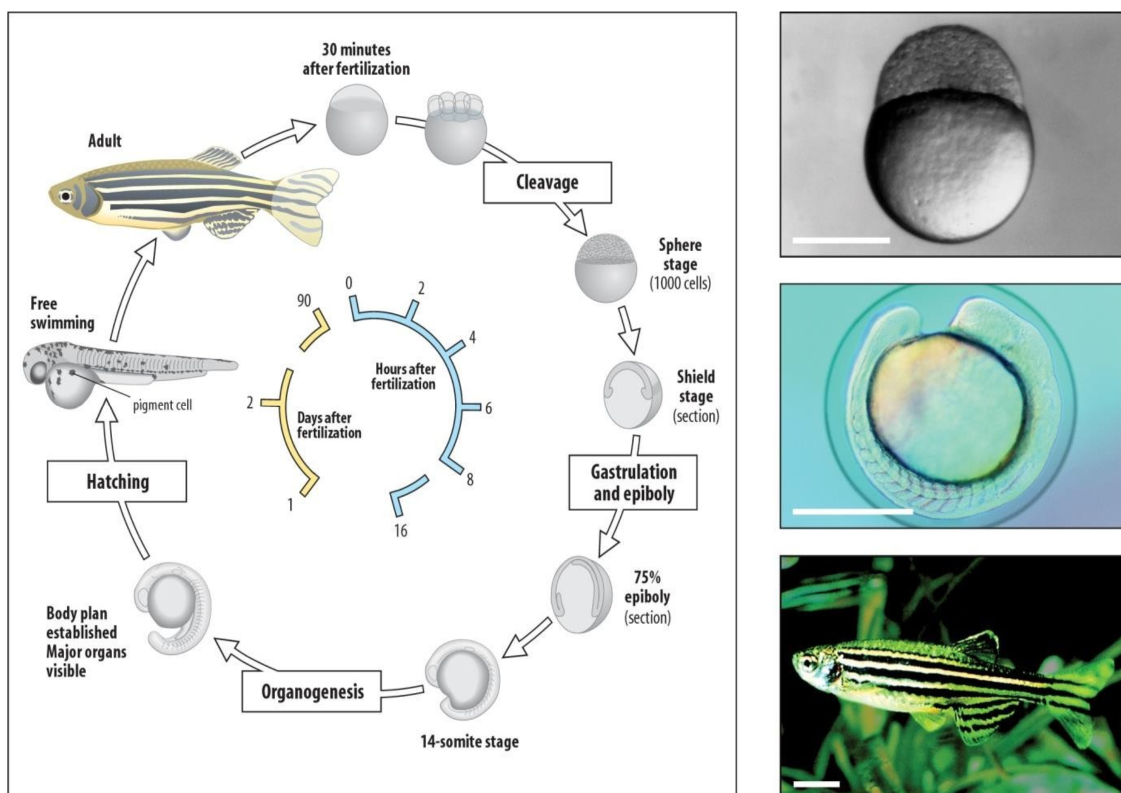
- **St. John's wort** (*Hypericum perforatum*) is well suited for its anti-depressant effects.
- **Valerian** (*Valeriana officinalis*) is a popular substitute to commonly prescribed medications for sleep problems because it is considered to be both safe and gentle.
- **Echinacea preparations**(from *Echinacea purpurea* and other *Echinacea* species) may progress the body's natural immunity (Bent S, 2008).

CARDIOVASCULAR DISEASES:

Cardio metabolic syndrome is related with multiple risk factors including insulin resistance, dyslipidemia, hypertension, and obesity. According to World Health Organization, every year about 2.8 million people die worldwide due to overweight or obesity. Occurrence of diabetes appears with projections to affect about 439 million adults by 2030, whereas cardiovascular diseases account for 30% of deaths annually, including both developed and developing countries. Because of their chronic degenerative nature, cardio metabolic-related disorders have long-lasting treatments, costly for both the patient and the health services, in addition to potentially harmful side effects caused by polytherapeutic regimens. In this context, herbal medicines have become the major source of bioactive molecules and emerged as potential therapeutic tools to fulfil a multiple-target strategy, especially because of their inherent large-scale structural diversity as compared with synthetic compounds (Chagas V.T.*et al*., 2015).

ZEBRAFISH, AS MODEL ORGANISM:

The **zebrafish** (*Danio rerio*) is a tropical freshwater species belonging to the minnow family (Cyprinidae) of the order Cypriniformes. The zebra fish is also a significant and extensively used vertebrate model organism in scientific research, and was the first vertebrates to be cloned. It is particularly notable for its regenerative abilities and has been modified by researchers to fabricate many transgenic strains



The main advantages of zebra fish embryos in drug testing and discovery are summarized as follows:

- ❖ Zebra fish and mammals demonstrate a high degree of similarity as it relates to molecular mechanisms of development and cellular physiology.

- ❖ Fertilization and development occurs externally, providing direct observation and manipulation of embryos in a wide range of laboratory conditions.
- ❖ The small sized embryos (0.1 mm in diameter) allows for distribution into 96 or 384- microtitre well plates. A single embryo can be maintained in 100 µl of embryo medium for days.
- ❖ A pair of zebra fish can produce 100–300 embryos. The high number of zebra fish progeny per clutch facilitates statistically considerable sample sizes at minimal cost and makes the zebra fish an excellent model for medium- and, possibly, high- throughput chemical screening required for preclinical drug discovery and toxicological evaluation.
- ❖ Embryonic development is rapid. For instance, embryos execute elusive maneuvers upon touch within 24 h post fertilization (hpf). Additionally, most major organs including the gut and the vasculature are in place by 2 days post fertilization and embryogenesis is complete 5 days after fertilization.
- ❖ Zebra fish embryos and early adults are optically transparent, a feature that facilitates direct observation of internal organs by light microscopy.
- ❖ During organogenesis, zebra fish embryos are permeable to small molecules and drugs, providing easy access for drug administration and vital dye staining.

ZEBRAFISH FOR CARDIOVASCULAR DISEASES:

Cardio vascular diseases remain a primary cause of morbidity and mortality, and many of these diseases arise from genetic defects that affect the development and maturation of the heart. Hence, understanding the

genetic, molecular, and cellular mechanisms that govern the formation, differentiation, and growth of the heart will greatly augment our understanding of heart disease and aid the identification of new approaches to repair or regenerate damaged heart muscle.

The advent of various genetic and cell biological tools such as forward genetic screens, transgenesis, lineage tracing, and cell transplantation, emerges the zebra fish, as a powerful vertebrate model to investigate a variety of biological processes. In addition, the zebra fish provides several advantages as a model to study cardiac development. First, the external development of the embryos permits a direct non invasive observation of the development of the heart as it happens and at cellular resolution. Secondly, zebra fish embryos do not initially rely on their cardiovascular system for oxygen needs. Therefore, zebra fish cardiac mutants can survive and continue to grow for several days, allowing a detailed analysis of their phenotype. Even though the zebra fish heart is simple in structure than its mammalian counterpart, genes responsible for essential steps of cardiac development are preserved throughout vertebrates (Shin J.T. *et al*., 2010).

REASON FOR SELECTION OF *Commiphora caudata* (Wt&Arn) Engl:

Burseraceae family comprises about 17-19 genera and 540 species-rich genus of flowering plants in the frankincense and myrrh family, The genus contains approximately 190 species of shrubs and trees, which are distributed throughout the (sub-) tropical regions of Africa, the western Indian Ocean islands, the Arabian Peninsula, India, and Vietnam. The genus is drought-tolerant and common throughout the xerophytic scrub, seasonally

dry tropical forests and woodlands of these regions. The common name myrrh refers to several species of the genus, from which aromatic resins are derived for various fragrance and medicinal uses by humans with deserving attention to *Commiphora caudata* (Wt&Arn) Engl. (Syn: *Proteum caudatum, Balsamodendrum caudatum*), which has been used in the treatment of numerous diseases, especially as analgesic, anti-inflammatory and wound healing but more often valued for the aromatic resins.

C.caudata is a large tree native from Indian subcontinent. It is popularly known as the **hill mango** or **green commiphora**, is the most abundant Asian species of *Commiphora* of flowering plants in the frankincense and myrrh family, Burseraceae in India**.**

Though there is a traditional and experimental evidence to support various claims and benefits of these plants still it needs proper evaluation and exploitation.

C.caudata is a traditional medicinal plant which is used in the treatment of diabetes, ulcer, inflammation, diarrhea, spasms, fever, strangury, arthritis, obesity, fever and mainly for the treatment of cuts and wounds, vitiated conditions of vata, pitta in siddha system of medicines and used as aphrodisiac, astringent. Analgesic, anti-inflammatory, antioxidant, hepatoprotective, diuretic, antidiabetic, antiulcer and antibacterial activities have been reported by researchers.

Leaves of the plant used traditionally to treat painful and inflammatory conditions. Leaf juice is applied on wounds. The leaves are traditionally used in the treatment of rheumatism, ulcers, diarrhoea and dysentry, spasms, to improve digestion and to increase appetite. Analgesic, antioxidant,

antiarthritic, antihyperlipidemic, Stem and bark are traditionally used in the treatment of rheumatism, ulcers, diarrhoea. A lotion prepared from the stem bark is used to treat skin conditions such as impetigo, eczema and shingles. The oleo gum resin of the tree is used as incense, carminative and to treat stomach troubles, wounds, hyperlipidemia, atherosclerosis, urinary infections, ascites, fistula, piles, swelling, ulcers, and pain. Snake bites and scorpion sting. The resin of the stem mixed with water to form mouthwashes to cure mouth ulcers. It was reported as astringent, antiseptic, aphrodisiac.

C.caudata is known to possess wide range of medicinal properties, which have been ascribed to the presence of bioactive compounds in different parts of the plant like flavonoids, steroids (guggulsterones), triterpenoids, proteins, carbohydrates and glycosides.

Though there is a traditional and experimental evidence to support various claims and benefits of these plants still it needs proper evaluation and exploitation.

Based on the above discussion we focus our study to utilize the vast economic potentiality, which can be fully established by its vast consumption. It is evident that there is a good level of experimental evidence to support claims and advantages of various medicinal herbs used in our traditional diet and medicines. In this view we selected the widely available plant *Commiphora caudata* (Wt & Arn) Engl. for our study.

In India it is available it has been recorded in dry deciduous and scrub forests of Tamilnadu, Karnataka, Kerala, Andra Pradesh and also in SriLanka .

This study aims to scientifically explore its important medicinal uses which have not been fully studied. These initiated us to investigate the leaves of this plant with strict scientific protocols. Review of literature showed lacunae exist in the pharmacognostic, phytochemical and pharmacological studies of *C.caudata*.

LITERATURE REVIEW

LITERATURE REVIEW

TAXONAMIC HIERARCHY:

CLASSIFICATION:

(Anonymous,www.eol.org)

Kingdom	:	Plantae
Phylum	:	Tracheophyta
Class	:	Magnoliopsida
Order	:	Sapindales
Family	:	Burseraceae
Genus	:	*Commiphora*
Species	:	*caudata*

PREFERRED SCIENTIFIC NAME:

Commiphora caudata (Wt & Arn) Engl.

OTHER SCIENTIFIC NAMES

Syn: *Amyris acuminata* Roxb.;

Amyris serratifolia Rottl. ex A. W. Benn.;

Balsamea caudata (Wight & Arn.) Engl.;

Balsamodendrum caudatum (Wight & Arn.) March.;

Balsamodendrum roxburghianum (Wight & Arn.) Wall. ex Voigt;

Commiphora roxburghii (Wight & Arn.) Alston;

Protionopsis caudata (Wight & Arn.) Bl.;

Protionopsis roxburghiana (Wight & Arn.) Bl.;

Protium caudatum Wight & Arn.;

PREFERRED COMMON NAMES:

English: Hill mango
Green commiphora

LOCAL COMMON NAMES: (Anonymus,www.eol.org)

Languages	Vernacular Names
Kannada (6)	Assuraada, hasuvaara, kalmaavu, konda maavu, konda mugur, kondamavu
Tamil (13)	Atakamikam, atakamikamaram, karpurakkiluvai, kattukkiluvai, kiluvai, malaikkiluvai, malaima, malankiluvai, paccaikkiluvai , perunkiluvai, urukkutanaiparpamakki, venkiluvai, vetkiluvai
Telugu (7)	Konda-mamidi, kondamaamidi, kondamukkadi, kondaraavi, netta maamidi, vaetapathri, vetapatri (for balsamodendron)
Sanskrit (2)	Ikkata, Ikkada
Malayalam(2)	Idinjil, Itinjil

ORIGIN AND DISTRIBUTION:

RANGE:

This species is has a restricted global distribution occurring only in India and Sri Lanka. Within India mainly Southern India, it has been recorded in dry deciduous and scrub forests of Andhra Pradesh, Karnataka, Kerala and Tamil Nadu.

Average income, frequency of cuttings and average pole length of live fences were produced from the trees *Commiphora caudata* which is

traditionally called as “Kiluvai” and highly contributed to income from live fence of the homegardens in sites. Income of the live fence was mainly generated by sales of pole or stick after pollarding the trees. Thus, when planted by farmers, live fences tend to be simple linear.

The tree is sometimes harvested from the wild for local medicinal use. It is occasionally used as an avenue tree and is often planted as an ornamental.

Special characters:High durability, low wind susceptibility, low susceptibility to pests, low wood quality.

GROWTH REQUIREMENT:

1.SOIL:

Requires a in a well-drained soil with rich in organic matter.

2.CLIMATE:

C.caudata usually growing in the full sun or sunny position on hilly granite rock outcrops in dry zone areas.

3.ALTITUDE:

The altitude is an important factor which limits the distribution and importance of several species Among the medicinal plants, the species *Commiphora caudata,* are considered to be ecologically well established in the low hills of many places of Southern India (450m above msl and 750m above msl and 950m above msl).

Commiphora caudata is also found in dry deciduous forest from the coast up to 1000-1200 meters in the hills.

The medicinal species were respectively determined as ecologically important due to their high perpetuation level.

The variation in local climate at different altitudes may cause this difference in structural organization of communities .

4. NUTRIENTS:

The soils are rich in organic matter, which increases content with increase in altitude.

Maximum value of organic matter has been found in *Commiphora caudata,* organic matter also leads to increase in cation exchangeable capacity and quantity of N,K and P. P availability is also indicative of the soil pH. High level of P has been found in soil having *C.caudata* plant.

There are trace elements that are required in micro-quantity for optimum growth of plants. *C.caudata* requires the amount of micronutrients in the order of Mn > Fe > Cu > Zn..

- **Flowering season :** From March to May.
- **Fruiting season :** From August to January.
- **Seeding season :** From August to January.
- **Leaves falling :** During the hot season.
- **Sex distribution:**

Commiphora caudata is bisexual (each flower of each individual has both male and female structures).

- **Mode of pollination :**

Commiphora caudata is pollinated by a wide variety of insects.

- **Seed dispersal :**

The seeds of *Commiphora caudata* are mainly dispersed by frugivorous birds and mammals.

PROPAGATION:

Seed and Cuttings. Cutting propagation is a very easy technique plantings of sticks or poles of trees (usually of only a major species) that are evenly spaced and periodically pollarded and trimmed.

PLANT COLLECTION AND AUTHENTICATION:

Leaves of the plant ***C.caudata*** selected for our study was collected from **Oricheri Village,Bhavani Taluk, Erode District**, Tamilnadu, India during the month of July 2016 and was authenticated by **Dr.Stephen,** Department of Botany, American college, Madurai and **Dr.Sasikala,** Director (Retd) of Siddha Central Research Institute, Arumbakkam, Chennai.

LEAF DRYING AND PULVERIZING:

The leaves were collected and shade dried. It was powdered in a mixer. The powder was sieved in a No.60 sieve and kept in a well closed container in a dry place.

WHOLE PLANT

ETHNOMEDICAL USES:

- *Commiphora caudata* (Common name:Kiluvai) is a traditional medicinal plant which is used in the treatment of diabetes, ulcer, inflammation , diarrhoea and spasms. (Selvamani P *et al.,*2013)
- *C. caudata* was practiced as village folklore medicine for treating ulcer, inflammation, diarrhea and spasms.(Deepa V S *et al*.,2009)
- The plant *Commiphora caudata* posses astringent, sweet, cooling, aphrodisiac, diuretic and antidiabetic activities. The *C.caudata* is used for fever, strangury , vitiated conditions of vata and pitta in siddha system of

medicines. *C.caudata* is a potential medicinal plant used traditionally in the treatment of rheumatism , ulcers , diarrhoea and diabetes.(Nisha P and Jyothi H.,2014)

- *C.caudata* employed in the treatment of various ulcers .(Ganesan S *et al*.,2008)
- Ethnomedical plants used for the treatment of cuts and wounds by Kurkuma tribes, Wayanadu district of Kerala, India. (Thom as B *et al*.,2014)
- Many of these compounds have been used in the treatment of hyperlipidemia, rheumatic disorders, obesity and ischaemic heart diseases. *C.caudata* has been used traditionally in the treatment of arthritis , diabetes and obesity.(Geetha K and Ganapathy S.,2013)
- *Commiphora wightii* reported as endemic and endangered (Prasad M N V *et al*.,2007)
- Analgesic, anti-inflammatory and antioxidant, hepatoprotective, diuretic, antiulcer and antibacterial activities were reported from this plant(Geetha K and Ganapathy S.,2013)

PHYTOCHEMISTRY:

- An exhaustive literature survey on the C*ommiphora* species revealed that the genus is a rich source of steroidal compounds, flavanoids, glycosides phenolics, etc.,(Selvamani P *et al.,*2013)

LEAVES

PHARMACOGNOSY:

Macroscopy:

Leaves were compound, alternative 3 to 7 foliolate, upper surface dark green, lower surface light green in colour. There is no characteristic odour and it has mucilaginous taste. Shape is ovate -oblong, length - 4.5 to 6.5 cm; width -2.2 to 3.5 cm; acuminate apex , slightly asymmetric base; entire margin, reticulate pinnate venation; pedicle length - 3.5 to 6.2 cm and glabrous texture , glossy above, subglaucous below. Leaflets ovate or elliptical, chartaceous and glabrous.

Microscopy of the leaves:

Leaflet Diagnostic features

The leaflets were dorsiventral with prominent midrib**.**

Leaflets - dorsiventral, mesomorphic,hypostomatic, glaborous; midrib adaxially projecting into a hump;adaxial part shallowly convex.

Vascular bundles of the **midrib** include one larger median bundle and one smaller, adaxial accessory bundle.

Lamina with uniserrate epidermal layers; mesophyll differentiated into a single layer of palisade cells and lobed aerenchymatous spongy parenchyma cells.

Vascular bundle of the lateral vein has adaxial bundle sheath extension.

Stomata actinocytic type; epidermal cells angular, straight and thick walled.Vein islets polygonal; vein termination branched many times.

The petiole is roughly circular in outline , petiole semicircular with adaxial depression.

Vascular strengths of the petiole many, arranged in a circle with adaxial opening. Petiole circular with a ring of vascular strands.

Secretory canals occur in the phloem region of leaf, veins and petioles. Large druses of calcium oxalate crystals abundant in the leaf.

ETHNOMEDICAL USES:

- Leaves of the plant used traditionally and tribal medicine of Kerala to treat painful and inflammatory conditions.The hill Pulayas of Kerala use the leaves to treat inflammation and pain.(Annu W *et al*.,2010)
- Leaf juice is applied on wounds (Thomas B *et al*.,2014)
- The leaves are useful in rheumatalgia (Nisha P,Jyoti H.,2014)
- The leaves are traditionally used in the treatment of rheumatism, ulcers, diarrhoea and spasms(Geetha K *et al.,*2014)
- The leaves are used to improve digestion and to increase appetite (Akhade MS *et al*.,2017)
- Leaves are used in the treatment of dysentry.(Gunasekaran M , Balasubramaniyam P.,2012)

PHYTOCHEMISTRY:

- It was identified that the active constituents present in the plant was steroids and triterpenoides and it was suggested as marker for this plant.(Akhade MS *et al*.,2017)
- TLC of methanolic *C.caudata* leaves:

  On pre-coated silicagel G60-F254 using the petroleum ether:Ethyl acetate : methanol in ratio 6:2:0.5 as mobile phase and E-Guggulsterone as standard. The λmax of E-guggulsterone is identified as242 nm.HPLC analysis was done using PDA detector so that the

same system can be used to quantify the steroid or triterpenoid compounds having different absorption,the percentage content of *E*-guggulsterone 0.059% .(Akhade MS *et al*.,2017)

- The leaves were reported to contain tannins, carbohydrates and oleo-gum resin.(Nisha P *et al*.,2014)
- The HCl extract of *C.caudata* leaves contains alkaloids, glycosides, flavanoids, saponins, carbohydrates. The H_2SO_4 extract of the *C.caudata* leaves contains alkaloids, terpenoids and carbohydrates, phenols, proteins (abundent).(Aejitha S *et al*.,2015)
- Phytochemical screening of *C.caudata* showed the presence of glycosides, flavonoids, starch, reducing sugars, proteins, aminoacids, steroids, tannins and terpenoids . (Selvamani P *et al*.,2013)
- The presence of flavonoids, tannins , polyphenols , alkaloids , terpenoids were reported (Meera S *and* Yashashwini Y C .,2016)
- Phytochemical studies of this plant showed the presence of flavonoids, glycosides, steroids, proteins, mucilage, tannins, terpenoids, and carbohydrates .(Geetha K and Ganapathy S, 2013)
- The potential activity of the extracts can be attributed to the presence of antioxidant principles such such as phenolic compounds which were reported from this plant.(Geetha K,Ganapathy S.,2013)
- The percentage yield of ethanolic extract was 8%w/w. The amount of phenols in ethanolic extract of *C.caudata* was found to be 18.67mg/g of crude drug. (Geetha K and Ganapathy S, 2013)

- Phytochemical study showed the presence of flavanoid compounds as a secondary metabolite in plants which is well known for its anti-inflammatory property.(Balasundar S *et al*.,2016)

PHARMACOLOGICAL ACTIVITIES:

- ✓ Hepatoprotective activity was reported for *C.caudata* leaves (Selvamani P *et al*.,2013)
- ✓ Pharmacological evidence reports that *C.caudata* have antidiabetic, antimicrobial, antibacterial and anticancer activities. (Selvamani P *et al*., 2013)
- ✓ EFFECT ON NERVOUS SYSTEM:

  It was demonstrated that *C. caudata* leaves was effective in reverting amnesia in scopolamine induced amnesia in rats(200,400mg B.W p.o) learning and memory enhancing activity was also proved dose dependently (Meera S and Yashashwini Y C.,2016)

- ANTIMALARIAL ACTIVITY:

  Invitro schizonticidal activity with *P.falciparum* (Prasad M N V *et al*.,2007)

- ANALGESIC EFFECT:

  IP injection of extract(250,500 mg) significantly inhibited acetic acid induced writhing response in mice 73.44% and 77% respectively when compared to aspirin 25mg/kg ,92.18%.(Annu W *et al*.,2009)

- ANTIOXIDANT ACTIVITY:
- ✓ Reduction in lipid peroxidation , increase of superoxide dismutase activity in brain homogenate , catalase activity, glutathione assay dose dependent manner proved its antioxidant effect . (Meera S and Yashashwini Y C.,2016)

- ✓ All the *invitro* assays of the ethanolic extract of the *C.caudata* leaves showed antioxidant activity by ability to scavenge free radicles. This study showed that C.caudata has potent antioxidant activity than *C.Var pubescence* (Deepa V S *et al.,*2009)
- ✓ The *C.caudata* posses higher antioxidant activity than *C.var pubescence* which may correlatable to the phenolic and flavanoid content of the respective plant extract. (Deepa V S *et al*.,2009).
- ✓ The antioxidant studies on the ethanolic extract of *Commiphora* species could be helpful in preventing or slowing the progress of various oxidative stress-related diseases (Deepa V S *et al*.,2009).
- ✓ *Invitro* antioxidant effect of ethanolic extract of leaves by anti- lipid peroxidation method showed total inhibition of $FeCl_2$ to ascorbic acid stimulated rat liver peroxidation at 50 µg/ml did not significantly increase the anti lipid peroxidation any further. From the above findings it was reported that *C.caudata* leaves has potent analgesic and anti inflammatory activity and justifice its use in traditional medicine to treat inflammatory and painful conditions and may be due to free radical scavenging activity. (Annu W *et.al.*,2010)

- ➢ ANTIARTHRITIC ACTIVITY:

  The ethanolic extract of *C.caudata* leaves was reported that the anti arthritic activity by complete Freunds adjuvant induced arthritic rats (200 and 400 mg/kg P.O). The ethanolic extract of *C.caudata* leaves posses potential antiarthritic activity. (Girija P *et al*., 2014, Reddy J S *et al*., 2014)

- ANTIHYPERLIPIDAEMIC ACTIVITY:

- ✓ The ethanolic extract of *C.caudata* leaves showed significant antihyperlipidaemic properties and it can be used in the treatment of vascular disorders including atherosclerosis (Geetha K and Ganapathy S.,2013)
- ✓ In a dose dependent manner the oral administration of EECCL (100 and 200 mg/kg body weight) for 21 days significantly lowered the serum total cholesterol(TC), triglycerides(TG) and low density lipoprotein-(LDL-C) levels while a marked increase in high density lipoprotein(HDL-C) levels were observed . (Geetha K and Ganapathy S.,2013)
- ✓ The alcoholic (propanol) extract of *C.caudata* leaves significantly reduces the lipid accumulation with 51.5% at 100 µg/ml through the quantification method of Oil Red O staining.(*Invitro* Antiadipogenic activity). The ethanolic extract of the *C. caudata* leaves proved to be beneficial in the management of hyperlipidaemia. (Geetha K *et.al*,2014)
- ✓ ANTIANAEMIC ACTIVITY:

  Ethanolic extract of *C.caudata* leaves with dose 400 mg/kg, p.o significantly increase the red bloodcells, haemoglobin and above the 400 mg/kg dose significantly decrease the white blood cells, erythrocyte sedimentation rate, SRF (Serum rheumatic factor) and CRP(c-reaction protein) when compared with arthritic control rats.(Girija P *et.al*,2017)
- ✓ Hematological parameters: The ethanolic extract of *C.caudata* leaves (200mg/kg)treated group reduces the WBC and increases the haemoglobin content significantlt and no effect on RBC when compared with arthritic control.(Girija P *et al.*,2017)

- ✓ HEPATOPROTECTIVE ACTIVITY:

  The oral administration of ethanolic extract of *C.caudata* (200mg/kg)for 21 days which reduces the AST,ALT and ALP levels (were observed) which indicates the hepatoprotective potential of the extract.(Geetha K and Ganapathy S.,2013).

- ➢ ACUTE TOXICITY STUDIES:

- ✓ The acute toxicity studies in ethanolic extract of *C.caudata* leaves was carried out using Albino rats as per the guidelines set by Organization for Economic Co-operation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)}.The *C.caudata* at the dose of 2g/kg B.W was given to 5 animals.the animals were continuously observed for 14 days for general behaviour and mortality. Mortality was not observed by the dose of 2 g/kg bodyweight of *C.caudata* leaves in the oral toxicity studies. From the results the efficiency test drug doses of 0.2,0.4g/kg body weight were chosen for the efficiency studies.(Girija P *et.al*,2017)

- ✓ The ethanolic extract of *C.caudata* leaves was found to be less toxic. (CTC 50 ranging from120- 720 µg/ml) on 3T3 L1 cells. (Geetha K *et.al*,2014)

- ➢ BEHAVIOURAL AND TOXIC EFFECT:

  Ethanolic extract of the leaf given p.o and observed behavioural changes,toxicity and mortality . LD_{50} was greaterthan 1500 mg p.o in mice. (Annu W *et.al.*,2010)

- ➢ ANTI INFLAMMATORY ACTIVITY:

- ✓ The aqueous extract of *C.caudata* leaves has proved for its significant

action in inflammation for oral administration is much better than external application in experimental evaluation on artificially induced inflammation. So it could be considered as a drug of choice for inflammatory conditions. (Balasundar S *et.al*,2016)

- ✓ The ethanolic extract of *C.caudata* leaves reduced the paw volume at 41.52%, 45.76% in first phase. 62.33%, 65.35% in second phase. (Selvamani P *et.al*,2013)
- ✓ Ethanolic extract of the *C.caudata* leaves and its formulation when evaluated (*C.caudata* leaves-25mg,*Commiphora berryi* bark-25mg, *Commiphora pubecense* -25 mg =final 300 mg/capsule) for CCl_4 induced hepatotoxicity in albino rats. It was observed that the percentage of hepatoprotective of *C.caudata* leaves was significant in cell necrosis, fatty change, hyaline regeneration, ballooning degeneration. (Balamurugan B *et.al.*,2010)
- ✓ Histopathological studies of the liver showed preservation of the structural integrity of the hepatocellular membrane in a dose dependent manner. Formulation showed significant activity than the individual drugs. (Balamurugan B *et al.*, 2010)
- ✓ Ethanolic extract of the leaves showed inhibition of carrageenan induced paw oedema in rats (250,500mg/kg) dose dependently (67 and 78%).Indomethacin (5mg/kg) showed 89% inhibition.(Annu W et al.,2010)
- ✓ The ethanolic extract of the *C.caudata* leaves inhibits the activation of β-cells by medicinal constituents because it contains substantial amounts of plant steroids which are reported to produce an anti inflammatory action.(Girija P *et al.*,2017)

LEAF OIL:

PHYTOCHEMISTRY:

- The yield of 2.7ml/kg from fresh leaves C.caudata was reported and by GC-MS analysis ,the presence of 15 compounds were reported. In which β-pinene(33.7%), cyclofenchene(17.8%), and α-terpineol (10.5%) were found to be major compounds. 10ml/kgaromatic oil with mango like odour volatile oil yield was reported . (Anjaneya Reddy *et al*.,2015) .
- Monoterpene hydrocarbons (51.54%) i.e. cychlofenchene (17.84%) and β-pinene (33.70%) were the major compounds.
- α-Terpineol (10.40%), verbenol (5.40%), 4-terpineol (3.79%), myrtenol (3.73%), myrtenal (3.45%), linalool acetate (2.61%), thujen-2-one (2.02%), β-linalool (1.48%), 2-pinene-4-one (1.37%) and 1,8-cineole (0.94%) were the oxygenated monoterpenes accounting for 35.19% of the oil.
- Other compounds identified in the oil were: sesquiterepene hydrocarbons, caryophyllene (1.66%) and oxygenated sesquiterpenes (11.61%) , caryophyllene oxide (9.82%) and ledol (1.79%).(Anjaneya Reddy *et al*.,2015)
- 28 compounds were reported by GC-MS in which verbenone(8.1%), 3-carene(9.9%), cyclofenchene(16.9%), dihydrocarveol(19.5%) are major compouns.(Anjaneya Reddy *et al*.,2015)

FRUIT:

PHYTOCHEMISTRY:

- It was reported that phytochemical screening of the fruit using 3 various solvents(Ethanol, Methanol and Aqueous) to contain the following constituents.

Ethanol –alkaloids, coumarins, flavanoids, terpenoids, phenols, cardiac glycosides, saponins, quinones, steroids.

Methanol-alkaloids, coumarins, flavanoids, terpenoids, phenols,cardiac glycosides, saponins, quinones, steroids.

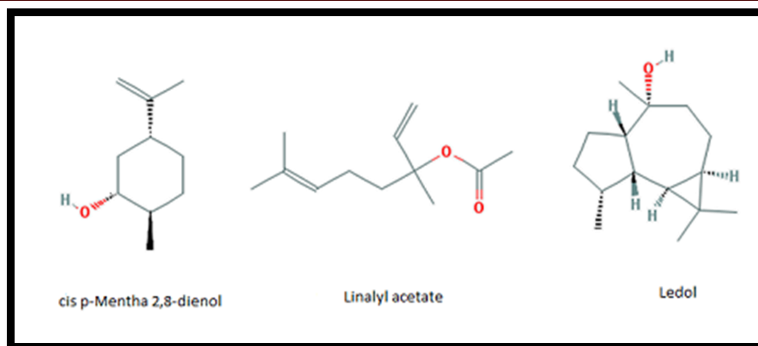
Aqueous-tannins, terpenoids, cardiac glycosides, quinones, steroids,carbohydrates.

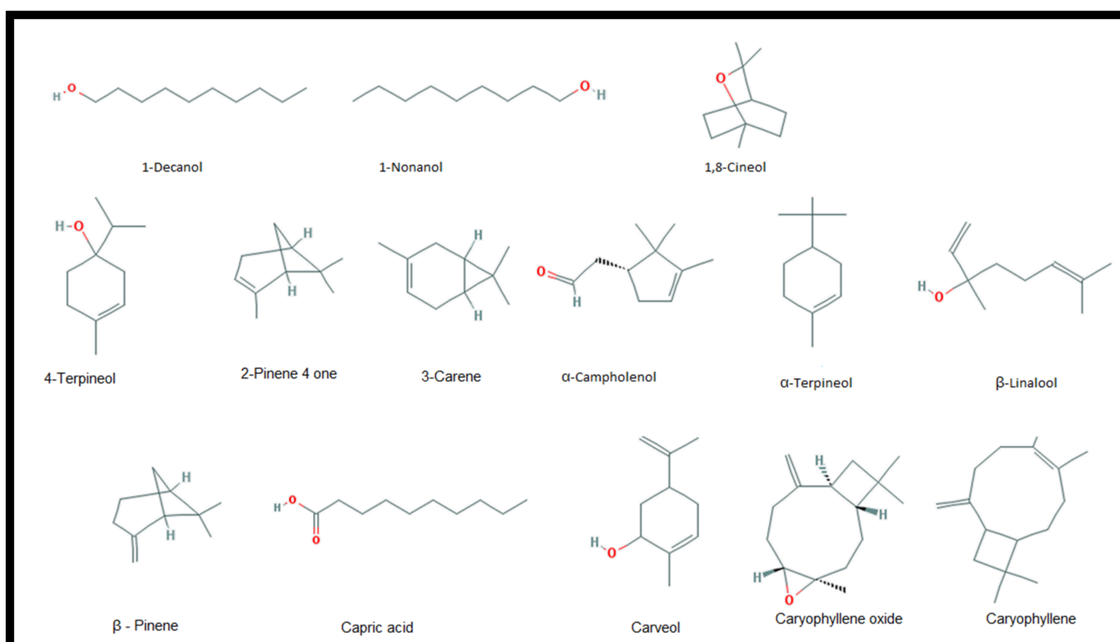
(Shaik A T and Balakumar B S.,2014)

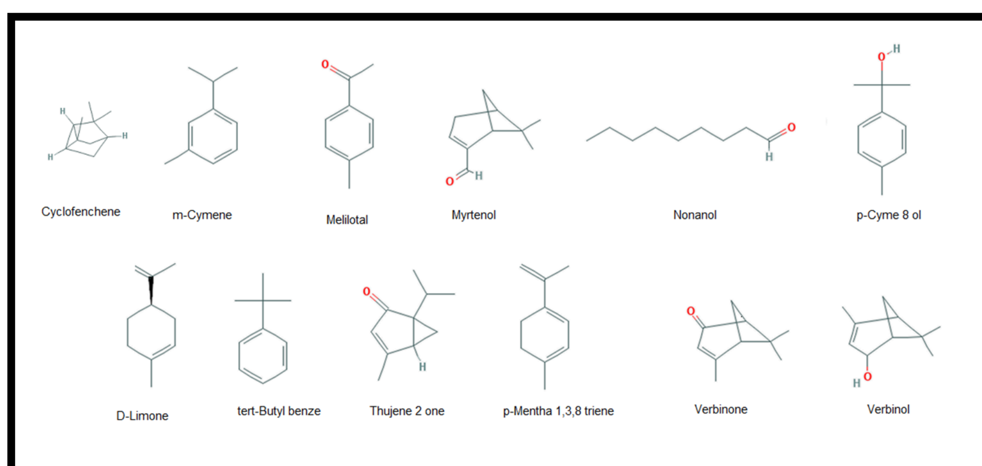
FRUIT OIL:

PHYTOCHEMISTRY:

- Monoterpene hydrocarbons (32.56) i.e.Cychlofenchene(16.97), β-Pinene(2.58) are the major compounds.
- Linalool acetate (1.15), Thujen-2-one (1.91), Myrtenol (0.86), β-Linalool (1.23), 1,8-Cineole (2.05) were the oxygenated monoterpens (50.32).
- 3-Carene(9.90), *tert-Butylbenzene* (1.11), D-Limonene (0.89), *p*-Mentha-1,3,8-triene (1.11), *m-Cymene* (2.62), Nonanal (0.91), Verbenone (8.18), Phenylacetone (3.75), 2(10)-Pinene-3-ol (2.51), *cis*-*p*-Mentha-2,8-dienol (0.90), Melilotal(1.12), α-Campholenal (1.00), 1-Nonanol (1.20), Dihydrocarveol (19.58) , *p-Cymene-8-ol* (6.05), Carveol (1.37), 1-Decanol (1.83), Capric acid (2.41), oxygenated hydrocarbons (4.24), aromatic compounds (8.67).
- Oxygenated sesquiterpenes (0.96) , caryophyllene oxide (0.96).


cis p-Mentha 2,8-dienol
Linalyl acetate
Ledol


1-Decanol
1-Nonanol
1,8-Cineol
4-Terpineol
2-Pinene 4 one
3-Carene
α-Campholenol
α-Terpineol
β-Linalool
β - Pinene
Capric acid
Carveol
Caryophyllene oxide
Caryophyllene


Cyclofenchene
m-Cymene
Melilotal
Myrtenol
Nonanol
p-Cyme 8 ol
D-Limone
tert-Butyl benze
Thujene 2 one
p-Mentha 1,3,8 triene
Verbinone
Verbinol

SEEDS:

ETHNOMEDICAL USES:

- The endosperm obtained from 4/5 fresh or dried seeds of *C.caudata* is taken 2 times a day for 2-3 days to relieve stomach ache. (Vikneshwaran D *et al*.,2008)

PHYTOCHEMICAL STUDIES:

- By HPLC analysis acetonitrile: water (86:14) with R_t 6 mts for E-guggulsterone with no interference with other constituents and found to be suitable for estimation of steroids and terpenoids using PDA actdetector. The percentage of E-guggulsterone present as 0.059%.(Akhade MS *et al*.,2017)
- The C.caudata plant extract contains 8.21± 5.0%(mg/ml) flavanoids, 20.8 ±0.2% (mg/ml)phenol. (Deepa V S *et al*.,2009)

STEM AND BARK

ETHNOMEDICAL USES:

- The stem and bark are traditionally used in the treatment of rheumatism, ulcers, diarrhoea and spasms.(Girija P *et al.,* 2017)

For Skin Diseases:

- A lotion is prepared from its stembark is used to treat skin conditions such as impetigo, eczema and shingles.(Prasad MNV *et al*.,2007)
- Bark is used in the treatment of diarrhoea (Gunasekaran M,Balasubramaniyam P)

ROOT:

PHYTOCHEMISTRY:

- It was reported that phytochemical screening of the root using various solvents.
  1. Aminoacids(Chloroform, Ethyl alcohol, Water)
  2. Anthraquinone(Absent in all solvents)
  3. flavanoids(Petroleumether, Chloroform, Ethanol, Water)
  4. glycosides(Petether, Chloroform, Ethylalcohol, Ethylacetate, Water)
  5. Proteins(Ethylalcohol, Ethylacetate)
  6. reducing sugars(Petether, Chloroform, Ethanol, Water)
  7. saponins(Water)
  8. starch(Petether, Chloroform, Ethyl acetate, Ethylalcohol, Water)
  9. tannins(Petether, Chloroform, Ethylalcohol, Water)
  10. terpenoids(Petether, Chloroform, Ethyl alcohol)
  11. steroids (Petether, Chloroform, Ethyl acetate, Ethanol, Water)
  12. (Nisha P and Jyoti H.,2014)
- The percentage yield of extractive values of root were reported as

  Petroleum ether (brown)-0.9, Chloroform (brown)-1.2

  Ethyl acetate (brown)-1.17, Ethyl alcohol (brown)-9.7

  Distilled water (brown) - 4.7.(Nisha P and Jyoti H.,2014)

PHARMACOLOGICAL ACTIVITY:

HEPATOPROTECTIVE ACTIVITY

- It was reported that 500µg/ml,40µg/ml protected antitubercular drugs and galactosamine hydrochloride induced hepatotoxicity in BRL3A Cell lines protection is 80.5%.(Nisha P and Jyoti H.,2014)
- *Invitro* hepatoprotective activity of ethanolic extract of *C.caudata* (wight &

Arn) Engl on the BRL 3A cell line study indicates that root extracts of plants have good potentials for use in hepatic diseases.(Nisha P and Jyothi H.,2014)

BARK:

Ethnomedical uses:

- The bark is traditionally used in the treatment of rheumatism, ulcers, diarrhoea and spasms.(Girija P *et al*.,2017)

PHARMACOGNOSY:

- The alcoholic extract of *C.caudata* bark was found to be.,19.5%w/w. (Geetha K *et al*., 2014)

GUM RESIN:

ETHNOMEDICAL USES

- The oleo gum resin of the tree is used as incense. (Anonymus, 1950)
- The gumresin from the bark is used for treating stomach troubles. (Latha P *et al*.,2005)
- The gum of the stem mixed with water to form mouthwashes to cure mouthulcers and the gum is used for wound healing.(Ganesan S *et al*.,2007)
- Resin is carminative. (Akhade M S *et al*.,2017)

PHYTOCHEMISTRY

- Guggulsterone-M, Guggulsterols-I, Guggulstrols Y, myrrhanol, myrrhanone A,Z and E-guggulsterones many related compounds were ferulates, furanosesquiterpenes, steroids, oxygenated alkenes, lignans,etc.(Prasad M N V *et al*.,2007)

PHARMACOLOGICAL ACTIVITY:

ANTIOXIDANT ACTIVITY

- The methanolic extract of the oleo gum resin showed NO (Nitric oxide) production inhibition.(Prasad M N V *et al*.,2007)
- This plant contain a substance like guggulsterone, which has been proved to be anti-inflammatory by the mechanisms may be associated with the inhibition of inflammatory mediator overproduction. (100µg/ml of *C.caudata* having 75.98% anti inflammatory activity compared to dexamethasone having 94.35% activity.)(Girija P *et al*.,2017)

BARK

PHARMACOLOGICAL ACTIVITY:

HYPOGLYCAEMIC ACTIVITY:

- Methanolic extract of *C.caudata* bark reduced blood glucose level by chronic treatment in alloxan induced diabetic rats (dose of 200,400 mg /kg B.W P.O) in duration dependent manner which indicating its antihyperglycaemic activity.Normoglycaemic blood level also not altered(normal rats) further strengthening the hypoglycaemic potential of that extract.

  (Srinivasa Reddy CH *et al*.,2017).
- The *C.caudata* bark did not show the significant inhibition of fat accumulation or fat droplet formation at the tested concentration(100µg/ml-4.8%).(Geetha K *et al.,*2014)

ACUTE TOXICITY STUDIES:

- The dose of 2000mg/kg of methanolic extract of bark was found to be safe and no toxicity and mortality was not observed.(Srinivasa Reddy CH et.al,2017)

ZEBRAFISH AND 3Rs:

Zebrafish research supports each of these principles through,

REFINEMENT: Using the results of zebrafish screening to design definitive mammalian studies that will provide the useful information (e.g., collection of additional endpoints, shifting timing of studies in the drug development process)

REPLACEMENT: Testing chemicals under REACH regulations in a non-mammalian model rather than rodents or rabbits

REDUCTION: Reducing the number of compounds that need to be tested in mammalian models by facilitating the choice of candidates with a greater likelihood of success (River C,2014).

ZEBRAFISH AN EXCELLENT MODEL ORGANISM:

The zebrafish is an excellent model organism for vertebrate developmental biology, which generates high-value knowledge on safety risks of novel drugs. The larval zebrafish possess advantages of whole organism phenotypic assays and those (rapid production of results with minimal resource engagement) of in vitro high-throughput screening techniques.

An attractive feature of zebrafish assays for pharmacological investigations is the potential to use of them in medium-to high- throughput screening mode, as they are smaller in size (5cm for an adult and 5mm for 7

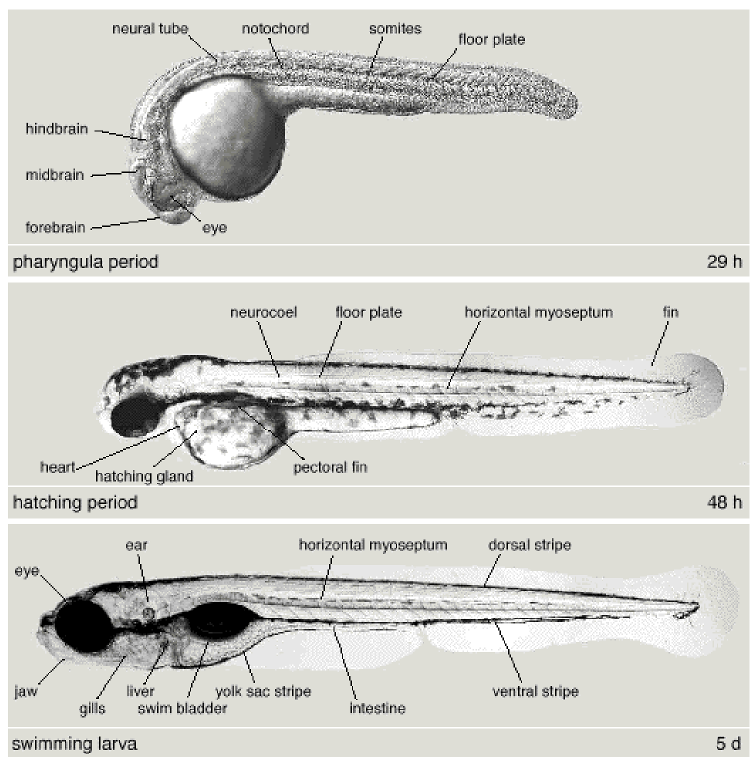
days postfertilization (d.p.f.) larvae) and robust freshwater tropical cyprinid that is easy to maintain in large stocks due to their highfecundity.

Experimental manipulation and direct observation of organ function can be easily performed as embryos are transparent and rapidly develop ex utero with most organs becoming fully functional between 3 and 5d.p.f.

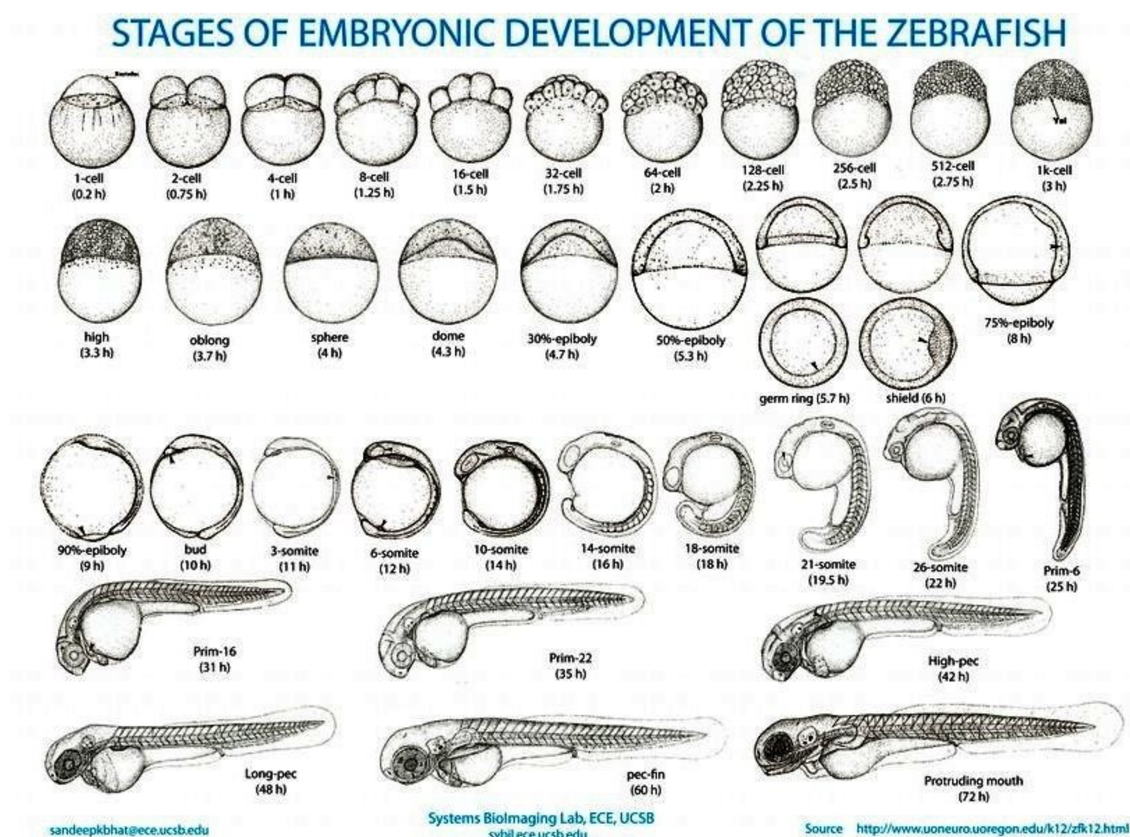
METHOD:

- Treatment with a range of concentrations of the test compound (e.g., 0.1 to 100μM)
- Stock solution of test compound in either DMSO or an aqueous vehicle added to zebrafish embryo medium
- Continuous exposure of zebrafish embryos/larvae in the treated embryo medium between approximately 4 and 120 hours post-fertilization (developmental periods generally analogous to the period of treatment in mammalian EFDstudies)
- Assessment of viability, growth, morphology and functional end points
- The results of these tests are then used for identification of potential developmental toxicity hazards, the data are used to predict whether the test compound is likely to produce any developmental toxicity in standard mammalian EFD studies or human exposures (River C, 2014).

ANATOMY OF ZEBRAFISH:



EMBRYONIC STAGES OF ZEBRAFISH:



- The organization of the genome and the genetic pathways controlling signal transduction and development appear to be highly conserved between zebrafish and humans.
- In contrast to rodents, the zebrafish larvae are not foetal but are closer to the juvenile state in that the nervous system is mature, vital organs are functioning and tissue architecture is fully developed at the time of theassays.
- Further, only milligrams of compound are needed for screening in 96-well plates as the larvae can live in as little as 50 ml of fluid.

- Finally, chemical screening is facilitated by the fact that zebrafish are reasonably tolerant to dimethylsulphoxide concentrations generally used in such technologies and small molecule compounds dissolved in the swimming medium can reach target tissues via passage through the skin of thelarvae.
- The zebrafish cardiovascular system can reveal decrease in heart rate and atrial– ventricular dissociation, which may signal human ether-a-go-go-related gene (hERG) channel blockade.
- Another area of interest is the CNS, where zebrafish behavioural assays have been further developed into screening platforms for assessment of locomotor activity, convulsant and proconvulsant liability, cognitive impairment, drug dependence potential and impaired visual and auditoryfunctions.
- Zebrafish also provide interesting possibilities for evaluating effects on bone density and gastrointestinal function.
- In addition, available knowledge of the renal system in larval zebrafish can permit identification of potential safety issues of drugs on this often neglected area in early developmental stages (Barros T.P.*et al*;2008).

ZEBRAFISH AS A TOOL FOR DEVELOPMENTAL TOXICITY ASSAY

The 5th-day zebrafish developmental toxicity assay is based on well-tested methods with demonstrated value in pharmaceutical and chemical compound screening. In addition, the assay has been designed to predict the outcome of standard developmental toxicity testing methods defined in guidance from the US FDA and ICH for pharmaceuticals and the US EPA and OECD for chemicals.

- potential to use of them in medium-to high- throughput screening mode, as they are smaller in size (5cm for an adult and 5mm for 7 days postfertilization (d.p.f.) larvae) and robust freshwater tropical cyprinid that is easy to maintain in large stocks due to their high fecundity.
- Experimental manipulation and direct observation of organ function can be easily performed as embryos are transparent and rapidly develop ex utero with most organs becoming fully functional between 3 and 5d.p.f.
- The organization of the genome and the genetic pathways controlling signal transduction and development appear to be highly conserved between zebrafish and humans.
- In contrast to rodents, the zebrafish larvae are not foetal but are closer to the juvenile state in that the nervous system is mature, vital organs are functioning and tissue architecture is fully developed at the time of the assays.
- Further, only milligrams of compound are needed for screening in 96-well plates as the larvae can live in as little as 50 ml of fluid.
- Finally, chemical screening is facilitated by the fact that zebrafish are reasonably tolerant to dimethylsulphoxide concentrations generally used in such technologies and small molecule compounds dissolved in the swimming medium can reach target tissues via passage through the skin of the larvae.
- The zebrafish cardiovascular system can reveal decrease in heart rate and atrial– ventricular dissociation, which may signal human ether-a-go-go-related gene (hERG) channel blockade.

- Another area of interest is the CNS, where zebrafish behavioural assays have been further developed into screening platforms for assessment of locomotor activity, convulsant and proconvulsant liability, cognitive impairment, drug dependence potential and impaired visual and auditory functions.

- Zebrafish also provide interesting possibilities for evaluating effects on bone density and gastrointestinal function.

- In addition, available knowledge of the renal system in larval zebrafish can permit identification of potential safety issues of drugs on this often neglected area in early developmental stages (Barros T.P.*et al*;2008).

AIM AND OBJECTIVE

AIM AND OBJECTIVE

Cardiovascular diseases (CVD) s is a group of diseases that involve the heart and blood vessels. They are the major cause of death, globally an approximate million people died from CVDs in 2012, representing 30% of all global deaths. Over three quarters of CVD deaths take place in low and middle-income countries. CVDs includes coronary artery diseases (CAD) such as angina and myocardial infarction, stroke, heart failure, hypertensive heart disease, rheumatic heart disease, cardiomyopathy, heart arrhythmia, congenital heart disease, valvular heart disease, carditis, aortic aneurysms, peripheral artery disease, thromboembolic disease, and venous thrombosis**(**Wikipedia**)**.

Doxorubicin (DOX) is a potent and extensively used anthracycline antibiotic for the treatment of cancers. However, the major impeding issue pertaining to the clinical application of DOX is related to its ability to induce untoward toxicity in heart tissues. The occurrence of fatal cardio toxicity is characterized by an irreversible cardiomyopathy which compromises the clinical efficacy of DOX and accounts for the major basis of the chemotherapy related morbidity and mortality (Ojha *et al*., 2016).

Since the origin of the human civilization, plants and herbs have been traditionally used in the treatment of various diseases and ailments. In this direction, the prospect of harnessing the potentials of plant-derived small molecules (Phytochemicals) are the natural constituents of herbs and plants.

Nowadays, essential oils are well known for their cosmetic, pharmaceutical, agricultural and industrial applications. The main focus of pharmaceutical and agricultural companies in the Mediterranean countries is

now the commercial production of known aromatic herbs and neglecting the utilization of trees like *Commiphora caudata*(Wt & Arn) Engl., which are globally distributed and have massive diversity and may provide new sources of flavors, perfumes and remedies for aging as well as other medical and agricultural applications.

AIM:

To study the Pharmacognostical, preliminary phytochemical Perspectives of The Leaves of*Commiphora caudata*. - *Invivo* Cardio protective Potential of Its steroid rich fraction on Doxorubicin induced Cardio toxicity in Zebra fish Larvae Model.

OBJECTIVE:

The objective of the study was divided into three parts.

PART 1: PHRAMACOGNOSTICAL STUDY

- Collection and authentication of plant
- Morphological study of the plant
- Microscopy of the leaf
    - Anatomical study using light microscope
    - SEM analysis
    - Powder microscopy
    - Microscopic schedules
- Physio-chemicalparameters
    - Ash values
    - Loss on drying
    - Extractive values

PART 2: PRELIMINARY PHYTOCHEMICAL SCREENING

- Qualitative analysis of the leaves for the presence of various phytoconstituents
- Determination of flavonoid content, total phenolic content of the leaf,
- Determination trace elements by SEM
- Preparation of ethanolic extract of the leaves (EECCL)
- To study the TLC of the EECCL
- To study the HPTLC profile of the EECCL to identify the presence of guggulsterone

PART 3: PHARMACOLOGICAL STUDIES:

The **3R's** ethical principle (**R**eduction, **R**efinement, and **R**eplacement) was implemented that help to minimize harms to vertebrate animals used in science.

- Collection of *Danio rerio* or zebra fish (Zf)Larvae
- To study the preliminary toxicological studies of the EECCL on the early development of Zf

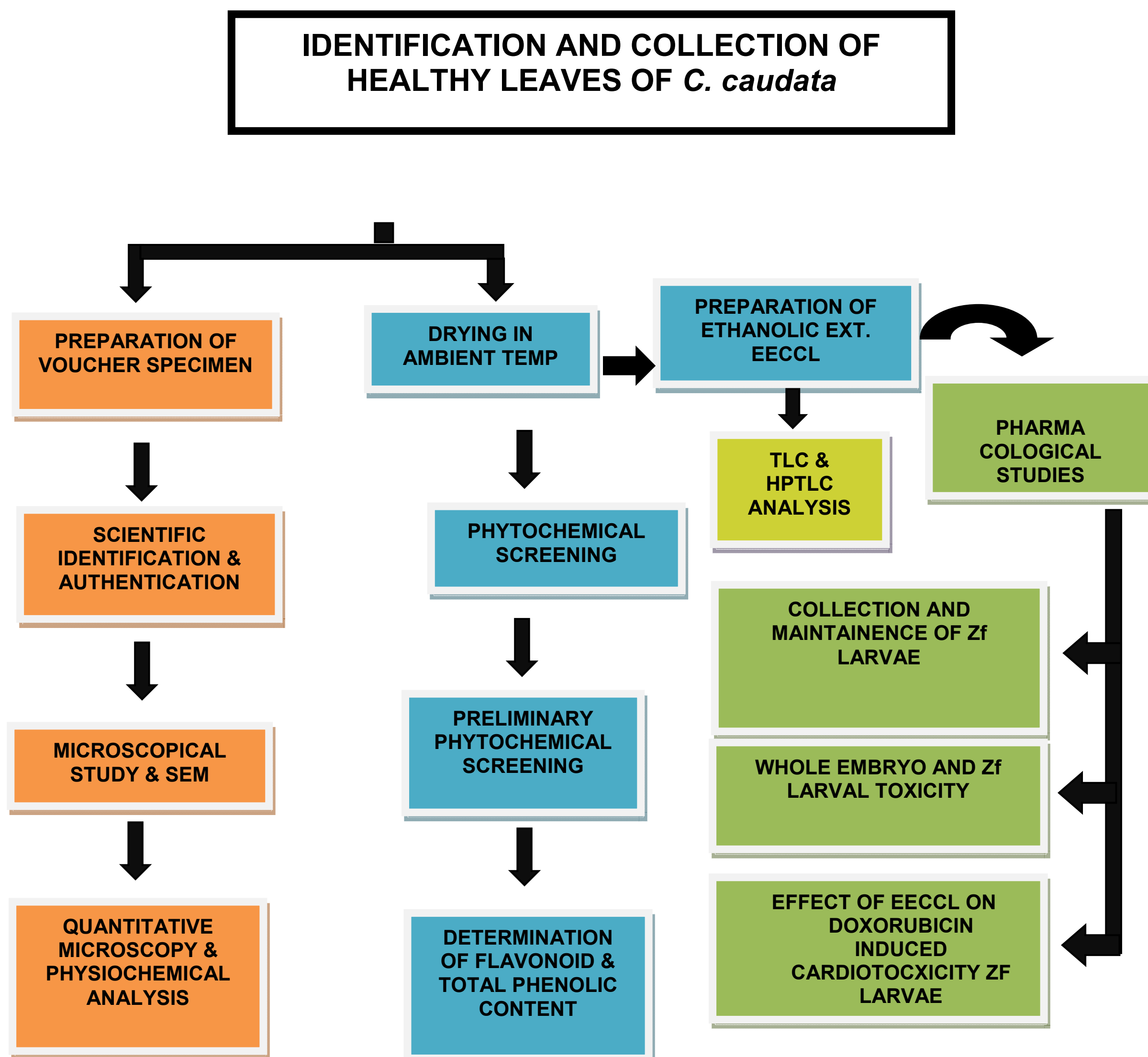
1. Whole embryo culture toxicity study
2. Larval toxicity study

- Observation of the overt morphological effects of Doxorubicin on heart including ventricular collapse and pericardial edema.
- Observation of blood cell circulation within tail blood vessels using high speed camera
- To evaluate the action of EECCL on Doxorubicin induced toxicity of Zf larvae model like heart fractional shortening measurement to assess heart rate and contractility.

MATERIALS AND METHODS

CHAPTER IV

MATERIALS AND METHODS- RESEARCH DESIGN



MATERIALS AND METHODS

4.1. PLANT COLLECTION AND AUTHENTIFICATION

Leaves of the plant ***C.caudata*** selected for the study was collected from Oricheri, Bhavani (Tk), Erode (Dt), Tamilnadu, India during the month of July 2016 and was authenticated by **Dr.Stephen,** Department of Botany, American college, Madurai and **Dr.Sasikala,** Director (Retd) of Siddha Central Research Institute, Arumbakkam, Chennai.

LEAF DRYING AND PULVERIZING

The leaves were collected and shade dried. It was powdered in a mixer. The powder was sieved in a sieve No.60 and kept in a well closed container in a dry place.

4.2. PHARMACOGNOSTICAL STUDIES

Morphological and micro morphological examination and characterization of medicinal plants have always been accorded due credentials in the pharmacognostical studies. Botanical identity of the plants is an essential prerequisite for undertaking the analysis of medicinal properties of any plant. A researcher may succeed in getting a new compound or may find many useful pharmacological active properties in the plant. If the botanical identity of the plant happens to be dubious or erratic, the entire work on the plant becomes invalid. Thus it is needless to stress the botanical identity of the crude drug is the threshold in the processes of pharmacological investigations. The researchers should be equipped with all possible diagnostic parameters of the plant on which the researchers plan to work.

4.2.1 MORPHOLOGICAL STUDIES OF *C.caudata:*

Leaf, bark, seed, fruit, and flower were studied individually for its morphological characters by organoleptic analysis.

4.2.2 MICROSCOPICAL STUDIES ON THE LEAF OF *C.caudata:*

COLLECTION OF SPECIMEN

Care was taken to select healthy plants and for normal organs. Leaf, Petiole specimens were collected from a healthy plant by making a cut with petioles. The materials were cut into pieces and immediately immersed in fixative fluid FAA (Formalin – 5ml + Acetic acid – 5ml +70% Ethyl alcohol – 90ml).

DEHYDRATION

After 24 hours of fixing, the specimens were dehydrated with graded series of ethyl alcohol and tertiary-butyl alcohol (Sass, 1940). The specimen is then kept in various grades of the fluid for about 6 hrs. Every time the fluid is decanted and immediately the specimens were flooded with next grade of fluid.

INFILTRATION WITH PARAFFIN WAX

After dehydration, the shavings of paraffin wax were added to the vial containing the plant material with pure TBA. The paraffin shavings are added every 30mts at about 40-45ºC four or five times. Then the vials were filled with wax without damaging the tissues. The vial filled with wax is kept open in warm condition to evaporate all TBA, leaving the specimen in pure molten wax. The specimen filled with pure molten wax for 2 or 3 times by decanting the old wax every time.

CASTING TO MOLD

A boat made out of chart board, by folding the margin, is used to prepare a mold of wax containing specimens. The paraffin along with the leaf and petiole specimen was poured into the boat. With the help of heated needles, the specimen were arranged in parallel rows with enough space in between the specimens. The block was then immersed in chilled water and allowed to cool for few hours.

SECTIONING

The paraffin embedded specimens were sectioned with the help of microtome**.** The thickness of the sections was 10-12µm. Dewaxing of the sections was by customary procedure. The sections were stained with **Toluidine blue** as per the method published by O'Brien *et al*(1964). Since toluidine blue is a poly chromatic strain, the straining results were remarkably good and some **cytochemical reactions** were also obtained. The dye rendered pink colour to the **cellulose** walls, blue to the lignified cells, dark green to **suberin**, violet to the **mucilage**, blue to the **protein** bodies etc. Where ever necessary sections were also stained with **safranin** and **fast-green** and potassium iodide (for starch). For studying the stomatal morphology, venation pattern and trichome distribution, **paradermal sections** (sections taken parallel to the surface of leaf as well as **clearing** of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) were prepared. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell components were studied and measured.

PHOTOMICROGRAPHS

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken. With Nikon labphot 2 Microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light were employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifigations of the figures are indicated by the scalebars. (Johansen DA, 1940, Purvis MJ *et al*., 1966).

4.2.3 MICROSCOPICAL STUDY OF LEAF USING SCANNING ELECTRON MICROSCOPE

SCANNING ELECTRON MICROSCOPE (SEM)

Movement of beam of focused electrons across an object forms a 3D image on a cathode - ray tube in a Scanning Electron Microscope and it reads both the electrons scattered by the object and the secondary electrons produced by it. The electromagnetic lenses are used in SEM and focusing is done by the current. On photographic plate of screen the image is projected which gives comprehensive, quasi 3-D representation of the objects gives the ultra-structure of plant cells. In addition, shows the unsuspected details and any undescribed characters. In other words the micrograph from SEM, shows the best possible structural details of the specimens. (Robards, 1970)

USAGE

SEM info was handled as conventional character (or) character complexes as “pure” information without being broken down (or) interpreted as individual character using computer processing. The SEM information can

be used somewhat at the superficial level just described to assist in solving taxonomic problem by confirming, changing (or) other grounds. It is also used often as diagnostic feature to avoid misleading by over simplified descriptions and one may find new kinds of microstructures not previously recognized and apparently simple structures may be extremely complex. Remarkably, poor conventional descriptions enabling taxonomic process of reducing a complex pattern to a few simple characters (Heywood VH, 1971). SEM plays a vital role when a specimen need to be satisfactorily defined in terms of characters. For most biological materials, maximum information is obtained by employing light and electron microscopy jointly and an attempt was made by applying SEM to the leaf of *C.caudata*, to pinpoint the positions of specific characters with in the cell, which can be easily seen in final image.

SEM SAMPLE PREPARATION

Sample for SEM analysis were mounted on the specimen stub using carbon adhesive sheet. Small sample were mounted with I sq. cm glass slide and kept in carbon adhesive sheet. Samples were coated with gold to a thickness of 100 AO using Hitachi vacuum evaporator. Coated sample were analyzed in a Hitachi Scanning electron Microscope 3000 H model.

4.2.4 POWDER MICROSCOPY MACERATION TECHNIQUE

Maceration is the process of separation of individual cells by selectively dissolving the pectic middle lamella between the cells. The middle lamella binds the cells with each other forming different tissues. The middle lamella is dissolved by employing a chemical that dissolves the lamella to free the cells to obtain their three dimensional view.

MACERATION FLUID

Jaffrey's maceration fluid is one that is commonly used for maceration (Johnsen DA, 1940). The fluid consists of equal volumes of 5% chromic acid and 5% nitric acid. The plant material is cut into small pieces and immersed in the maceration fluid. The fluid with the materials is kept at 55ºC for 3-5 hrs. Then the material is washed thoroughly with water and placed on a glass slide in a drop of Safranin (0.5%) for 15-20 min. The stain is drained carefully and mounted with a drop of dilute glycerine. The cells are spread well with a needle and the material is covered with cover slip. The slide so prepared is examined under the microscope to study different components of the macerate.

4.2.5 MICROSCOPIC SCHEDULES (Wallis TE. 1953, Wallis TE, 1965, Iyengar MA, 1994, Anonymous,2001)

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

A.VEIN ISLET NUMBER AND VEIN TERMINATION NUMBER

The term vein islet in used to denote the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands. The number of vein islets per sq.mm. Area is called vein islet number.

Vein terminal number may be defined as the number of vein terminals present in one sq., mm. area of the photosynthetic tissue.

B. DETERMINATION OF VEIN ISLET NUMBER AND VEIN TERMINATION NUMBER

Small square portion from the lamina region of the leaf was cleared in chloral hydrate, stained and mounted on a slide. A camera Lucida is set up and by means of a stage micro meter the paper is divided into squares of $1mm^2$ using a 16mm objective. The stage micro meter is then replaced by the cleared preparation and the veins are raced in four continuous squares, either in a square 2mm x 2mm (or) rectangle 1mm x 4 mm.

When counting, it is convenient to number each vein-islet on the tracing. Each numbered area must be completely enclosed by veins, and those which are incomplete are excluded from the count if cut by the top and left-hand sides of the square (or) rectangle but included if cut by the other two sides. Ten readings for vein islet and vein termination number were recorded.

C.STOMATAL INDEX

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

Stomatal Index = S / S+E x100

Where, S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area

D.DETERMINATION OF STOMATAL INDEX

The procedure adopted in the determinations of stomatal number was observed under high power (45 X).The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the above formula.

4.2.6 PHYSIOCHEMICAL PARAMETERS (Anonymous, 1996, 1998, 2001)

DETERMINATION OF ASH VALUES

ASH VALUE

The ash values were determined by using air dried powder of the leaf as per the official method.

TOTAL ASH

Two grams of the air dried leaf powder was accurately weighed in a silica crucible separately. The powder was scattered into a fine even layer on the bottom of the crucible and incinerated by gradually increasing the temperature not exceeding 450°c, until free from carbon. Then it was cooled and weighed for constant weight. The percentage of ash with reference to the air dried powder was calculated.

WATER SOLUBLE ASH

The ash obtained from the total ash procedure was boiled with 25ml of water for 5 minutes and the insoluble matter was collected on an ash less filter paper and washed with hot water. Then it was ignited for 15 minutes at a temperature not exceeding 450°c. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried powder.

ACID INSOLUBLE ASH

The ash obtained from the total ash was boiled for five minutes with 25ml of dilute hydrochloric acid. The insoluble matter was collected in a tarred sintered glass crucible. The residue was washed with hot water, dried and weighed. The percentage of acid insoluble ash with reference to the air dried drug was calculated.

DETERMINATION OF LOSS ON DRYING

For the determination of loss on drying, the method described by Wallis was followed. One gram of dried powdered leaf was accurately weighed in a tarred Petri dish, previously dried under the conditions specified in IP'96. The powder was distributed as evenly as practicable, by gentle sidewise shaking. The dish was dried in an oven at 100 – 105°C for 1 hour. It was cooled in desiccators and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken.

EXTRACTIVE VALUES

Petroleum ether soluble extractive value

Five gram of the coarsely powder was macerated separately with 100ml of petroleum ether in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of petroleum ether. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°c and weighed. The percentage of the petroleum ether soluble extractive value was calculated with reference to the air dried powder.

Ethanol soluble extractive value

Five gram of the coarsely powder was macerated separately with 100ml of ethanol in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of ethanol. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of the ethanol soluble extractive value was calculated with reference to the air dried powder.

Water soluble extractive value

Five gram of the coarsely powder was macerated separately with 100ml of chloroform water in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of chloroform water. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at $105^{O}C$ and weighed. The percentage of the chloroform water soluble extractive value was calculated with reference to the air dried powder.

4.3 PHYTOCHEMICAL STUDIES [Anonymous, 1998, Chaudhri RD, 1999, Kokate CK, 2005, Agarwal, 2007, Horbone JB, 1973].

4.3.1 PRELIMINARY PHYTOCHEMICAL SCREENING TEST FOR ALKALOIDS:

VARIOUS PROCEDURES TO LIBERATE ALKALOIDS

- ❖ Powdered drug was mixed thoroughly with 1ml of 10% ammonia solution and then extracted for 10 minutes with 5 ml methanol, under reflux. The filtrate was then concentrated.
- ❖ Powdered drug was mixed thoroughly with 1ml of 10% sodium carbonate solution and then extracted for 10 minutes with 5ml methanol, under reflux. The filtrate was then concentrated.
- ❖ Powdered drug was ground in a mortar for about 1 minute with 2ml of 10% ammonia solution and then thoroughly mixed with 7 gram basic Aluminum oxide. The mixture was then loosely packed into a glass column and 10ml chloroform was added, eluted, dried and methanol was added.
- ❖ Powdered drug was shaken for 15 minutes with 15ml of 0.1 N sulphuric acid and then filtered. The filter was washed with 0.1 N sulphuric acid 1 to a

volume of 20 ml filtrate:1ml concentrated ammonia was then added. The mixture was then shaken with two portions of 10ml diethylether.The ether was dried over anhydrous sodium sulphate filtered and evaporated to dryness and the resulting residue was dissolved in methanol.

- Powdered drug was mixed with one gram of calcium hydroxide and 5ml of water, made into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. 20ml of 90% alcohol was added, mixed well and then refluxed for half an hour on a water bath. It was then filtered and the alcohol was evaporated. To that dilute sulphuric acid was added.

The above made extracts were tested with various alkaloid reagents as follows.

1. **MAYER'S REAGENT**
2. **DRAGENDORFF'S REAGENT**
3. **HAGER'S REAGENT**
4. **WAGNER'S REAGENT**

TEST FOR CARBOHYDRATES

MOLISCH'STEST

The aqueous extract of the powdered material was treated with alcoholic solution of α - naphthol in the presence of sulphuric acid.

FEHLING'S TEST

The aqueous extract of the powdered material was treated with Fehling's A and B solution and heated on a boiling water bath.

BENEDICT'S TEST

The aqueous extract of the powdered drug was treated with Benedict's reagent and heated over a water bath.

TEST FOR GLYCOSIDES GENERAL TEST

TEST A

200 mg of the powdered drug was extracted with 5ml of dilute sulphuric acid by warming on a water bath, filtered and neutralized with 5% sodium hydroxide solution. Then 0.1ml of Fehling's solution I and II were added, until it becomes alkaline and heated on a water bath for 2 minutes.

TEST B

200 mg of the powdered drug was extracted with 5ml of water instead of sulphuric acid. Boiled and equal amount of water was added instead of sodium hydroxide solution. Then 0.1ml of Fehling's solution I and II were added, until it becomes alkaline and heated on a water bath for 2minutes.

TEST FOR ANTHRAQUINONES

BORNTRAGER'S TEST

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

MODIFIED BORNTRAGER'S TEST

About 0.1gram of the powdered leaf was boiled for two minutes with dilute hydrochloric acid and few drops of ferric chloride solution was added, 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 and shaken well.

TEST FOR CYANOGENETIC GLYCOSIDES

Small quantity of the powdered leaf was placed in a stoppered conical flask with just sufficient water to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place.

TEST FOR CARDIAC GLYCOSIDES

KELLER KILLIANI'S TEST

About 1gram of the powdered leaf was boiled with 10ml of 70% alcohol for two minutes, cooled and filtered. To the filtrate 10ml of water and 5 drops of solution of lead sub acetate were added and filtered. The filtrate was then extracted with chloroform and the chloroform layer was separated and evaporated to dryness. The residue was dissolved in 3ml of glacial acetic acid containing a trace of ferric chloride. To this 3ml of concentrated sulphuric acid was added along the sides of the test tube carefully.

RAYMOND TEST

To the alcoholic extract of the leaf, hot methanolic alkali was added

LEGAL'S TEST

To the alcoholic extract of the powdered drug, pyridine and alkaline sodium nitro pruside solution were added.

COUMARIN GLYCOSIDES

A small amount of powdered leaf was placed in test tube and covered with a filter paper moistened with dilute sodium hydroxide solution. The covered test tube was placed on water bath for several minutes. Then the paper was removed and exposed to UV light.

TEST FOR PHYTOSTEROLS

The powdered leaf was first extracted with petroleum ether and evaporated. The residue obtained was dissolved in chloroform and tested for sterols.

SALKOWSKI TEST

Few drops of concentrated sulphuric acid were added to the above solution, shaken well and set aside.

LIBERMANN – BURCHARD'S TEST

To the chloroform solution few drops of acetic anhydride was added and mixed well. 1ml of concentrated sulphuric acid was added through the sides of the test tube and set aside for a while.

TEST FOR SAPONINS

About 0.5gram of the powdered leaf was boiled gently for 2 minute with 20 ml of water and filtered while hot and allowed to cool. 5ml of the filtrate was then diluted with water and shaken vigorously**.**

DETERMINATION OF FOAMING INDEX

One gram of the coarsely powdered leaf was weighed and transferred to 500 ml conical flask containing 100 ml of boiling water. The flask was maintained at moderate boiling, at 80-90°C for about 30 minutes. Then it was cooled and filtered into a volumetric flask and sufficient water was added through the filter to make up the volume to 100 ml (V_1). Ten Stoppard test tubes were cleaned (height 16 cm, diameter 1.6 cm) and marked from 1 to 10. Measured and transferred the successive portions of 1, 2, 3ml up to 10ml and adjusted the volume of the liquid in each tube with water to 10ml. Then

the tubes were Stoppard and shaken lengthwise for 15 seconds, uniformly and allowed to stand for 15 minutes and measured the length of the foam in every tube.

TEST FOR TANNINS

To the aqueous extract of the powdered leaf, few drops of ferric chloride solution were added.

GOLD BEATER'S SKIN TEST

2% hydrochloric acid was added to a small piece of gold beater skin and rinsed with distilled water and placed in the solution to be tested for five minutes. Then washed with distilled water and transferred to a 1% ferrous sulphate solution.

TEST FOR PROTEINS AND FREE AMINOACIDS

MILLON'S TEST

The acidulous alcoholic extract of the powdered leaf was heated with Millon's reagent.

BIURET TEST

To the alcoholic extract of the powdered leaf 1ml of dilute sodium hydroxide was added. Followed by this one drop of very dilute copper sulphate solution was added.

NINHYDRIN TEST

To the extract of the powdered drug, Ninhydrin solution was added, and boiled.

TEST FOR MUCILAGE

To the aqueous extract of the powdered leaf, Ruthenium red solution was added.

TEST FOR FLAVONOIDS SHINODA TEST:

A little amount of the powdered leaf was heated with alcohol and filtered. To the alcoholic solution a few magnesium turnings and few drops of concentrated hydrochloric acid were added, and boiled for 5 minutes.

ALKALINE REAGENT TEST

To the alcoholic extract of the powdered leaf, few drop of sodium hydroxide solution was added.

ZINC HYDROCHLORIDE TEST

To the alcoholic extract, mixture of zinc dust and concentrated Hydrochloric acid was added.

TEST FOR TERPENOIDS

The powdered leaf was shaken with petroleum ether and filtered. The filtrate was evaporated and the residue was dissolved in small amount of chloroform. To the chloroform solution tin and Thionyl chloride were added.

TEST FOR VOLATILE OIL

About 100gram of fresh leaves, were taken in a volatile oil Clevenger apparatus and subjected to hydro distillation for four hours.

TEST FOR FIXED OIL

A small amount of the powdered leaf was pressed in between in the filter paper and the paper was heated in an oven at 105°C for 10 minutes.

4.3.2. FLUORESCENCE ANALYSIS

Powdered leaf material Of *Commiphora caudata* was subjected to analysis under UV light after treatment with various chemical and organic reagents like.

Ethanol, Ethyl acetate, Chloroform, Water, 50% sulphuric acid, 10% sodium hydroxide, 50% nitric acid and dried leaf powder. (Horbone JB, 1973).

4.3.3 ESTIMATION OF FLAVONOID CONTENT

[Chang C C *et al*., 2002, Mabry T J*et al*., 1970 and Siddiquie M A *et al.,* 2010].

The flavonoid content of plant extract was estimated by aluminium chloride method. In this method, aluminium chloride complexes with flavonoids of C3-C5 hydroxyl group and to produce intense colour in acidic medium. The intensity of the colour is proportional to the amount of flavonoids and can be estimated as quercetin equivalent at wavelength of 415nm.

MATERIALS REQUIRED

- Ethanolic extract of leaves of ***C.caudata***
- 10%w/v Aluminium chloride
- 1M Potassium acetate
- 95%v/v ethanol

PROCEDURE

0.5ml of the extract (1mg/ml) was transferred to a test tube. To this solution, 0.1ml of aluminium chloride, 0.1ml of potassium acetate, 1.5ml ethanol were added and made up to 5ml with distilled water. The mixture was allowed to stand for 30 min with intermittent shaking. The absorbance was measured at 415nm.

The calibration curve was generated using quercetin as a standard at different concentrations (5-50µg/ml). The reaction mixture without aluminium chloride was used as a blank. The flavonoids content was expressed as mg of quercetin equivalent per gram of extract.

4.3.4. ESTIMATION OF TOTAL PHENOLIC CONTENT (Singleto VL *et al*., 1979, Gouthamchandra K *et al.,*2010)

PRINCIPLE

The total phenolic content of the extract was determined by Folin&Cio-calteu's phenol reagent. This reagent consists of phosphotungstate and phosphomolybdate mixture which is reduced to mixture of blue molybdenum and tungsten oxides while phenolic content of the extract was oxidized. The intensity of colour is proportional to the amount of phenolic content of the extract and which was measured at 765nm. The total phenolic content in the extract was expressed as milligrams of gallic acid equivalent (GAE) per gm of extract.

MATERIALS

- Ethanolic extract of leaves of *C.caudata*
- 10%w/v sodium carbonate solution
- Gallic acid
- Folin & Cio-calcateu's phenol reagent

PROCEDURE

0.5ml and 1ml of extract was transferred into separate test tube. To this solution, FCR 0.5ml and 1ml of sodium carbonate were added and final volume made up to 10ml with distilled water. The mixture was allowed to stand for 1hr with intermittent shaking. The absorbance was measured at 765nm. A calibration curve was generated using Gallic acid as a standard at different concentrations (2, 4, 6, 8, 10µg/ml). The reaction mixture without sample was used as a blank. The total phenolic content was expressed as milligrams of Gallic acid equivalent (GAE) per g of extract.

4.3.5. DETERMINATION OF TRACE ELEMENTS IN THE LEAF OF *C.caudata* BY ENERGY DISPERSIVE X-RAY SPECTROMETER (EDS)

The SEM allows the observation of materials in macro and submicron ranges. SEM is capable of generating 3-D images for analysis of topographic features. When SEM is used along with EDS the analyst can perform an elemental analysis on specimens of microscopic sections or contaminants that may be present.

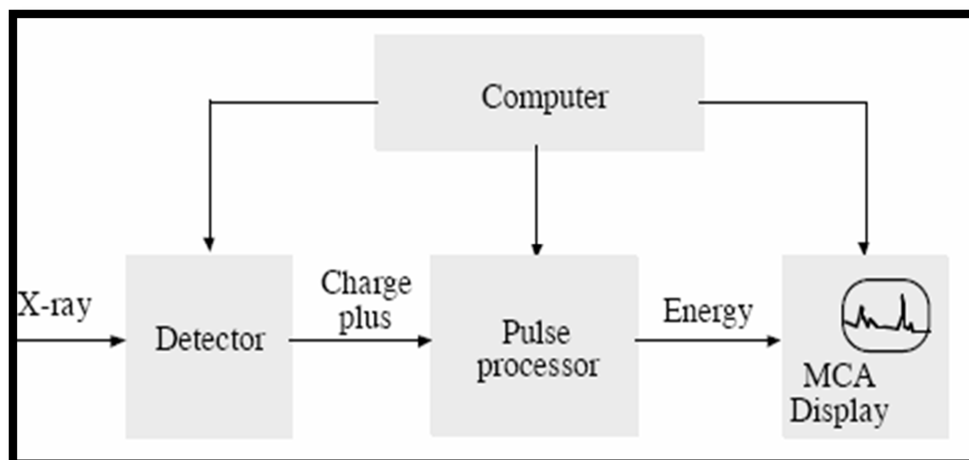
EDS ANALYTICAL CAPABILITIES

Backscattered electron images in the SEM display compositional contrast that results from different atomic number elements and their distribution. EDS is used to find particular elements are and their Atomic %. The Y-axis shows the counts (number of X-rays received and processed by the detector) and the X-axis shows the energy level of those counts [Bob Hofner].

By Viewing 3-D images of specimens solves some of the problem in an analysis and it is also necessary to detect different elements associated with the specimen. This is accomplished by using the “built-in” spectrometer called an Energy Dispersive X-ray Spectrometer.

EDS SYSTEM COMPRISES OF 3 BASIC COMPONENTS

- An X-ray Detector - detects and converts X-ray into electronic signals.
- A Pulse Processor - measures the electronic signals to find out energy of each X- ray detected; and
- A Multiple Channel Analyzer - interprets and displays analytical data.



EDS is an analytical technique in which the specimen emits X-rays due to the bombardment of electron beam on it which is used to identify the elemental composition of the specimen due to the ejection of electrons from the atoms on the specimen surface. To explain further, when the sample is bombarded by the electron beam of the SEM, electrons are ejected from the atoms on the specimen's surface. A resulting electron vacancy is filled by an electron from a higher shell, and an X-ray is emitted to balance the energy difference between the two electrons. The EDS X-ray detector measures the number of emitted X-rays emitted versus their energy. The energy of the X-ray is characteristic of the element from which the X-ray was emitted. A spectrum of the energy versus relative counts of the detected x-rays is obtained and evaluated for the determinations of the elements.

4.3.6. PREPARATION OF ETHANOLIC EXTRACT FROM THE LEAVES OF *C.caudata* (EECCL)

Weighed quantity of fresh leaves was extracted with 90% v/v ethanol by cold maceration for three days and the solvent was evaporated under reduced pressure using Buchi Rotovapor apparatus. Residue was collected and stored in refrigerator until use.

4.3.7 IDENTIFICATION OF COMPOUNDS PRESENT IN THE ETHANOLIC EXTRACT OF LEAVES BY HPTLC ANALYSIS

High performance thin layer chromatography (HPTLC) is a modern adaptation of TLC with improved versatility, separation efficiency and detection limits. HPTLC is a useful tool for identification of plant extract because each plant species produces a distinct chromatogram, with unique marker compounds used for the plant identification. It is used as a quality control tool since comparison of chromatograms of different lots can demonstrate the similarities and differences between the test samples and their standard chemical markers. HPLTC it is a reliable method for quantification of nanogram level even when present in complex formation. HPTLC fingerprint analysis is used for rapid identity check, for monitoring purity of drugs, for detection of adulterants, for determining whether a material is derived from adefined botanical species and also to know whether the constituents are clearly characterized (Wagner, H *et al*., 1996).

Instrument used	:	CAMAG TLC Scanner 3
Software	:	win CATS Planar Chromatography Manager
Sample application	:	Linomat 5

Detection	:	at 254nm in TLC Scanner 3
Stationary phase	:	HPTLC plates silica gel 60 F 254
Sample preparation	:	100mg per ml of sample was prepared in ethanol solvent.
Mobile phase	:	Toluene : Acetone (9:1)
Sample solution	:	5µL
Standard solution	:	5µL
Drying device	:	Oven
Temperature	:	60°C
Volume	:	10.0ml
Scanning speed	:	20mm/s
Time	:	5minutes
Wavelength	:	254 nm
Lamp	:	D2&W
Measurement type	:	Remission
Measurement mode	:	Absorption

4.4 PHARMACOLOGICALSTUDIES

4.4.1. PRELIMINARY TOXICOLOGICAL STUDIES OF EECCL ON THE EMBRYO AND LARVAE OF ZEBRAFISH

Toxicology through intensive studies has traditionally focused on the effects of chemicals on living organisms which was done by one chemical at a time. Such approaches show the mode of action of many chemicals and provide a detailed mechanistic understanding of the molecular targets of toxicity for some as the cost of this approach is high. Toxicology studies rely

on the utility of vertebrate animals which is an expensive undertaking in both time and cost with debatable predictive power in case of safety aspects for human. (Bucher, JR2002).

Role of zebra fish in high throughput screening:

In the last two decades safety pharmacology has become a most important part of the non- clinical safety assessment in finding new chemical entities (Bass, A *et al*., 2004).

The relative novelty of this discipline has granted it the flexibility to incorporate new experimental tools (Claude, JR and Claude, N 2004).

In addition to requiring small amount of compounds, the time and cost effectiveness of invitro assays have led to their use by the pharmaceutical industry for high or medium throughput safety screens (Suter, W.2006).

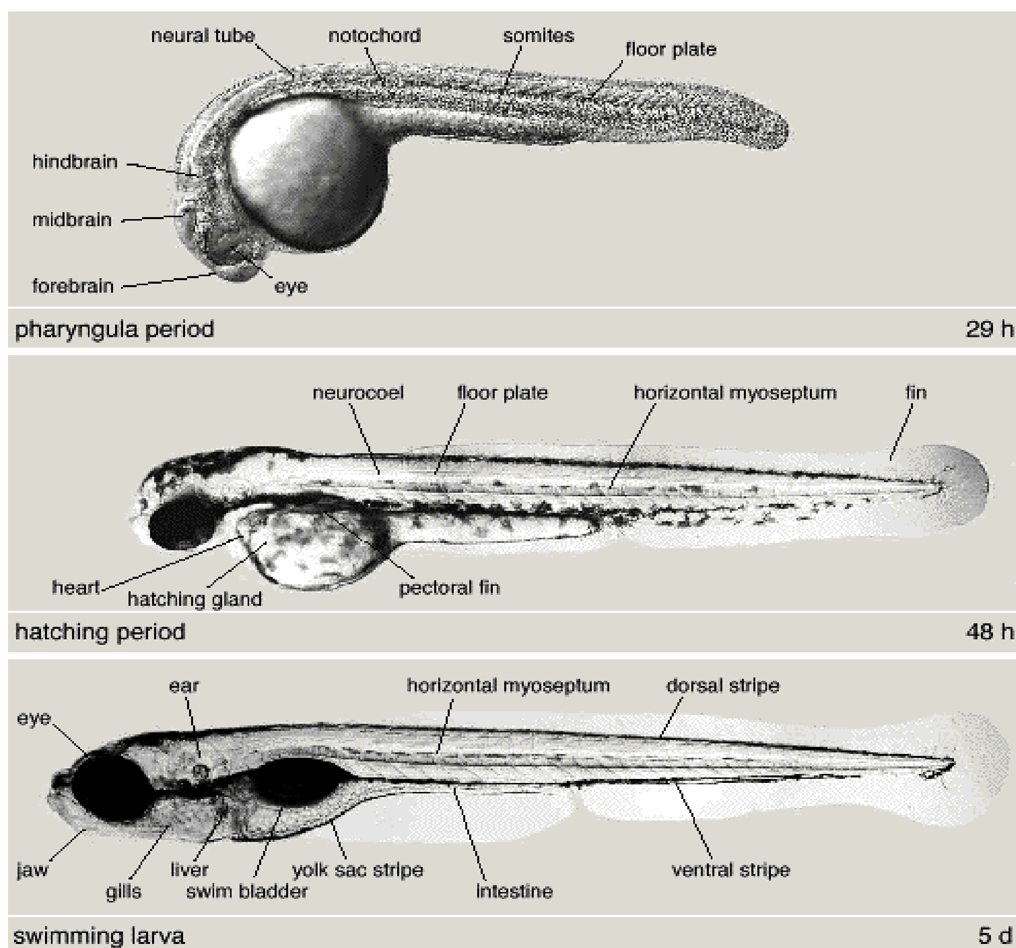
One of the limitations encountered with *in vitro* studies is that they are not fully representative of *in vivo* models. Therefore, safety pharmacology needs an *in vivo* model with the capacity for higher throughput screening. Thezebra fish model system is done from medium to high throughput because of many advantages, as they are small in size, cheap to maintain and fecundity as a single spawning produces 100 – 200 eggs. Larvae, which are only 1-4 mm long, can live for seven days in a single well of a standard 96 or 386 – well microtitre plate by the support of nutrients stored in the yolk sac.

Administration of drugs in zebra fish:

Larvae of zf can absorb small molecules diluted in the surrounding water through their gills and skin. Drugs can be given orally after this stage because zebra fish begin to swallow at 72(hpf).Drugs can also be delivered by oral intubation in case of adult zf. Compared to testing in other animals

models, statistically significant numbers of zebra fish can be used for each assay and small amounts (mg) of drugs are required.in addition the transparency of zebra fish larvae for several days of post fertilization (dpf) enables in vivo observation of live or whole fixed specimens, including the visualization of vital dyes, fluorescent tracers, antibodies and riboprobes. By 120 hpf, zebra fish develop discrete organs and tissues, including brain, heart, liver, pancreas, intestines, bone, muscles, nerve systems and sensory organs. These organs and tissues have been shown to be similar to their mammalian counter parts at the anatomical, physiological and molecular levels. Although conventional in vitro assays using cultured cells can be used to evaluate potential drug toxicity effects, results are frequently not predictive .Results in vivo will involve drug absorption, distribution, metabolism excretion (ADME). To streamline the drug development timeline prioritize drug candidates for animal testing and reduce unnecessary costs for mammalian studies, drug screening assays using zf are becoming increasing popular .(Mc Grath P and Chun-Qi Li 2008).

MORPHOLOGY OF ZEBRA FISH LARVAE



Anatomy and cardiovascular development in Zebra fish:

As in all vertebrates, the heart is the first organ to function in zf. It develops rapidly and is fully formed by 2 dpf, compared with 12 dpf in the mouse and 35 dpf in the human embryo at 3 weeks gestation. Zf hearts, like those of humans, start out as linear heart tube and start to loop 24 – 36 hour post fertilization (hpf).

Zf cardiac contraction begins at 22 hpf initially as a peristaltic wave which then develops into co – ordinate contractions of the atrium and ventricle by 36 hpf. Blood is first sent from the heart to gills where it is oxygenated. It then passes to the head before moving posteriorly to the rest of the body.

Despite the lack of a pulmonary circulationand its two chambered heart, there is significant conservation between mammalian and zf cardiovascular at morphologic, physiologic and genetic level, making it a useful model for studying cardiac development (Dooley, K and Zon, Li 2000).

A. WHOLE EMBRYO CULTURE TOXICITY STUDY

Materials

Fertile eggs, E_3embryo medium (standard medium), dimethylsulphoxide (DMSO), glass petridishes, research microscope (laboratory microscope with microphotography), incubator, micropipette, EECCL of *C.caudata* and standard podophyllotoxin.

Collection of eggs

Eggs were collected from natural spawning and reared in embryo medium at pH 7.2, and kept in an incubator at 28±0.5^{0}Cfor our assay. The developmental assay stage of the embryos was determined using microscope. (Hisaoka, KKand Battle, HI (1958). At around 2- 4h post fertilization, only the fertilized eggs (blastula stage) were selected. The fertilized eggs were collected and rinsed several times with tap water.

EXPERIMENTAL DESIGN:

The eggs were transferred to each of the glass petri dishes (3 per dish) containing different concentration of EECCL (0.5, 0.75, 1 and 2µg/ml) dissolved in 1% DMSO at 28°C as well as DMSO control. Embryo medium served as the over-all control. Standard podophyllotoxin of concentration 10µg/ml was taken as positive control .occasional stirring was done to ensure even distribution of the chemical. The maximal acceptable toxicant concentration (MATC) was calculated accordingly by scoring the malformations. (Dave, G et *al*., 1987)

The development of blastula eggs was monitored at specified time points (12, 36, 60, & 80 hrs.) under microscope. Endpoints were used for assessing the effect of drug during the major organ is visible included edema, eye, malformations, bent tail, undulated notochord, twisted notochord and death. Malformations were also noted and described among the juveniles from the control 1% DMSO treated and standard podophyllotoxin.

B. LARVAL TOXICITY STUDY

Materials

Zf larvae of 5 dpf ,E3 embryo medium ,1% DMSO, glass petri dishes ,research microscope (labscope microscope with microphotography) , incubator, micropipette, volatile oil of *C.caudata* in various concentration and standard podophyllotoxin.

Experimental design

Healthy 5 dpf larvae was selected and used for larval toxicity study. About 5 larvae were released in the embryonic medium (10ml) taken in a petridish, in triplicate. Various concentration of EECCL (0.5, 0.75, 1 and 2µg/ml) dissolved in 1% DMSO and tested. Podophyllotoxin (10µg/ml) was used as standard toxin.

4.4.2 EFFECT OF EECCL IN DOX INDUCED CARDIO TOXICITY ON ZEBRAFISH EMBRYOS

- The embryos, 1 dpf were arrayed into 96 well plates (n=3) containing 200µl of E3 medium buffered with Hepes (pH7.2).
- The embryos were treated with DMSO (vehicle control), 100 µM Doxorubicin, Doxorubicin + EECCL (0.5 ng, 1 ng 1.5 ng), Doxorubicin + Metaprolol (standard drug) and untreated.

- Microscopic examination of all treated and untreated zf showed various phenotypic changes, the end points like pericardial edema, heart atrium, ventricle and blood circulation within the tail blood vessels were observed.
- Heart rate and Contractility recorded using high speed camera and a custom analysis algorithm, and the fractional shortening of the zebra fish hearts were calculated.
- Quantification of Fractional shortening(FS) as follows,

$$\mathbf{VID_d - VID_s / VID_d * 100}$$

VID_d – Diastolic ventricular internal dimension

VID_s – Systolic ventricular internal dimension (Liu *et al.,* 2015).

- Statistical significance was analyzed using one way ANOVA.

RESULTS

CHAPTER V
RESULTS

5.1 PHARMACOGNOSY(PLATE 1)

5.1.1 MORPHOLOGICAL STUDIES OF *C.caudata* leaves

Commiphora caudata can be found in Southern India and Sri Lanka, usually growing in the full sun on hilly granite rock outcrops in dry zone areas. It is a small to medium-sized deciduous tree which is said to be able to reach height of 10-20m, but usually is less high. The tree has a smooth, succulent green bark, which partly flakes off with age, giving rise to a characteristic patchwork of green and brown patches. Its sap has a strong resinous scent. The tree has medicinal properties. The fruit is a globose fleshy drupe with 2 to 6 valves and 1 seed that is black and has 4 wings. Remnants of branches can form a kind of thorns on the trunk.

Macroscopic characters:

LEAVES: (PLATE 2,3)

Leaves were compound

Shape	:	Ovate, oblong
Arrangement	:	Alternative, leaflets 3 to 7 foliolate, broadly Ovate, lanceolate, leaves imparipinnate,
Texture	:	Glabrous, glossy above, subglaucous below and caudate.
Size	:	4.5 to 6.5 cm long and 2.2 to 3.5 cm wide.
Pedicle	:	length - 3.5 to 6.2 cm
Color	:	Upper surface dark green, lower surface light green in colour

Margin	:	Entire
Base	:	Slightly asymmetric
Apex	:	Acuminate
Petiole	:	Slender 1.5-2 cm long.
Venation	:	Reticulate pinnate
Leaves falling	:	During the hot season.

FLOWERS (PLATE 4)

Colour	:	The flowers have a greenish to cream-yellow pedestal with Cream to reddish petals.
Arrangement	:	Flowers are polygamous, the flowers are 4-merous and grouped in few- flowered and axillary paniculate dichasial cymes with solitary seeds
Odour	:	Fragrant
The calyx	:	Cup-shaped with 4 lobes.
Size of calyx tube	:	2 mm long and 3 mm in diameter
Colour	:	yellow - pinkish and glabrous.
Shape of lobes	:	Triangular and 1.5 mm long and wide
The corolla	:	4 yellow-pinkish-colored and glabrous petals. The petals are oblong and recurved at the end 5 mm long and 2 mm wide.
The androecium	:	composed of 8 stamens. Free. The filaments are 1.5-2 mm long and connate and the anthers are oblong
The pistil	:	composed of a 1 mm long style and a 2-lobed stigma. Ovary bilocular with two ovules per locule, axile placentation.
Flowering season	:	From March to May.

FRUITS(PLATE 5)

Shape : Globose, fleshy **drupe**

Size : 1.5 cm

Fruiting season : From August to January

BARK(PLATE 6)

Colour : greyish,new bark-bright green

Texture : smooth, papery, branchlets glabrous

SEEDS (PLATE - 7)

Colour : Black

Arrangement : Solitary with 4 radiating wings

Seeding season : From August to January.

WOOD:

The heartwood is grey with darker streaks

The sapwood is white

5.1.2 MICROSCOPY OF THE LEAF

5.1.3 Microscopic characters:

T.S.of midrib(PLATE 8)

Transverse section of midrib of leaflet shows prominent midrib a small projecting hump on the adaxial side and mucous cell is found at the centre.

Palisade tissue is not continuous over the midrib region. The ground tissue is made up of closely arranged round to oval parenchyma cells.

T.S.of Lamina(PLATE 9)

Leaf is dorsiventral in nature. Adaxial epidermal cells are angular, straight and thick walled covered by a thick cuticle. Mesophyll is differentiated in to palisade tissue occur beneath the epidermis as single layered, columnar and closely arranged cells and lobed aerenchymatous spongy parenchyma

PLATE 1 & 1A

HABIT AND HABITAT OF *Commiphora caudata*

PLATE 2

BRANCH SHOWING LEAF ARRANGEMENT OF *C.caudata*

PLATE 3

DORSAL VIEW OF *C.caudata*

PLATE 3A

VENTRAL VIEW OF *C.caudata*

PLATE 4

FLOWER BUDS OF *C.caudata*

PLATE 5

FRUITS OF *C.caudata*

PLATE 6

BARK OF *C.caudata*

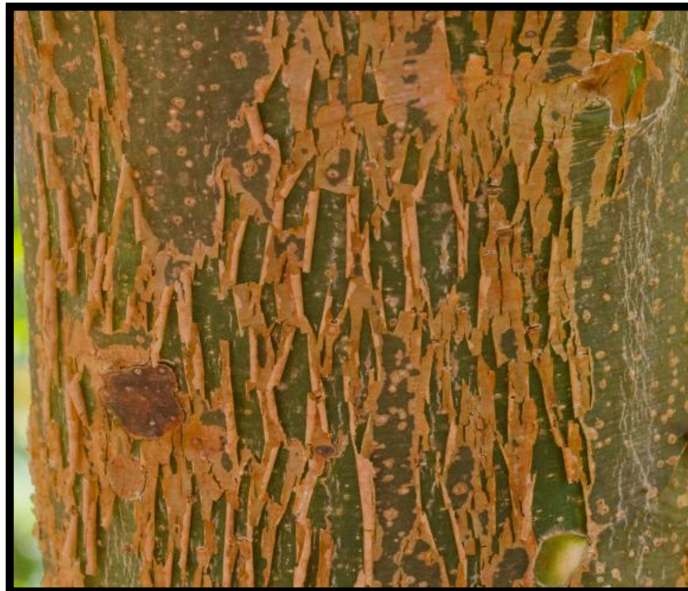


PLATE 7

DIAGRAMMATIC REPRESENTATION OF *C.caudata*

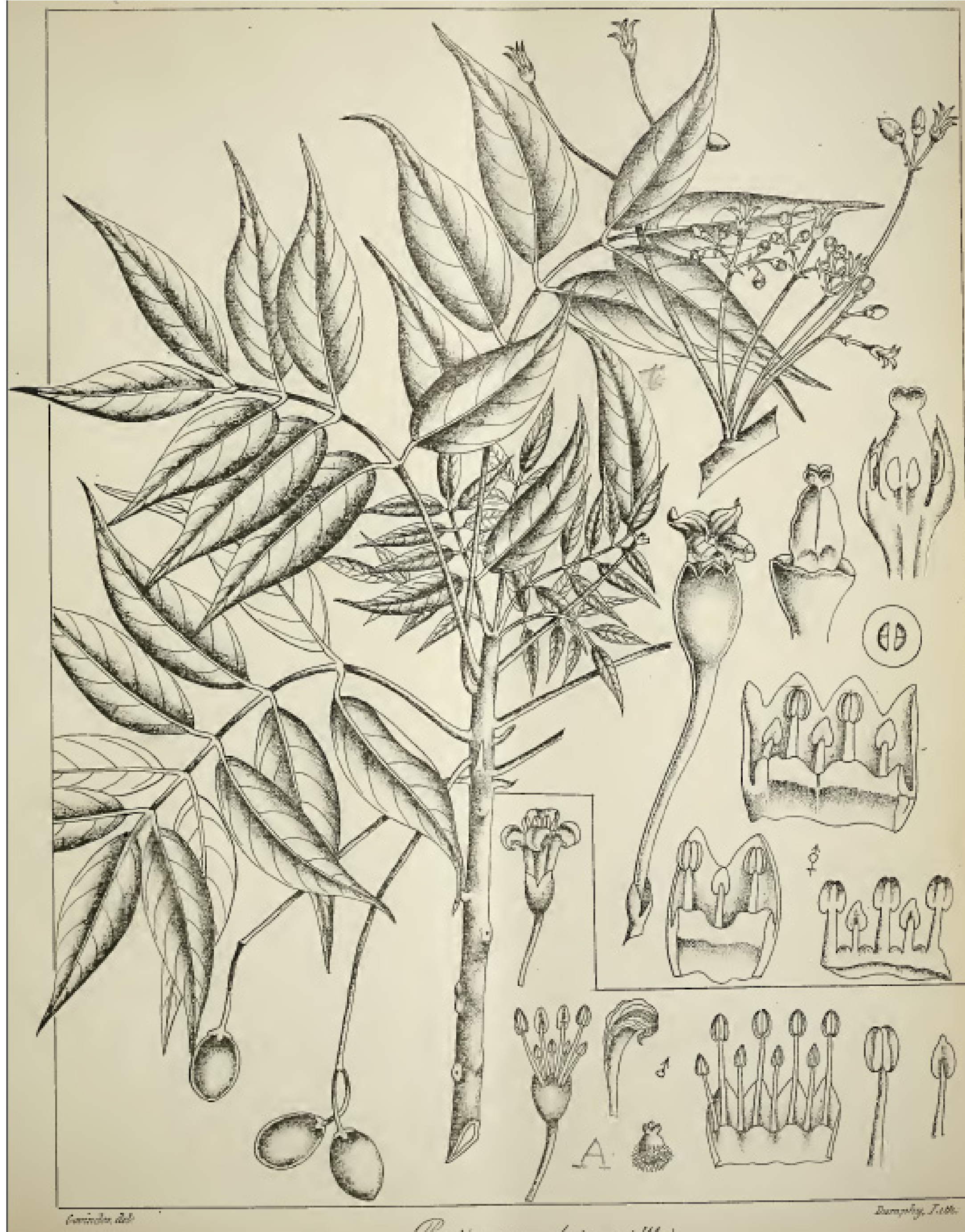


PLATE 8

T.S OF LEAF THROUGH MIDRIB [MICROTOME SECTION]

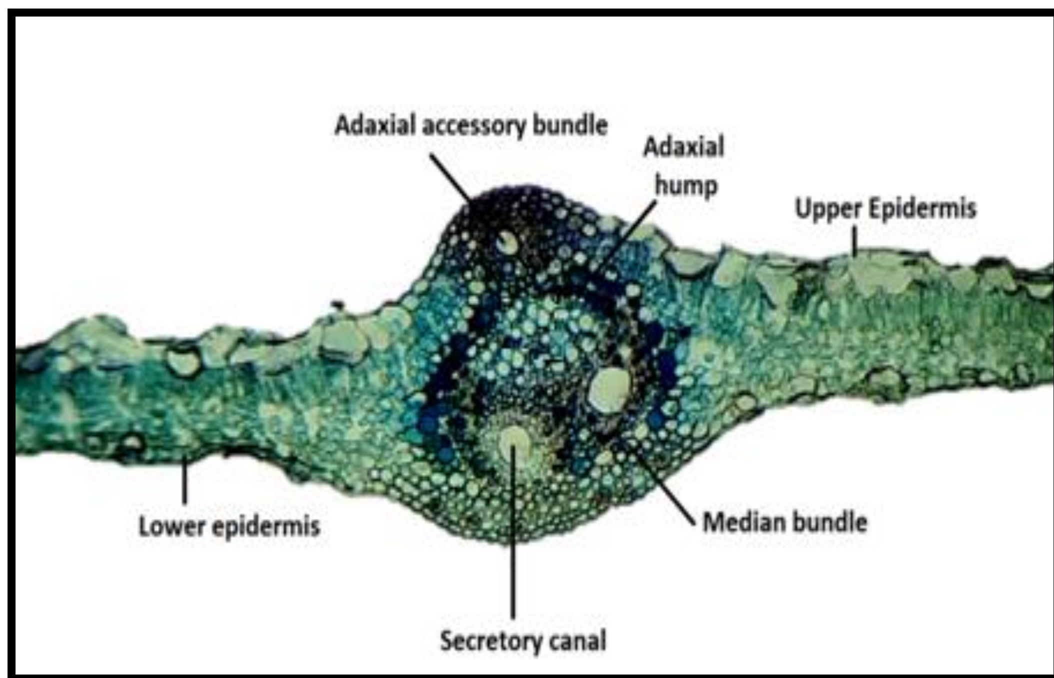


PLATE 8 A

T S OF MIDRIB - ENLARGED

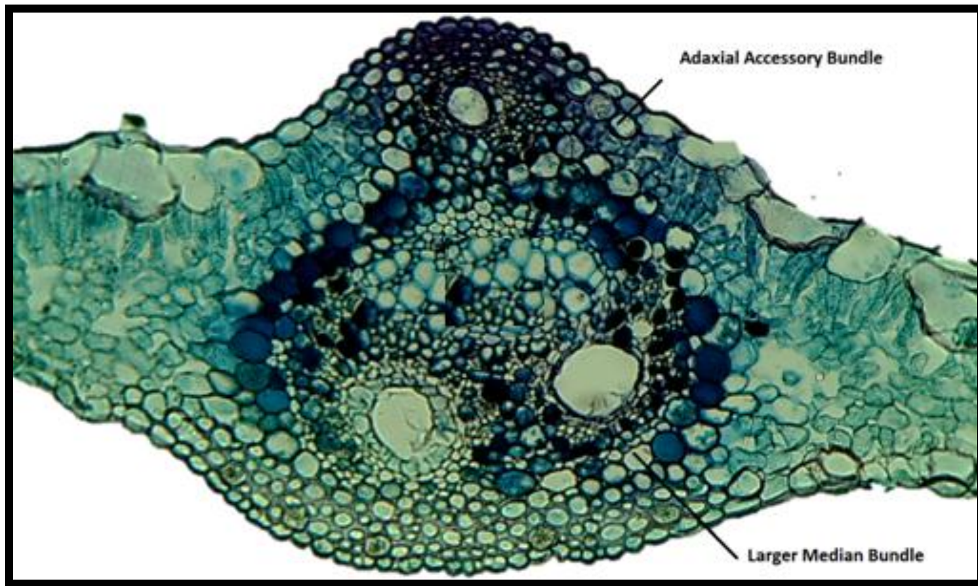


PLATE 8 B

T S OF MIDRIB [HAND SECTION]

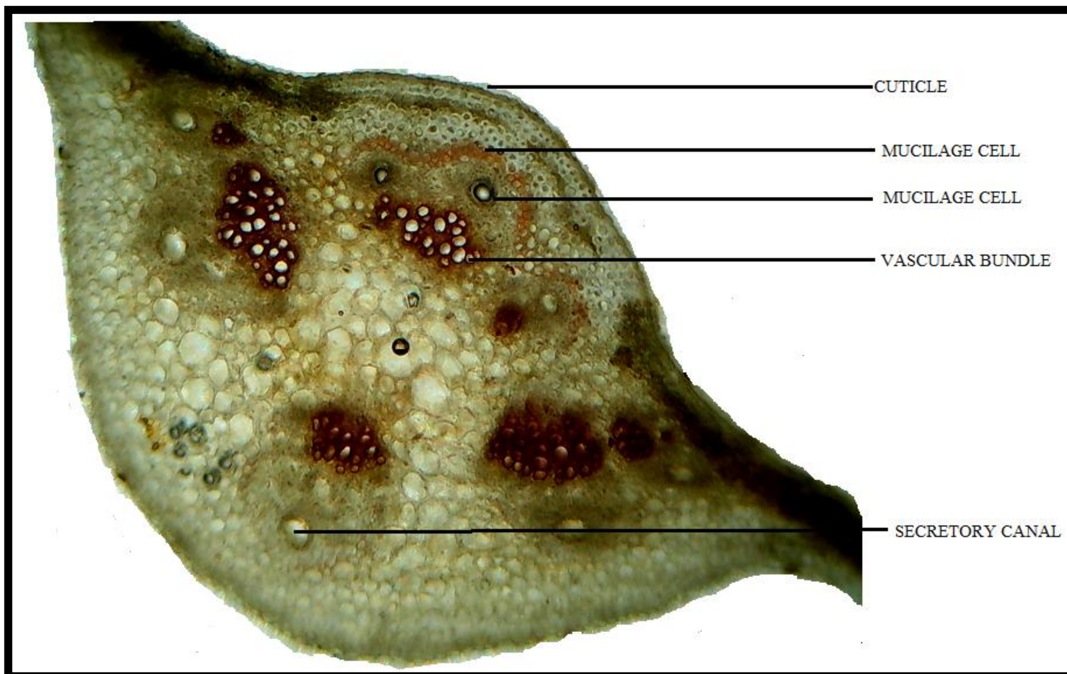


PLATE 9

T.S OF LAMINA [MICROTOME SECTION]

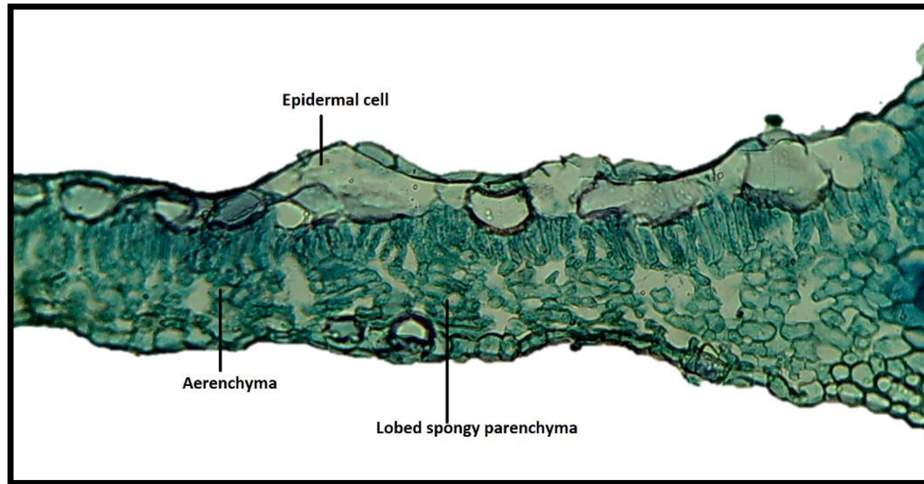


PLATE 9 A

T S OF LAMINA [HAND SECTION]

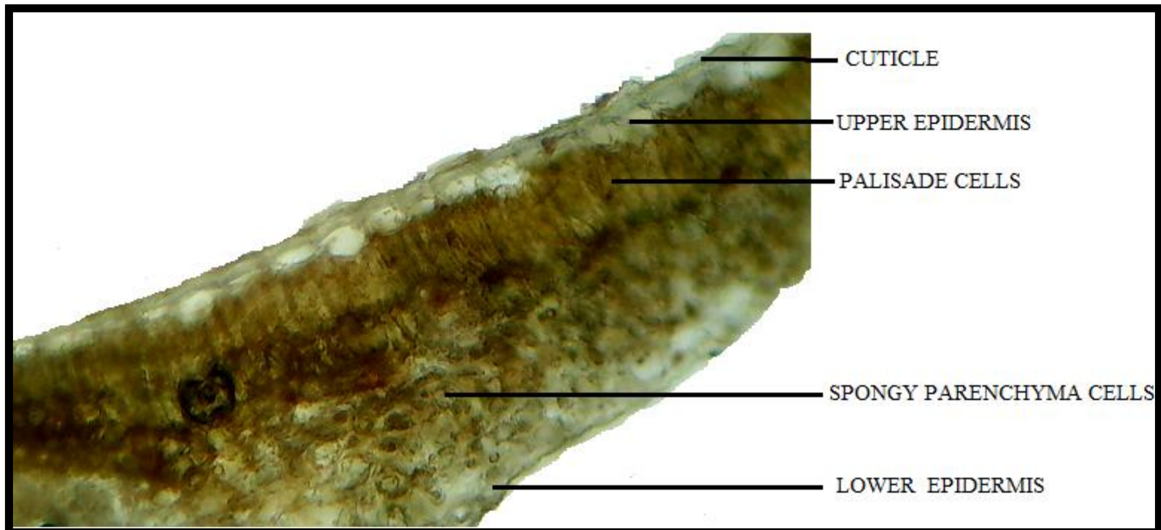


PLATE 10

UPPER EPIDERMIS SURFACE VIEW OF STOMATA OF *C.CAUDATA*

PLATE 10 A

LOWER EPIDERMIS SURFACE VIEW OF STOMATA OF *C.CAUDATA*

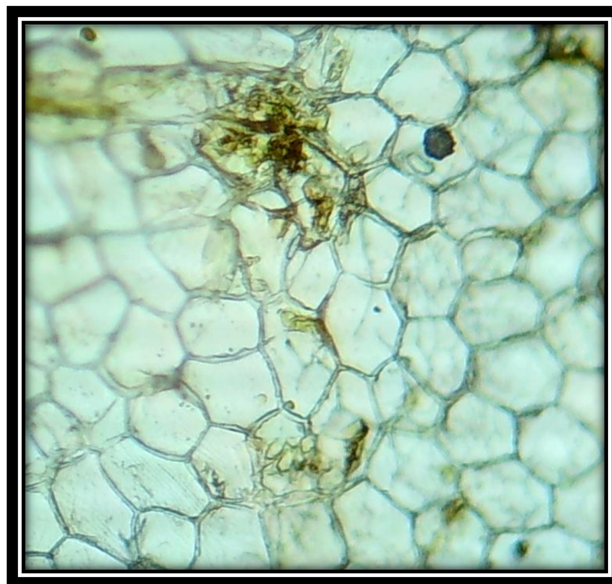


PLATE 11 A

T.S OF PETIOLE [MICROTOME SECTION]

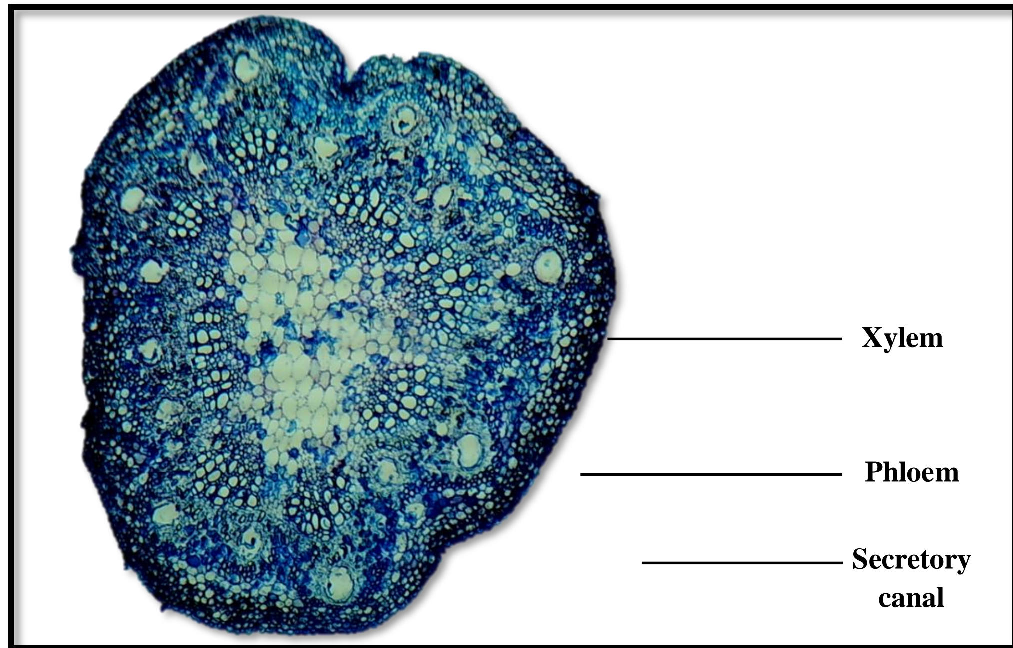


PLATE 11 B

T.S OF PETIOLE [HAND SECTION]

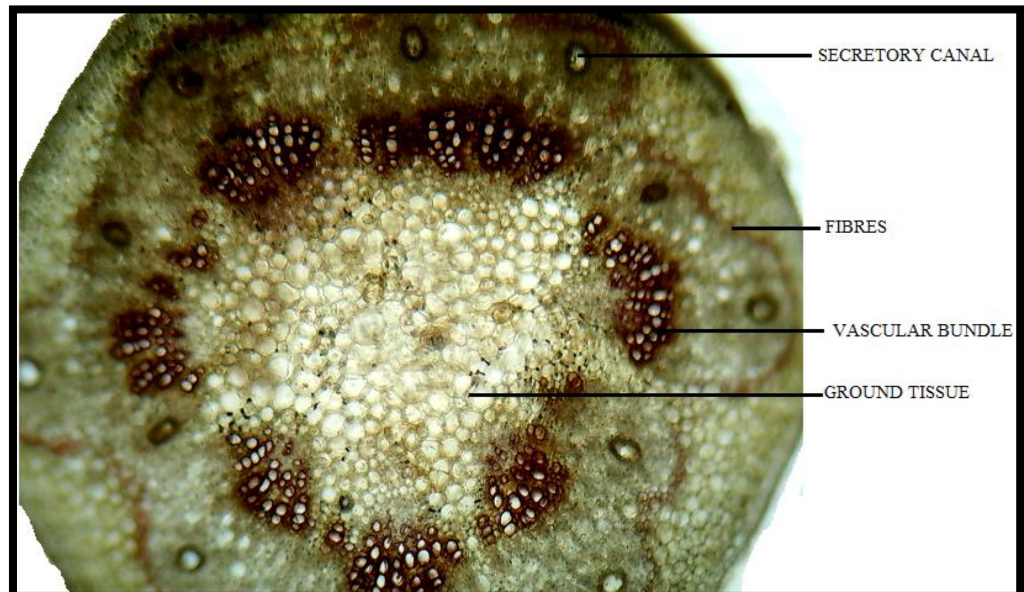
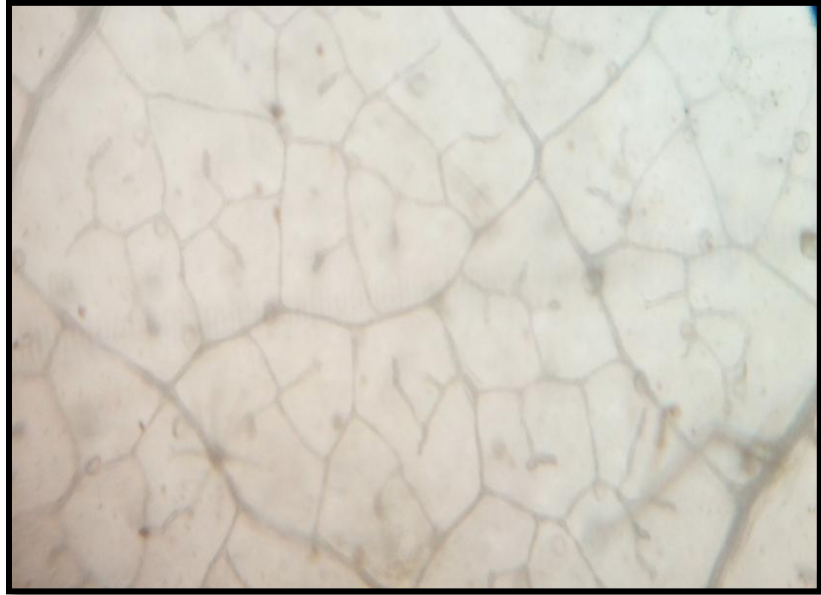


PLATE 12

VENATION OF *C.caudata*



cells.

A noteworthy feature is the presence of secretory cavities lined with epithelium cells occur below the epidermis on either side of the leaf.

Vascular bundle: One larger median bundle and one smaller adaxial accessory bundle. Secretory cavities occur in the phloem region.

Stomata are actinocytic**. (PLATE 10)**

T.S. of petiole(PLATE 11)

Transverse section of petiole is nearly circular in outline. Epidermis is made up of single layer of rectangular thick walled cells covered by a thick cuticle. The inner region is composed of ring of vascular bundle to 12 layers of parenchyma cells. Secretory canals situated below the epidermis in the cortical region.

5.1.3 SCANNING ELECTRON MICROSCOPY (SEM) (PLATE 13):

The Scanning Electron Microscopy with its relatively highly resolution and great depth of focus, provide topographical and morphological information. SEM studies showed the unicellular trichome and its occurrence and arrangement. Actinocytic stomata was predominantly observed.

5.1.4 POWDER MICROSCOPY(PLATE 14)

Green in colour, odour aromatic, taste mucilaginous. Microscopically showed fibres, actinocytic stomata, xylem vessels, phloem with companion cells, secretary cells, parenchyma cells.

5.1.5 MICROSCOPIC SCHEDULES

As per the methods described in materials and methods, microscopic schedules were carried out and the results tabulated from the Tables 1- 3

PLATE 13

SEM OF *Commiphora caudata* LEAF [STOMATA]

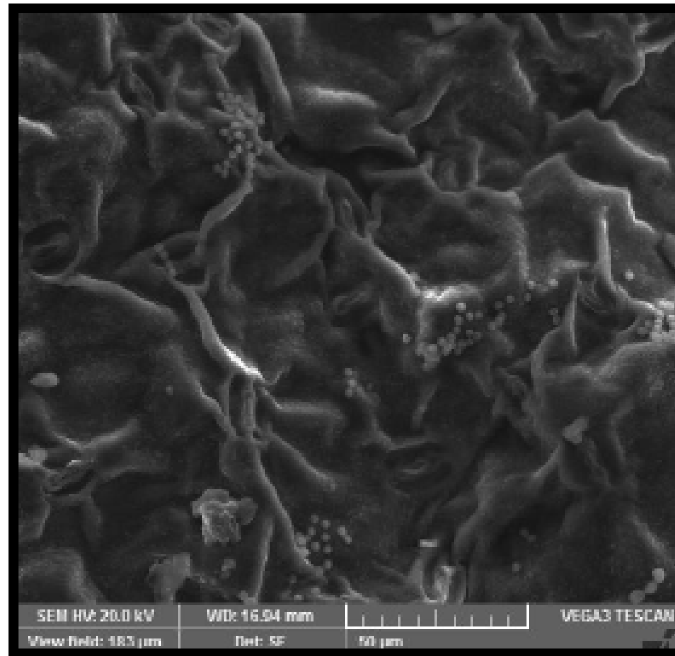


PLATE 13 A

SEM OF *C.caudata* [EPIDERMIS CELLS]

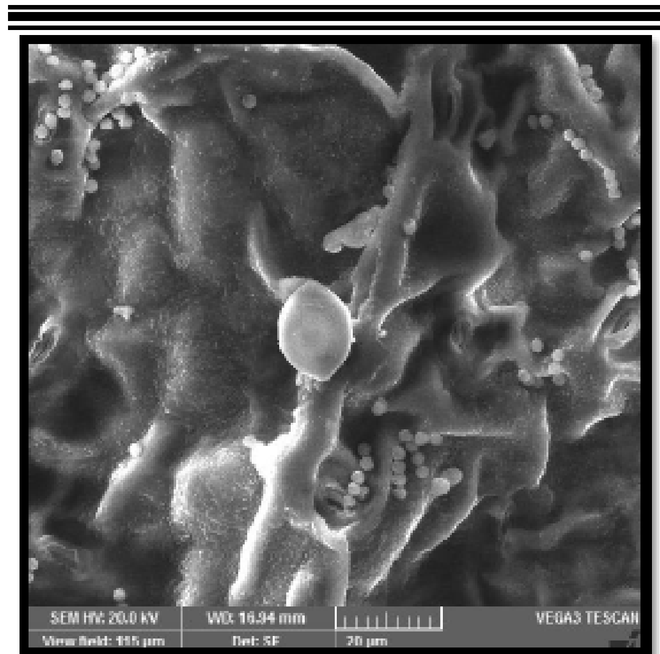


PLATE 13 B

SEM OF *C.caudata*

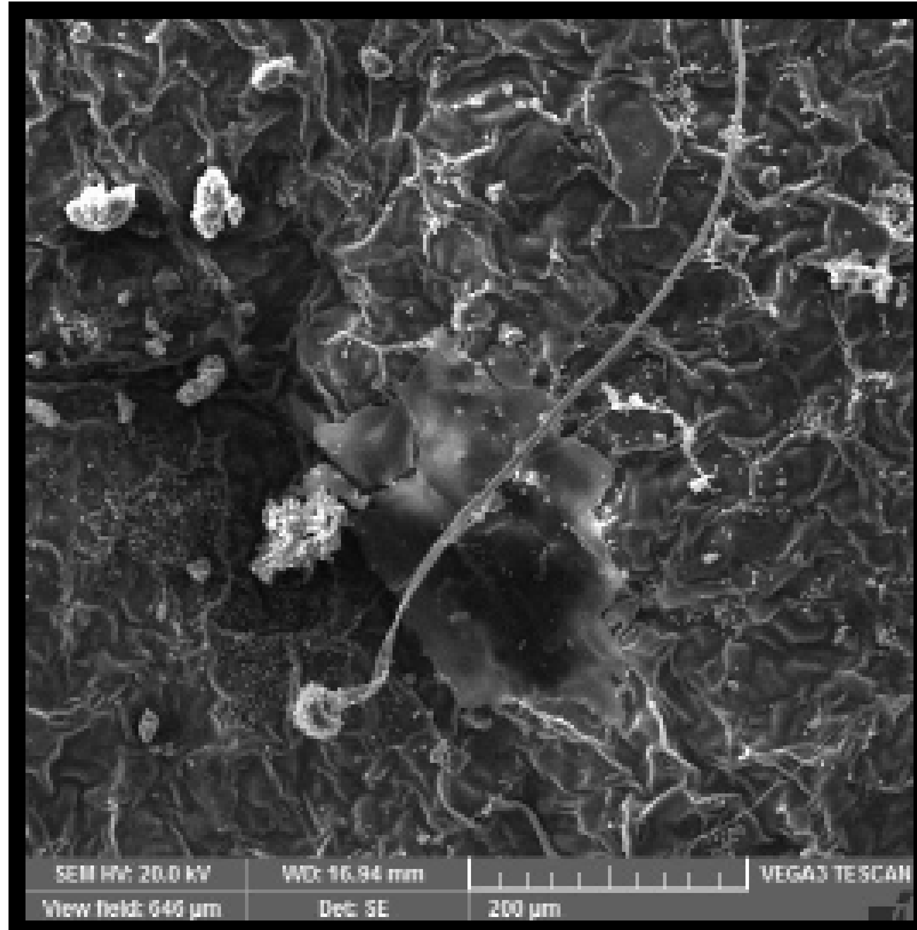


Table – 1

VEIN ISLET AND VEIN TERMINATION NUMBER OF *C.caudata* LEAVES

OBSERVATION NUMBER	VEIN ISLET NUMBER	VEIN TERMINATION NUMBER
1	5	3
2	4	2
3	6	1
4	6	1
5	5	1
6	4	2
7	5	1
8	6	2
9	4	1
10	5	4
Minimum	**4**	**1**
Average	**5**	**1.8**
Maximum	**6**	**4**

Table – 2

STOMATAL NUMBER OF *C.caudata* LEAVES

OBSERVATION NUMBER	LOWER EPIDERMIS	UPPER EPIDERMIS
1	11	6
2	13	9
3	12	7
4	12	7
5	13	7
6	11	8
7	11	8
8	12	7
9	13	9
10	11	6
Minimum	**11**	**6**
Average	**12**	**7.5**
Maximum	**13**	**9**

Table – 3

STOMATAL INDEX OF *C.caudata* LEAVES

OBSERVATION NUMBER	UPPER EPIDERMIS	LOWER EPIDERMIS
1	18	10
2	14	12
3	17	12
4	13	11
5	15	10
6	15	10
7	14	12
8	15	11
9	22	16
10	16	10
Minimum	**13**	**10**
Average	**15.90**	**11.40**
Maximum	**22**	**16**

5.1.1 PHYSIO CHEMICALPARAMETERS

As per the methods described in materials and methods, physicochemical parameters were carried and the results were as follows.

Table – 4
ASH VALUES OF THE OF *C.caudata* LEAVES

OBSERVATION NUMBER	TOTAL ASH (%)	ACID INSOLUBLE ASH (%)	WATER SOLUBLE ASH (%)
1	10.5	2.5	
2	8.8	2.6	
3	9.5	2.5	
4	9.4	2.6	
5	9.3	2.9	
6	9.1		7.1
7	10.3		7.5
8	10.1		7.3
9	10.5		7.6
10	9.6		7.2
Minimum	**8.8**	**2.5**	**7.1**
Average	**9.71**	**2.62**	**7.24**
Maximum	**10.5**	**2.9**	**7.8**

FIGURE 1

DIAGRAMMATIC REPRESENTATION T.S. OF MIDRIB

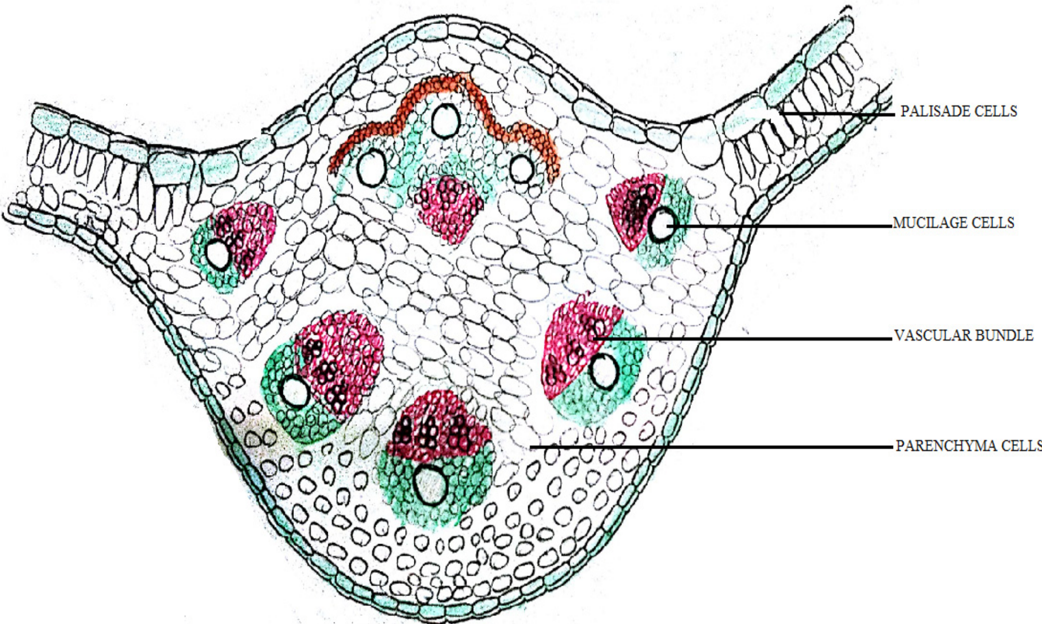
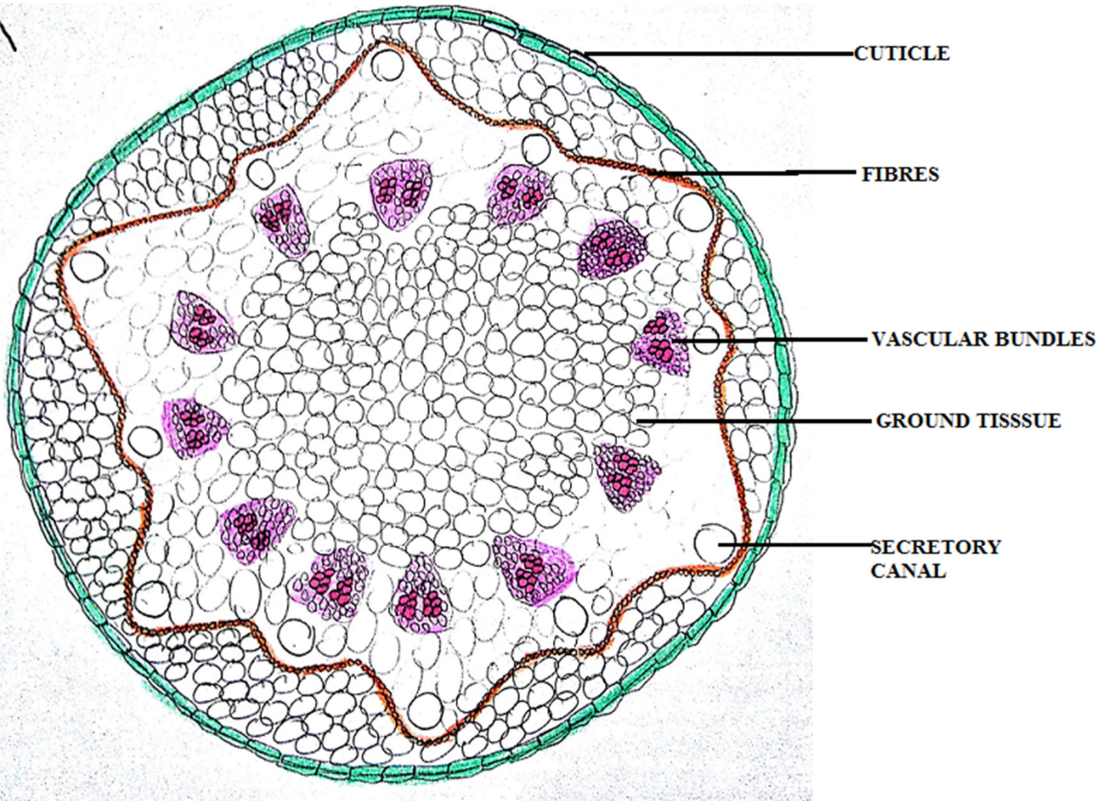


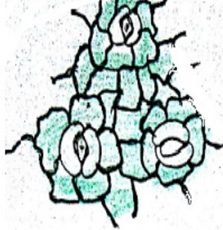
FIGURE 2

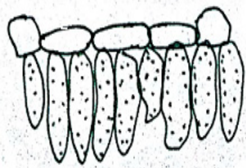
DIAGRAMMATIC REPRESENTATION T.S. OF LAMINA

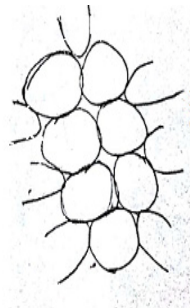


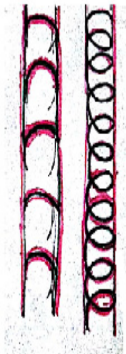
PLATES 14

POWDER MICROSCOPY OF *C.caudata*









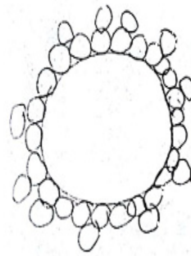


Table – 5

LOSS ON DRYING (LOD) of *C.caudata* LEAVES

OBSERVATION NUMBER	LOD %w/w
1	5.7
2	5.5
3	5.5
4	4.9
5	5.2
Minimum	**4.9**
Average	**5.36**
Maximum	**5.7**

Table – 6

EXTRACTIVE VALUES FOR *C.caudata* LEAVES

SOLVENTS	EXTRACTIVE VALUE (%)
Petroleum ether	10.15
Ethanol	8.2
Water	10.5

5.2 PHYTOCHEMICAL STUDIES

5.2.1 PRELIMINARY PHYTOCHEMICAL SCREENING QUALITATIVE PHYTOCHEMICALTESTS:

Preliminary phytochemical screening of the powdered mature leaves was performed and the results are as follows (Table 7)

TEST FOR ALKALOIDS

Mayer's test : No appearance of cream precipitate shows the **absence** of alkaloids

Dragendorff's test : No appearance of reddish brown precipitate shows the **absence** of alkaloids

Hager's test : No appearance of yellow precipitate shows the **absence** of alkaloids

TEST FOR CARBOHYDRATES

Molisch's test : Appearance of purple colour shows the **presence** of carbohydrates.

Fehling's test : Formation of reddish brown precipitate shows the **Presence** of free reducing sugars.

Benedict's test : Formation of reddish brown precipitate shows the **Presence** of free reducing sugars.

TEST FOR GLYCOSIDES

General test

Test A : No red colour precipitate shows the **absence** of glycosides

Test B : No appearance of red colour shows the **presence** of glycosides

Keller Killani's test : No reddish brown colour ring at the junction shows the absence of cardiac glycosides

Borntrager's test : No pink colour shows the **absence** of anthraquinone glycosides

Modified Borntrager's test : No pink colour in ammoniacal layer shows the **absence** of anthraquinone glycosides.

TEST FOR PHYTOSTEROL

Salkowski's test : Appearance of red colour in lower layer shows the **presence** of sterol.

Liebermann – Burchard's test : Brown ring at the junction of two layers and green colour in the upper layer shows the **presence** of sterols

TEST FOR SAPONINS : Frothing occurs indicates the **presence** of Saponins

TEST FOR TANNINS

Ferric chloride test : Appearance of bluish black colour shows the **Presence** of tannins

Gold beater skin test : Appearance of brown colour shows the **presence** of tannins

TEST FOR PROTEINS AND FREE AMINOACIDS

Millon's test : Appearance of red colour on heating shows the **presence** of proteins

Biuret test : Appearance of violet colour shows the **presence** of proteins

Ninhydrin test : Formation of violet colour shows the **presence** of amino acids.

TEST FOR TERPENOIDS

Appearance of pink colour shows the **presence** of terpenoids

TEST FOR FLAVONOIDS

Shinoda test	:	Purple colour shows the **presence** of flavonoids
Alkaline reagent test	:	Yellow - orange colour shows the **presence** of flavonoids .
Acid test	:	Yellow – orange colour shows the **presence** of flavonoids
Zinc hydrochloride test	:	Red colour shows the **presence** of flavonoids.

TEST FOR VOLATILE OIL

Volatile oil was not obtained shows the **absence** of volatile oil

TEST FOR FIXED OIL

No translucent greasy spot shows the **absence** of fixed oil

Table – 7

PRELIMINARY PHYTOCHEMICAL SCREENING OFLEAVES OF *C.caudata*

S.NO	TEST	OBSERVATION
I.	**ALKALOIDS**	
	Mayer's reagent	-
	Dragandorff's reagent	-
	Hager's reagent	-
II.	**CARBOHYDRATES**	
	Molisch's test	+
	Fehling's test	+
	Benedict's test	+
III.	**GLYCOSIDES**	
	Anthroquinone glycosides	-
	Borntrager's test	-
	Modified Borntrager's test	-
	Cardiac glycosides	
	Keller Kiliani test	-
	Raymond test	-
	Legal test	-
IV.	**STEROLS**	
	Salkowski test	+
	LibermanBurchard's test	+
V.	**SAPONINS**	+
VI.	**TANNINS**	
	Ferric chloride	+
	Gold Beater's skin test	+
VII.	**PROTEINS AND FREE AMINO ACIDS**	
	Millon's test	+
	Biuret test	+
	Ninhydrin test	+
VIII.	**MUCILAGE**	+
IX.	**TERPENOIDS**	+
X.	**FLAVONOIDS**	
	Shinoda test	+
	Alkali test	+
	Acid test	+
	Zn/Hcl test	+
XI.	**VOLATILE OIL**	_
XII.	**FIXED OIL**	_

5.2.2 FLUORESCENCE ANALYSIS

The fluorescence analysis of the leaf powder of ***C.caudata*** was studied.

The results were as follows (Table -8**)**

Table -8

FLUORESCENCE ANALYSIS

Reagent	Observation
Powder as such	Dark Green
Powder + 50% Hydrochloric acid	Yellowish Green
Powder + 50% Nitric acid	Light brown
Powder + Petroleum ether	Light orange
Powder + 50% Sulphuric acid	Dark brown
Powder + 1N NaOH in water	Dark green
Powder + 1N NaOH in methanol	Dark Green
Powder +5% Ferric chloride solution	Greenish brown
Powder + Picric acid	Fluorescence green
Powder + Chloroform	Green

5.2.3 ESTIMATION OF FLAVONOID CONTENT

Flavonoid content of **extract** in terms of quercetin by aluminium chloride was found to be **7.8 mg/g**.

5.2.4 ESTIMATION OF TOTAL PHENOLIC CONTENT

Total phenolic content of extract in terms of Gallic acid was found to be **18.8 mg/g**.

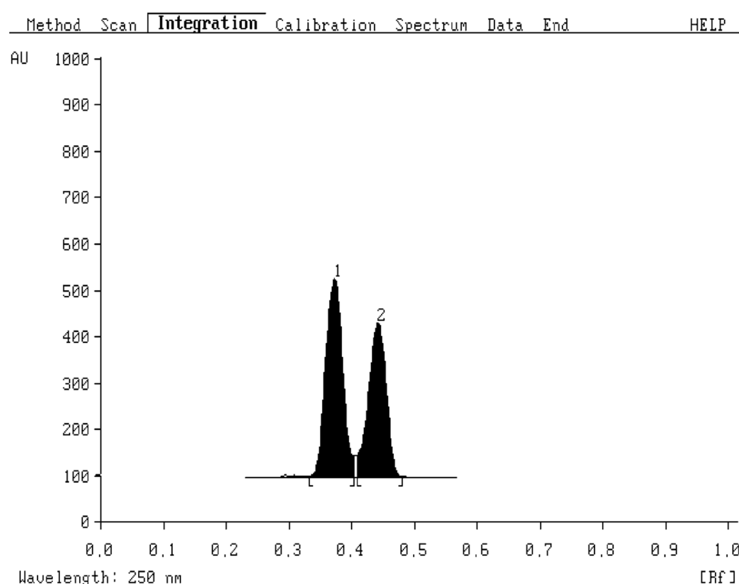
5.2.5 IDENTIFICATION OF COMPOUND PRESENT IN THE EECCL BY HPTLC ANALYSIS

Sample Information: Sample ID: Ethanol extract of *C. caudate* leaf

About 5µl of standard quinine, 5µl of EECCL was applied as a band using CAMEG Linomat sample applicator on aluminium sheets pre-coated with silica gel 60 GF 254 HPTLC plates used as a stationary phase. The plates were developed in the mobile phase Toluene: acetone (9:1) to a distance of 80 mm in CAMAG trough glass chamber. The tracks were scanned using WIN CATS 1.43 software at 254nm. The fingerprint profiles were recorded and presented. The plot showed 12 spots after development with the mobile phase.

HPTLC profile for the following compounds revealed that,

HPTLC PROFILE (FIGURE 4) OF STANDARD GUGGULSTERONE MIXTURE



Chromatogram of standard guggulsterone mixture (1000ng/spot); peak 1is of E-guggulsterone (*R*f:0.38 ± 0.02),peak of Z-guggulsterone (*R*f:0.46 ± 0.02); mobile phase; toluene–acetone (9.0:1.0,v/v).

HPTLC PROFILE OF EECC LEAVES: FIGURE 5

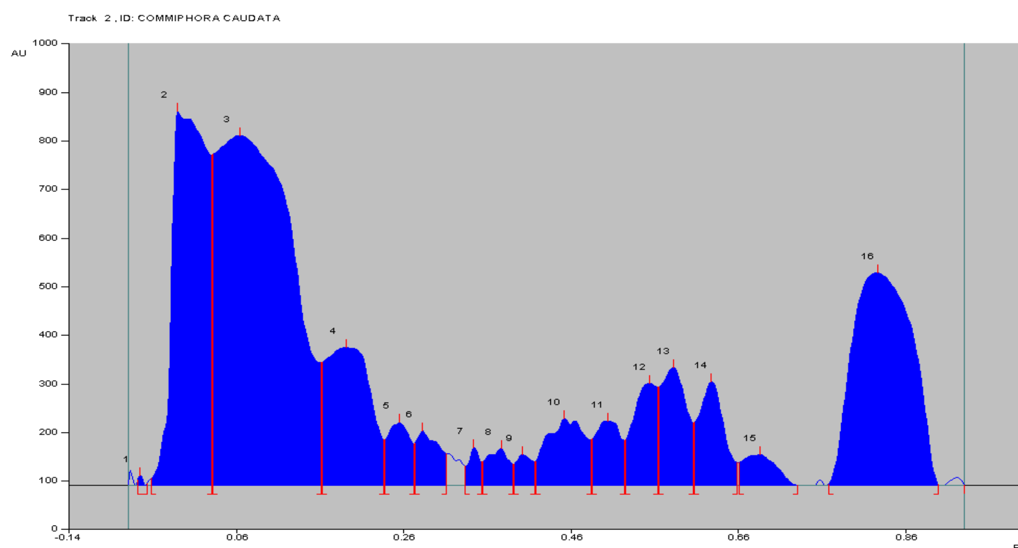


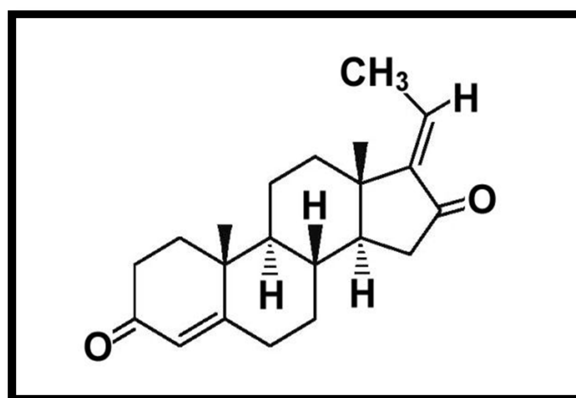
TABLE 9

HPTLC PROFILE OF C.caudata

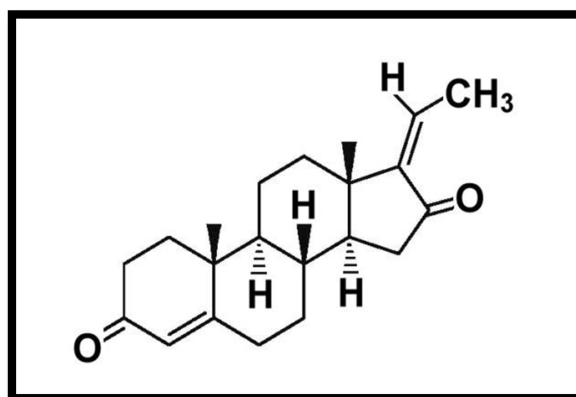
COMMIPHORA	RF	LAMBDA MAX				
		V BAND	IV BAND	III BAND	II BAND	I BAND
	0.25	233	270	329		
	0.37	240	315	424	660	
	0.40	240	315	424	660	
	0.45	240	315	424	660	
	0.50	240	306	370	413	660

S.NO	COMPOUND	RF VALUE	AMOUNT PRESENT
1	E-Guggulsterone	0.38 ± 0.02	0.051%w/w

STRUCTURE OF E - GUGGULSTERONE

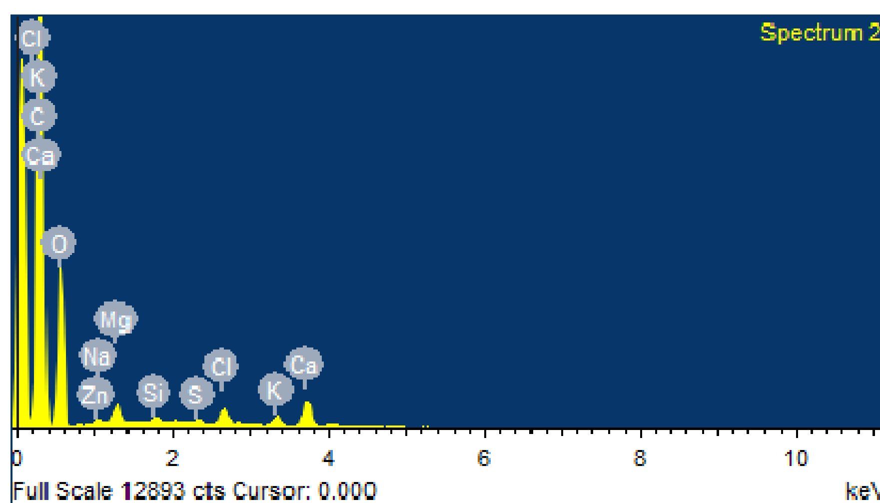


STRUCTURE OF Z-GUGGULSTERONE



5.2.6 DETERMINATION OF TRACE ELEMENTS IN THE LEAF OF *C.caudata* BY ENERGY DISPERSIVE X-RAY SPECTROMETER (EDS)

FIGURE – 6



ENERGY DISPERSIVE X-RAY SPECTRUM FOR *C.caudata* LEAVES

Estimation of the elements like Ca, O, Al and Si showed the following mg weight percentage and atomic percentage.

Table-9

***C.caudata* LEAVES ELEMENTS WEIGHT & ATOMIC PERCENTAGE**

Element	Weight%	Atomic%
C K	61.16	68.63
O K	35.85	30.20
Na K	0.11	0.06
Mg K	0.48	0.27
Si K	0.14	0.07
S K	0.10	0.04
Cl K	0.53	0.20
K K	0.36	0.12
Ca K	1.05	0.35
Zn K	0.22	0.05
Totals	100.00	

PHARMACOLOGICAL STUDIES

5.3.1 WHOLE EMBRYO CULTURE TOXICITYSTUDY

Effect of EECCL on the developmental stages of Zf embryo was carried out. The eggs were cultured in the embryonic medium. The maximal acceptable toxicant concentration (MATC) was calculated by scoring the malformation 1 % DMSO and podophyllotoxic (0.010µg/ml) were used as control and standard toxin. No malformations are incidence of mortality was observed up to the 0.5 – 1µg/ml concentration level (score 0). But medium to strong edema, eye malformation, bent tail, weak undulated notochord and twisted notochord were observed from 1 to 2 µg/ml concentration up to 80 hpf. No mortality was observed in this concentration level. Total mortality was observed in the standard podophyllotoxin at 0.01µg/ml concentration

(score40).

TABLE 11

SCORES FOR THE WHOLE EMBRYO TOXICITY

<table>
<tr><th>Conc. µg/ml</th><th>Hours</th><th>score</th></tr>
<tr><td>0.5 – 0.7</td><td>All</td><td>Nil</td></tr>
<tr><td rowspan="4">1</td><td>12</td><td>1</td></tr>
<tr><td>36</td><td>1</td></tr>
<tr><td>60</td><td>2</td></tr>
<tr><td>80</td><td>2</td></tr>
<tr><td rowspan="4">2</td><td>12</td><td>5</td></tr>
<tr><td>36</td><td>5</td></tr>
<tr><td>60</td><td>10</td></tr>
<tr><td>80</td><td>13</td></tr>
</table>

5.3.2 ZEBRA FISH LARVAL TOXICITY STUDY

Larval toxicity study was carried out on zf larvae of 5 dpf cultured in E3 embryonic medium. Five larvae per group was taken and treated with 0.5, 0.75, 1 and 2 µg was taken as a test trail and mortality percentage was calculated.

From the experiment it was observed that there was no mortality in 0.5 and 0.75µg/ml concentrations. But 5% and 10% mortality was observed at 1 and 2 µg/ml concentrations respectively. No mortality was observed in the control. 100% mortality was observed in the standard podophyllotoxin at 0.5 µg/ml concentration.

TABLE 12

ZEBRAFISH LARVAL TOXICITY STUDY

CONCENTRATION (µg/ml)	NUMBER OF LARVAE	AFTER 24h	CORRECTED MORTALITY	MORTALITY
0.5	20	NIL	NIL	NIL
	20			
	20			
0.75	20	NIL	NIL	NIL
	20			
	20			
1	20	NIL	NIL	5
	20	NIL	NIL	
	20	1	5	
2	20	1	10	10
	20	1	10	
	20	NIL	NIL	
Control (vehicle)	20		NIL	NIL
	20			
	20			
	20	NIL		
Standard podophyllotoxin	20	20	100	100
	20	20	100	
	20	20	100	

FIGURE 7

ZEBRAFISH LARVAL TOXICITY

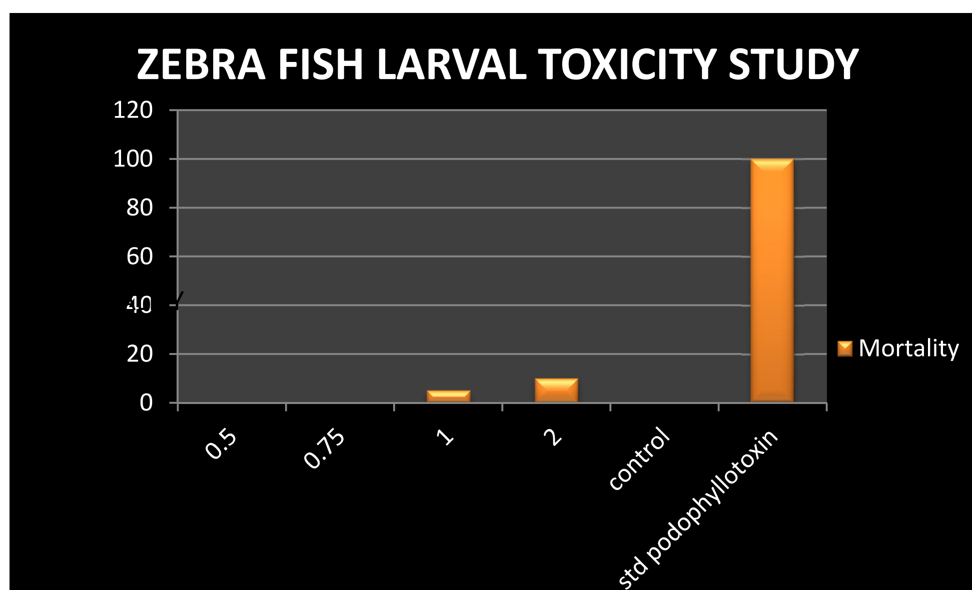


PLATE – 15

VEHICLE CONTROL ZF LARVAE [NORMAL]

PLATE – 16

DOX INDUCED CARDIOTOXIC ZF LARVAE SHOWING PERICARDIAL EDEMA

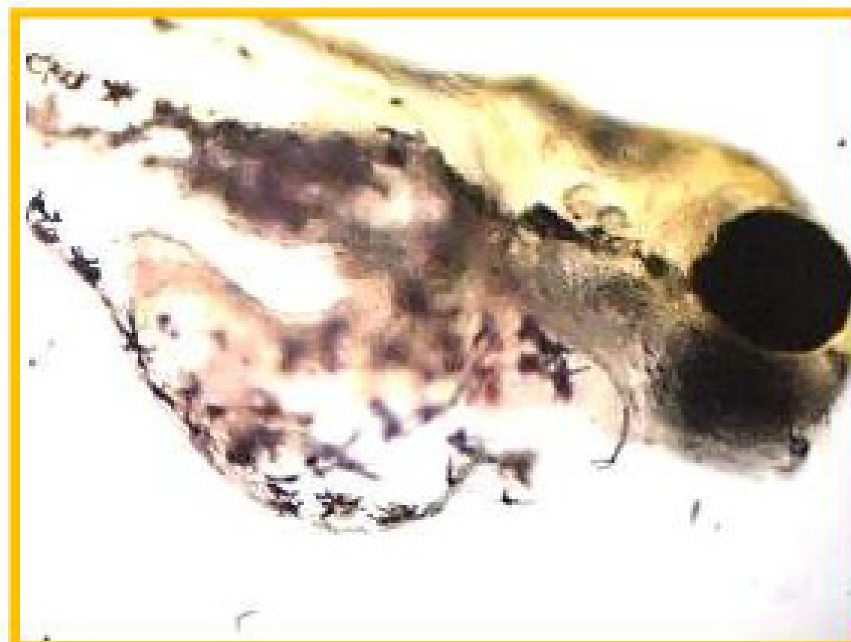
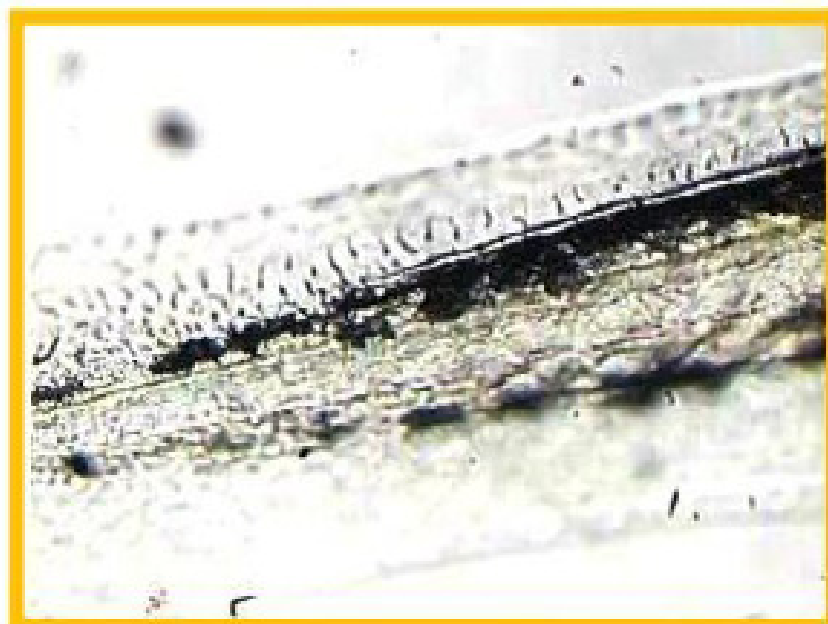


PLATE - 17

EFFECT OF EECCL ON DOX INDUCED CARDIOTOXIC ZF LARVAE



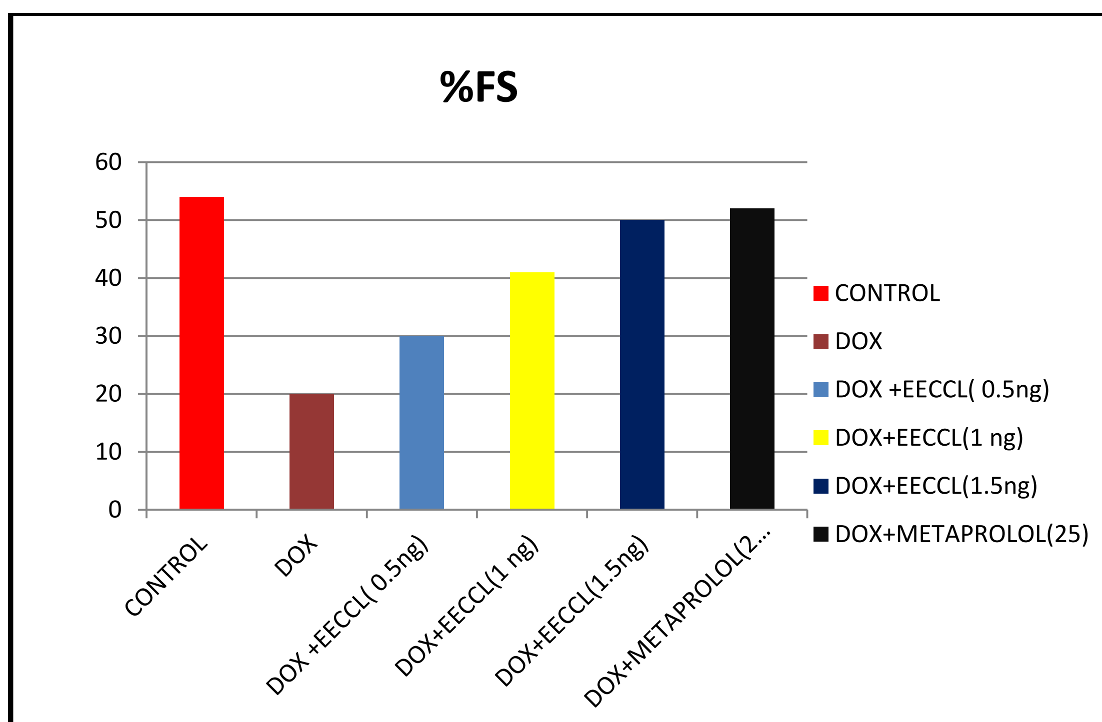
5.3.3 EFFECT OF EECCL ON DOXORUBICIN INDUCED CARDIOTOXICITY ON ZEBRAFISH MODEL

TABLE 13

DRUG	%FS
CONTROL	54 ± 1.22
DOX	20± 1.08
DOX +EECCL(0.5ng)	30±1.15
DOX+EECCL(1 ng)	41±1.04
DOX+EECCL(1.5ng)	50±0.99
DOX+METAPROLOL(25)	52

FIGURE 8

EFFECT OF EECCL ON DOXORUBICIN INDUCED CARDIOTOXICITY ON ZF LARVAE MODEL



DISCUSSION

CHAPTER VI

DISCUSSION

The dissertation covers a study on widely available member of the family Burseraceae known botanically as *Commiphora caudata (Wt & Arn)Engl.* which is commonly called as **'Hill mango'**, **'Green Commiphora'** in English and **'Kiluvai'** in Tamil. The leaves of *C.caudata* really do not have any match as a cheap natural and easily available plant. It was reported that Burseraceae which family members have phytoconstituents useful in the treatment of various diseases and was also claimed that these plants merit detailed study which can prove useful in the discovery of lead compounds leading to novel and more efficacious drugs.

The plant *Commiphora caudata* is traditionally known to be useful to treat a range of ailments the most important being, diabetes, ulcer, diarrhoea, inflammations and spasms (Selvamani P *et al.,*2013*,*Deepa V S *et al.,*2009*,* Nisha P and Jyothi H.,2014) , various types of ulcers (Ganesan S *et al.,*2008), rhumatalgia (Nisha P and Jyoti H ., 2014) , anti-inflammatory , mouth ulcer and mainly used in the treatment of cuts and wounds (Thomas B *et al*.,2014). It is also act as astringent, sweet, cooling, aphrodisiac, diuretic and antidiabetic activities and is used for fever, strangury , vitiated conditions of vata and pitta in siddha system of medicines. It is a potential medicinal plant has been used traditionally in the treatment of arthritis, diabetes and obesity.(Geetha K and Ganapathy S.,2013).
It is used in Ayurveda and Siddha traditional medicines.

Leaves of the plant used traditionally to treat painful and inflammatory conditions (Annu W *et al*.,2010), wounds (Thomas B *et al*.,2014), rheuma-

talgia (Nisha P and Jyoti .,2014, Geetha K *et al.,*2014), ulcers, diarrhoea and spasms (Geetha K *et al.,*2014), dysentery (Gunasekaran M , Balasubramaniyam P.,2012), to improve digestion and to increase appetite (Akhade MS *et al*.,2017), the seeds are to relieve stomach ache. (Vikneshwaran D *et al*.,2008) The stem and bark are traditionally used in the treatment of rheumatism, ulcers, diarrhoea and spasms.(Girija P *et al.,* 2017), lotion of its stembark is used to treat skin conditions such as impetigo, eczema and shingles. (Prasad MNV *et al*.,2007), bark is used in the treatment of diarrhoea (Gunasekaran M, Balasubramaniyam P.,2012).

Phytochemical investigation of *C.caudata* showed that a rich source of steroidal compounds, etc.,(Selvamani P *et al.,*2013), steroids and triterpenoides and it was suggested as marker for this plant.(Akhade MS *et al*.,2017).

E-guggulsterone 0.059%. (Akhade MS *et al*.,2017) the main active principle of this plant.

Flavanoids, glycosides, phenolics (phenols and polyphenols), tannins, carbohydrates (starch,reducing sugars), proteins and oleo-gum resin, alkaloids, glycosides, saponins , alkaloids, terpenoids, aminoacids, mucilage, antioxidant principles are also present in this plant.

Pharmacological evidence reports that hepatoprotective, antidiabetic, antimicrobial, antibacterial and anticancer activities.(Selvamani P *et al*.,2013, Annu w *et al.,*2010, Girija P *et al.*,2017,etc.,),analgesic, anti inflammatory(Selvamani P *et al*.,2013) and antioxidant (Annu w *et al.,*2010), diuretic, antiulcer and antibacterial activities were reported from this plant (Geetha K and Ganapathy S.,2013) , nitric oxide scavenging, free radical scavenging

(Deepa V S *et al*.,2009), antimalarial (Prasad M N V *et al*.,2007), antiarthritic (Girija P *et al*., 2014, Reddy J S *et al*., 2014) , antihyperlipidaemic(Geetha K and Ganapathy S.,2013 ,Geetha K *et.al*,2014), antianaemic (Girija P *et.al*, 2017), Cardioprotective, gastroprotective and anti-ulcerogenic and is used for wound healing hyperlipidemia, rheumatic disorders, obesity and ischaemic heart diseases. (Geetha K *et al*,2014).

PHARMACOGNOSTICAL STUDIES:

Morphological and micromorphological examination and characterization of medicinal plants have always been accorded due to credentials in the pharmacological studies. There was no detailed pharmacognostical work has been carried out including botanical identity based on micro morphology in this leaves of this plant.

The application of morphological studies in drug analysis is pertinent in the field of crude drug authentication. It was studied for the leaf, Interpretation of the morphological characteristics based on different parameters, for the plant organs give a guideline for the diagnosis of the original plant and its adulterants.

Colour, size, shape, margin, texture, arrangement were observed and compared with previous data.

Microscopic techniques help to magnify the fine structure of minute objects and there by confirm the structural details of the plant drug. Though the microscopical evaluation cannot provide complete profile, still it can offer supporting evidences which when combined with other analytical parameters can be used to obtain full evidence for standardization and evaluation of herbal drugs. Consideration must therefore be given to the types of cells and

cell inclusions and the manner in which they are distributed in different organ of the plants. The habit and habitat and the various morphological characters of the various parts have been studied after proper identification and authentication.

PHARMACOGNOSTICAL STUDIES

Macroscopy of the leaves were compound, alternative 3 to 7 foliolate, upper surface dark green, lower surface light green in colour. There is mango like odour and it has mucilaginous taste. Shape is ovate - oblong, length - 4.5 to 6.5 cm; width -2.2 to 3.5 cm; acuminate apex , slightly asymmetric base; entire margin, reticulate pinnate venation; pedicle length - 3.5 to 6.2 cm and glabrous texture , glossy above, subglaucous below. Leaflets ovate or elliptical, chartaceous and glabrous.

Microscopy of the leaves The leaflets were dorsiventral with prominent midrib**.** Vascular bundles of the **midrib** include one larger median bundle and one smaller, adaxial accessory bundle. **Lamina** with uniserrate epidermal layers; mesophyll differentiated into a single layer of palisade cells and lobed aerenchymatous spongy parenchyma cells. Vascular bundle of the lateral vein has adaxial bundle sheath extension. **Stomata** actinocytic type; epidermal cells angular, straight and thick walled.Vein islets polygonal; vein termination branched many times. **The petiole** is roughly circular in outline , petiole semicircular with adaxial depression. Vascular strengths of the petiole many, arranged in a circle with adaxial opening. Petiole circular with a ring of vascular strands. **Secretory canals** occur in the phloem region of leaf, veins and petioles.

Large druses of calcium oxalate crystals abundant in the leaf. The fibres are arranged around the mucilage cells and vascular bundles.

Scanning Electron Microscopy (SEM):

The Scanning Electron Microscopy with its relatively highly resolution and great depth of focus, provide topographical and morphological information. SEM studies showed the unicellular trichome and its occurrence and arrangement.

Actinocytic stomata was predominantly observed.

The plant drugs are generally used in the powdered form where the macro morphology is generally destroyed, so the diagnosis of the plant through the microscopicalcharacter is essential. The powdered crude drugs can be identified based on the presence or absence of different celltypes.

In powdered microscopy observed actinocytic stomata, wavy walled epidermal cells, spiral annular vessels, phloem cells, sieve tube and companion cells, fibres, lobed spongy parenchyma cells**.**

Quantitative microscopy includes certain measurements to distinguish some closely related species which are not easily differentiated by general microscopy. The **stomatal number** is the oldest technique but a simple method of diagnosis of fragmentary leaf parts. The **stomatal index** is the percentage of stomata in relation to the epidermal cells. Both are very specific criteria for the identification and characterization of leafy drugs. **Vein islet and vein termination number** are another simple technique for distinguishing fragmentary specimens at specific levels. It is used as the distinguishing character for the leaf of the same species or different one (Table 1). The ash content of the crude drug is generally taken to be the residue remaining after incineration. If usually represents the inorganic

salts naturally occurring in the drug and adhering to it, but it may also involve the inorganic matter added for the purpose of adulteration. There is a considerable difference within narrow limits in the case of individual drug. Hence ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information related to its adulteration with inorganic matter. The ash or residue yielded by an organic chemical compound is a rule to measure the amount of inorganic matter, which is present as impurity. In most cases the inorganic matter is present in small amounts which are difficult to remove in the purification process and which are not objectionable if only traces are present. Ash values are helpful in determining the quality and purity of the crude drug in especially in powdered from. The **acid insoluble ash** is of more value to detect the earthy matter adhering to the drug. In this way one can obtain evidence of the presence of foreign matter, which likely to occur with root, rhizomes and also in pubescent leaves. **The water soluble ash** is used to detect the presence of matter exhausted by water. Insufficient drying favours spoilage by molds and bacteria and makes possible the enzymatic destruction of activeprinciples.

Extractive values of crude drugs determine the amount of active constituents in a given amount of medicinal plant material when exhausted with solvents. It is employed for that material for which no chemical or biological assay method exist. As mentioned in different official books **[Anonymous,1996 and Horborne JB,1973]** the determination of water-soluble and alcohol soluble extractive, is used as means of evaluating crude drugs which are not readily estimated by other means. The extraction of any crude with a particular solvent yields a solution containing

different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of the drug and solvent used. The use of single solvent can be the means of providing preliminary information on the quality of a particular drug sample. The **water soluble extractive** values play an important role for the evaluation of crude drugs. It can be used to indicate poor quality, adulteration with any unwanted material or incorrect processing of the crude drug during the drying, storage etc. The **alcohol soluble extractive** is also indicative for the same purpose as water soluble extractive values (Table-6).

Loss on drying at 105°C is determined as the presence of excess moisture is conductive to the promotion of mold and bacterial growth, and subsequently to deterioration and spoilage of the drug.

THE PHYTOCHEMICAL STUDIES :

The preliminary phytochemical screening reveals the presence of carbohydrates, proteins and amino acids, steroids, phytosterols, mucilage , alkaloids, flavonoids, terpenoids, tannins, saponins, sterols. Fixed oil and volatile oil was found to be absent.

The reaction of drugs in powdered form in ordinary light and with filtered UV light is of importance in several cases by the luminosity in UV light by **fluorescent analysis**. Many flavonoids showed distinctive colours under UV light.

Hence this parameter can also be used as a diagnostic tool for the standardization of herbal drugs for the detection of adulterants in crude drugs (Horborne JB., 1973).

The total flavonoid content was found to be 7.8 mg/g and the total phenolic content was found to be 18.8 mg/g .

Identification of inorganic minerals of the leaves of *C.caudata* by energy dispersive X- ray spectrometer (EDS) showed the presence of C (61.16), O (35.85), Na (0.11), Mg(0.48), Si (0.14), S(0.14), Cl(0.53), K(0.36), Ca(1.05), Zn(0.22).

HPTLC analysis of EECCL was performed and the presence of E and Z guggulsterones and also 17, 20 dihydroguggulsterone were identified and quantified as 0.051 % w/w. Previous studies on composition of EECCL are the same mentioned above.

PHARMACOLOGICAL STUDIES:

- ✓ Previous acute toxicity study revealed that mortality was not observed by the dose of 2 g/kg body weight of *C.caudata* leaves in the oral toxicity studies. From the results the efficiency test drug doses of 0.2,0.4g/kg body weight were chosen for the efficiency studies.(Girija P *et.al*,2017)

WHOLE EMBRYO CULTURE TOXICITY STUDY

Effect of EECCL on the developmental stages of Zf embryo was carried out. The eggs were cultured in the embryonic medium. The maximal acceptable toxicant concentration (MATC) was calculated by scoring the malformation. 1 % DMSO and podophyllotoxin (0.010µg/ml) were used as control and standard toxin. No malformations or incidence of mortality was observed up to the 0.5 – 1µl/ml concentration level (score 0). But medium to strong edema, eye malformation, bent tail, weak undulated notochord and twisted notochord were observed from 1 to 2 µl/ml concentration up to 80 hpf. No mortality was observed in this concentration level. Total mortality was observed in the standard podophyllotoxin at 0.01µg/ml concentration (score40).

ZEBRA FISH LARVAL TOXICITY STUDY

Larval toxicity study was carried out on zf larvae of 5 dpf cultured in E3 embryonic medium. Five larvae per group was taken and treated with 0.5, 0.75, 1 and 2 µl was taken as a test trail and mortality percentage was calculated.

From the experiment it was observed that there was no mortality in 0.5 and 0.75µl/ml concentrations. But 5% and 10% mortality was observed at 1 and 2 µl/ml concentrations respectively. No mortality was observed in the control. 100% mortality was observed in the standard podophyllotoxin at 0.5 µl/ml concentration.

EFFECT OF EECCL ON DOXORUBICINORUBICIN INDUCED CARDIO-TOXICITY ON ZEBRAFISH MODEL

Zebrafish embryos 1 dpf were treated with DMSO(vehicle control), doxorubicin, doxorubicin + EECCL(0.5 ng, 1ng, 1.5ng) and doxorubicin + Metaprolol for 2 days and % of Fractional shortening was found to be 54%, 20%, 30%, 41%, 50% and 52% respectively. The fig 6 shows the effect of EECCL to overcome doxorubicin induced decreases in cardiac contraction and circulation is dose dependant. From the measurement of fractional shortening, the suppressive effect of EECCL on doxorubicin induced cardiotoxicity was proved. Various clinical parameters like cardiac contractility, heartbeat, pericardial edema and blood flow in tail blood vessels were analysed using high speed video microscopy and found to be normal in test and standard. The results were found to be statistically significant and $p < 0.001$.

Previous investigation revealed that E-guggulsterone inhibited platelet aggregation and protected against myocardial ischemia induced by iso-

preternol , further suggested as this activity may be due to antioxidant property (Chander R *et al*., 2003). In another study it was concluded that EECCL has significant antihyperlipidemic properties and can be used in the treatment of vascular disorders including cardio vascular disorders like atherosclerosis . The potential activity of the extracts can be attributed to the presence of antioxidant principles such as phenolic compounds which were reported from the plant (Kodali G and Seru G 2013).

Before clinical use of EECCL identified in our screen, further experiments are needed to test different routes of administration, document any long – term toxicities, and confirm efficacy in preventing the development of cardiomyopathy**.** More detailed characterization of the mechanism mediated cardioprotection may facilitate the role of the pathway screening performed in Zf embryos to identify novel therapies for doxorubicin induced cardiotoxicity.

CONCLUSION

CHAPTER VII

CONCLUSION

The present investigation highlights the pharmacognostical, phytochemical studies of the leaves of *Commiphora caudata* (Wt & Arn) Engl. (Family:Burseraceae) and *Invivo* cardioprotective potential of its ethanolic extract on doxorubicin induced cardiotoxicity in zebrafish larvae model.

It is commonly called as "Hill mango"in English and "Kiluvai" in Tamil, widely cheap and easily available plant. Ethnomedical information revealed that it is used in various ailments for long period of time. The tremendous economic potentiality of this cash crop remains neglected by the scientists, technologists, physician, traders, administrators, policy makers, farmers etc.

The morphological evaluation showed the adherence of general characters to the family. Leaf is dorsiventral in nature.

Detailed microscopical characters of the leaves showed the presence of the mucilage cells of the midrib. Palisade tissue is not continuous over the midrib region. The ground tissue is made up of closely arranged round to oval parenchyma cells. T.S. of Lamina showed Adaxial epidermal cells are angular, straight and thick walled covered by a thick cuticle, mesophyll is differentiated in to palisade tissue occur beneath the epidermis as single layered, columnar and closely arranged cells and lobed aerenchymatous spongy parenchyma cells.

A noteworthy feature is the presence of secretory cavities lined with epithelium cells of the leaf and one larger median bundle and one smaller adaxial accessory bundle. Secretory cavities occurs below the phloem region. Stomata: Stomata are actinocytic.

Transverse section of petiole is nearly circular in outline and epidermis is covered by a thick cuticle. The inner region is composed of ring of vascular bundle to 12 layers of parenchyma cells. Secretory canals situated below the epidermis in the cortical region.

Scanning Electron Microscopic (SEM) observation SEM study of leaf provides detailed surface information and it showed the presence of few unicellular trichomes.

Quantitative parameters, Vein islet and termination numbers, stomatal number, stomatal index, palisade ratio, loss on drying, ash values, extractive values were determined and presented.

Preliminary phytochemical screening showed the presence of carbohydrates, proteins and amino acids, flavonoids, saponins, tannins, terpenoids, phytosterol, mucilage and absence of alkaloids,volatile oil, fixed oil.

Quantitative inorganic elemental analysis by Energy dispersive X-Ray analysis (EDS) showed the presence of C (61.16), O (35.85), Na (0.11), Mg (0.48), Si (0.14), S (0.10), Cl (0.53),K (0.36), Ca(1.05) and Zn (0.22).

The pharmacologically important phytoconstituents polyphenols as total phenolic 7.8mg/g content and flavonoids 18.8 mg/g were determined.

The TLC and HPTLC profile of EECCL was presented. It showed the presence of some pharmacologically active important component is E -Guggulsterones as 0.051%w/w.

The **3R's** ethical principle (**R**eduction, **R**efinement and**R**eplacement) was implemented that help to minimize harms to vertebrate animals used in science.

In our investigation we used Zebrafish larvae, which is emerging novel preclinical *in vivo* model that support rapid decision making in the early phases of drug discovery process. It is amendable to medium to high throughput screening (HTS) because of numerous advantages. The properties of the genome of zf have established it as an excellent model system that is relevant to studies of human diseases.

We performed preliminary toxicological studies of EECCL on the embryo and larvae of zebrafish and found no pronounced retardation in Zf embryo development when exposed to normal concentration (0.5 to 2 ng/ml) which showed that EECCL of leaf would propose no hazard to early life stages of *Danio rerio* but standard toxin podophyllotoxin showed 100% mortality at 0.01 µg/ml.

Larval toxicity was carried out on Zf larvae of 5 dpf, it was observed that there was no mortality in 0.5µg and 0.75 µg/ml concentrations. 5, 10, 100% mortalities were observed in 1, 2 ng/ml of EECCL and 0.5 µg/ml of podophyllotoxin respectively. (Previous investigation on acute toxicological study using EECCL showed no mortality up to 2g/kg in rats.)

EECCL prevented the overt morphological effects of doxorubicin on the heart including ventricular collapse and pericardial edema. Further it completely rescued cardiac contractility as measured by fractional shortening and potently protected the heart from toxic effects of doxorubicin dose dependently.

This may be due to the presence of gugulsterone which possesses cardioprotective activity,

This result is supporting the previous report of protection of EECCL in cardiovascular diseases like atherosclerosis

The leaves of *C.caudata* may be further investigated for the development as novel nontoxic preventive/treatment interventions for life threatening cardiovascular diseases. But to confirm our findings further investigations of these effects to mammalian model is necessary. Further pharmacokinetic studies are also required to understand the post metabolism ingredients along with the clinical efficacy and safety in human.

E-Guggulsterone is the bioactive constituents of EECCL and are endowed with immense pharmacological value. These conclusions could open a new window on the use of this plant in Ayurveda. This review clearly authenticates the Sanskrit definition of the term “guggul” which means one that protects against diseases. It is superbly reflected and proved by the diverse medicinal uses of this herbal drug.

REFERENCES

REFERENCES

1. Aejitha S, Kasthuri P K, Geethamani P, 2015. **"C**omparison of the corrosion inhibition efficiencies of mild steel in different acidic mediums using C*ommiphora caudata* plant extract**".** *IJATES;* **3(5***)* **:** 75-85**.**

2. Agarwal S S and Paridhavi. 2007**,** 'Herbal Drug Technology'**,** *1stedn Universities Press (India) Private Ltd* **,** *Hyderabad'***.**

3. Akhade MS**,** Agrawala PA and Laddhaa KS**,** 2017. **"**Quantification of steroids and triterpenoids from Indian medicinal plants by HPLC**".** *IJAPBR;* **2(3):** 28-32

4. .Al-HarbiM.M **,** Qureshi S **,** Raza M **,** Ahmed M M **,** Afzal M**,** Shah A H **,** *1997.* **"**Gastric antiulcer and cytoprotective effect of *Commiphora molmol* in rats**" .** *J Ethnopharmacol* **55 :**141-150.

5. Alpana Kulhari **,** Arun Sheorayan **,** Ashok Chaudhury **,** Susheel Sarkar **,** Rajwant K. Kalia . 2015**, "** Quantitative determination of guggulsterone in existing natural populations of *Commiphora wightii (Arn.) Bhandari* for identification of germplasm having higher guggulsterone content **".** *Physiol Mol Biol Plants ;* **21(1):** 71–81. DOI 10.1007/s12298-014-0271-1

6. Annu W**,** Latha P G**,** Shaji J**,** Anuja G I**,** Suja S R**,** Sini S**,** Shyamal S**,** Shine V J**,** Shika P**,** Rajasekharan S**,** 2010**. "**Anti inflammatory**,** analgesic and anti-lipid peroxidation studies on the leaves of *Commiphora caudata (Wt & Arn)* Engl" **.** *Ind J Nat Prod Resour ;* **Vol 1:** 44-48.

7. Anonymous.1996, Indian Pharmacopoeia', **Vol II**, *Ministry of Health and Family Welfare, New Delhi*, **A-53:** 54, 89.

8. Anonymous ,1998. **'**Quality control Methods for Medicinal Plant Materials**'**, *WHO* **,** 28-35.

9. Anonymous, 2001. "The Ayurvedic Pharmacopoeia of India", Part I, I[st] edn, Government of India, Ministry of Health and Family Welfare, Indian Systems. **1**, 140-145.

10. Anonymous , **"**www.eol.org**"**, dated 03.02.2018

11. Anonymous**,** www.pitchandikulam-herbarium.org / contents/ medicinal. php?id=83 dated 1.12.2017

12. Ashish Kumar, Jnanesha A C,2016. **"**Conservation of Rare and Endangered Plant Species for Medicinal Use**" .** *IJSR ;* **5(12):** 1370-1372.

13. Balasubramaniam Balamurugan **,** Pandiyan Rajesh **,** Palanisamy Selvamani **,** Subbiah Latha **,** 2010**. "** Hepatoprotective activity on *Commiphora* species. And its polyherbal formulation **".** *Int J Biol Med Res ;* **1(3):** 30-35.

13. Balasundar S**,** Ravi rao S**,** Subramanya P**,** 2016 **. "**Anti inflammatory activity of folklore medicinal plant *Commiphora caudata (Wt & Arn)* **".** *Int. J. Res. Ayurveda pharm ;* **7 (supple3):**102-106**.**

14. Baranitharan M and Dhanasekaran S., 2014. **"**Mosquito larvicidal properties of commiphora caudata (Wight & Arn.) (Bursaceae) against *Aedes aegypti* (Linn.), *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say)**" .** *Int.J.Curr.Microbiol.App.Sci ;* **3(6) :** 262-268.

15. Barros T.P**,** Alderton W.K**,** Reynolds H.M**,** Roach A.G**,** Bergams S **,** 2008**. "**Zebrafish: an emerging technology for in vivo pharmacological assessment to identify potential safety liabilities in early drug discovery**".** *B.J.Pharmacol. ;* **154:** 1400-1413.

16. Bass A, Kinter L & Williams P , 2004. **"**Origins, practices and future of safety pharmacology**". ***J Pharmacol Tox met ;* **49:**145 –151.

17. Bent S. 2008,"Herbal Medicine in the United States: Review of Efficacy, Safety, and Regulation**",***J Gen Intern Med* Jun; **23(6):**854–859.

18. Binu Thomas, Rajendran Arumugam, Aravindhan Veerasamy , Sivalingam Ramamoorthy , 2014. **"**Ethnomedicinal plants used for the treatment of cuts and wounds by Kuruma tribes, Wayanadu districts of Kerala, India **".** *Asian Pac J Trop Biomed;* **4(Suppl 1):** S 488-S 491 , doi: 10.12980 / APJTB.4. 2014B571 2014.

19. Bucher J.R, 2002, **"** The National Toxicological Program Rodent Bioassay: designs, interpretations, and scientific contributions **".** *Ann. N.Y. Acad. Sci.;* **982:** 198 –207.

20. Chang CC , Yang M H and Chern J C, 2002. **"**Estimation of total flavonoid content in propolis by two complementary colorimetric method**".** *J Food Drug Analysis;* **10(3):** 178-182.

21. Chagas. V.T, Franca. L.M, Malik. S, Paes. A, 2015 "A prominent source of bioactive molecules against cardiometabolic diseases", **Vol. 6,** Front. pharmacol. Doi. 10.3389 / f phar. 2015.00259.

22. Chaudhri R D , 1999. **'***Herbal Drug Industry***'** Eastern publishers**',** New Delhi**.** 489- 501

23. Claude J R & Claude N, 2004. **"**Safety Pharmacology: An essential interface of pharmacology and toxicology in the non-clinical assessment of new pharmaceuticals**" .** *Toxicology letters;* **151:** 25 – 28.

24. Deepa V S , Suresh Kumar P , Latha S, Selvamani P, Srinivasan S , 2009. "Antioxidant studies on the ethanolic extract of *Commiphora* spp.". *Afr. J. Biotechnol;* **8 (8):** 1630-1636.

25. Dooley K, Zon LI, 2000. "Zebrafish: A model system for the study of human disease". *curr opin genet dev;* **10(3) :** 252-256.

26. Ganesan S, Ramar pandi N and Banumathy N (2007–2008). "Ethnomedicinal Survey of Alagarkoil Hills (Reserved forest) , TamilNadu , India ". *e J Ind Med;* **1:** 1–18.

27. Ganesan S, Venkatesan G and Banumathy N, 2006. "Medicinal plants used by ethnic group thottianaickans of semmalai hills (reserved forest), Tiruchirappalli district , Tamilnadu ". *.Ind J Trad knowl;* **5(2):** 245-252.

28. Geetha Kodali and Ganapaty Seru, 2013. "Antihyperlipidemic activity of *commiphora caudata* leaves in atherogenic diet induced rats". *IJBPR;* **4(4):** 250-255.

29. Geetha Kodali, Sreekanth Kakarla and Ganapaty Seru, 2014. "Screening of Crude Plant Extracts for anti-adipogenesis activity in 3T3-L1 cells". *J of Pharm Res;* **8(1):** 81-86.

30. Girija P, Umasankar K, Venkateshwar Rao J, Sharvana Bhava B S, Venkateshwarlu E, 2014. "Anti-arthritic activity of ethanolic extract from the leaves of Commiphora caudata (Linn.) in complete Freund's adjuvant-induced arthritic rats". *Nig J Exp Clinic Biosci;* **2(1):** DOI:10.4103/2348-0149.135729

31. Goran Dave, Birthe Damgaard, Magne Grande ,Jarl Erik Martelin,Bjorn Rosander,Tomas Viktor, 1987 . "Ring test of an embryo-larval toxicity test with zebrafish (brachudanio rerio) using chromium and zing as toxicants".

Env Tox Chem; **6:** 61-71.

32. Gouthamchandra K**,** Mahmood R and Manjunatha H **,** 2010**. "**Free radical scavenging antioxidant enzymes and from *Clerodendrum infortunatum* L**".**
33. Graham Dellaire **,** Kumar S. Sarkar **,** Matthew Dai **,** Howard H. Chen **,** David E. Sosnovik **,** Jordan T. Shin **,** Daniel A. Haber **,** Jason N. Berman **,** Wei Chao **,** Randall T. Peterson , 2015**. "**Visnagin protects against doxorubicin-induced cardiomyopathy through modulation of mitochondrial malate dehydrogenase**" . 6 (266 266ra170).**
34. Gunasekaran M & Balasubramanian P , 2012**.** Ethnomedicinal uses of Sthalavrikshas (temple trees) in Tamil Nadu, southern India**" .** *Ethnobot Res. Apps;* **10(1547);**253-268.
35. Harborne JB 1973**, "**Phytochemical Analysis- A guide to modern techniques of plant analysis**",** London**.**
36. Heywood VH. 1971, 'The Systemic Association, Scanning Electron Microscopy, Systemics and evolutionary applications', Proceedings of an International Symposium held at the Department of Botany, University of Reading, Academic Press, London. **4:** 1 -16.
37. Hisaoka K K and Battle H I**,** 1958**. "**The normal developmental stages of the zebrafish**,** *brachydanio reiro* (Hamilton – Buchanan*)***".** *J of Morphology;* **102*:*** 311 – 328*.*
38. Iyengar M A **,** Nayak S G K **,** 1994**. "***Pharmacognosy Lab Manual"***,** Manipal press**,** Manipal **,** 78-87.
39. Jan M Spitsbergen and Michael L Kent **,** 2003 **. "**The State of the Art of the Zebrafish Model for Toxicology and Toxicologic Pathology Research—Advantages and Current Limitations**".** *Toxicologic Patho;* **31(Suppl.):** *62–87***.** DOI: 10.1080/01926230390174959.

40. Jaya Sankar Reddy V, Deval Rao G , Rajya Lakshmi G , 2014. "A review on anti-arthritic activity of some medicinal plants". *JGTPS;* **5(4):** *2061-2073.*

41. Johansan, DA, 1940, "Plant micro technique", *McGraw Hill Book Co;* **27(93):** 126 – 154.

42. Johnson Gritto M , Nandagopalan V , Doss A , Lakshmi Prabha A , 2015. "Phenological Behaviour of Some Tree Species of Pachamalai, Tamilnadu India". *J. Adv Bot. Zoo. ;* **3(4).**

43. Kannan Chandrasekar , Varadha Rajan , Chellappan David Raj , Narayanasamy Lakshmanan Gowrishankar , 2009. "Antiulcerogenic activity of *Commiphora caudata* bark extract against ethanol-induced gastric ulcer in rats". *J Pharm. Res ;* **2(4):** 701-703.

44. Kiran Sharma , Amit Lather , Vikash Kumar , Vaibhav Tyagi , Neetu Lather , "A study on standardization of triphala sodhita guggulu (*commiphora mukul* hook)" . *WJPR ;* **3(2):**2156-2162.

45. Kokate C K, Purohit A P and Gokhale S B , 2005. '*Pharmacognosy'*, ***32th*****edition,** Nirali Praksahan. 99 , 100 , 111 , 112.

46. Latha S , Selvamani P , Pal T K , Gupta J K , Ghosh L K , 2006 . " Pharma cognostical studies on leaves of *Commiphora caudata (Wt & Arn) Engl.* ". *Anc. Sci. Life* , **XXVI (1&2):** 19-25.

47. Lingassamy Arul Pragasan & Narayanaswamy Parthasarathy , 2009 . " Lists of species, Angiosperms, tree species in tropical forests of southern Eastern Ghats, Tamil Nadu, India".**5(3):** 542–569.

48. Liu Y , Asnani A , Zou L , Bentley V. L , Yu M , Wang Y , 2015, "Visnagin protects against doxorubicin – induced Cardiomyopathy through

modulation of mitochondrial malate dehydrogenase", *www. Science Translational Medicine. Org* **6 (266).**

49. Mabry TJ, Markham KR and Thomas MB. 1970, "The systemic identification of flavonoids", Springer verlay , Newyork.
50. McGrath, P and Chun – Qi Li, 2008. "Zebrafish a preclinical model in assessing drug induced toxicity" *Rev drug dis today*, **13(9).**
51. Meera Sumanth and Yashashwini Y. , 2016. " Evaluation of learning and memory enhancing activity of *commiphora caudata* leaves in rats ". *WJPR ;* **5(5):** 1223-1236.
52. Muralidharan R and Narasimhan D, 2012 . "Plants used for topical application from Gingee hills, Tamil Nadu, India". *Curr Bot* , **3(4):** 49-52.
53. Nisha Pothan and Jyoti Harindran, 2014 . "In Vitro Hepatoprotective Activity of Ethanolic Extract of *Commiphora Caudata (Wight&Arn) Engl* on the BRL- 3A Cell Line" *. IAJPR ;* **4(03).**
54. O' Brien, TP, Feder, N and MC Cull, ME, 1964, "Polychromatic staining of plant cell walls by toluidine Blue – O' *Protoplasma*, **59**: 364 –373.
55. Ojha. S, Taee H.A, Goyal S, Mahajan U.B, Patil C.R, 2016. "Cardioprotective potentials of plant derived small molecules against Doxorubicin associated cardio toxicity". *Oxidative. Med. Cellular longevity.* **1-19:** http://dx.doi.org/10.115 5/ 2016/5724973.
56. Poonia Priyanka , Mittal Sanjeev K. , Gupta Vivek Kumar , Singh Jitender , Sweety , 2014. "Gum Guggul: An Ayurvedic Boom". *IJPPR ;* **6(2):** 347-354

57. Pramod Kumar Sairka , Anjana Sharma , Shukla N P . "Estimation of guggulsterone E and Z in the Guggul based commercial formulations using High Performance Thin Layer Chromatography (HPTLC) " .

58. Prasad M N V , Padmalatha K , Jayaram K, Raju N L , Jaime A.Teixeira da Silva, "Medicinal plants from Deccan Ecoregion , India : Traditional knowledge , Ethnopharmacology , cultivation , utilization , Conservation and Biotechnology- Oppurtunities and Impediments ". Medicinal and aromatic plant science and biotechnology © 2007 Global science books.

59. Prasanna Anjaneya Reddy L , Narasimha Reddy B , Bhakshu M D L , Venkata Ratnam K Veeranjaneya Reddy L , 2015. "Chemical Composition, Antimicrobial and Antioxidant Activities of Essential Oils from Leaves and Fruits of *Commiphora caudata Engl." .* Int J of *Pharmacog and Phytochem Res;* **7(1):** 38-44 .

60. Prerna Sarup , Suman Bala and Sunil Kamboj , 2015 . Article ID 138039. "Pharmacology and Phytochemistry of Oleo-Gum Resin of *Commiphora wightii* (Guggulu)". 1-14. http://dx.doi.org/10.1155/2015/138039.

61. Purvis M J , Collier D C and Wallis D , 1966. "Laboratory techniques in Botany", Butterworth & Co Ltd . 2nd edn, 82-169.

62. Ramesh Chander, Farhan Rizvi , Khanna A K and Ram Pratap , 2003. "Cardioprotective activity of synthetic guggulsterone (E and Z-isomers) in isoproterenol induced myocardial ischaemia in rats:A comparative study " . *Ind J Clinic Biochem ;* **18(2):** 71-79.

63. Ramesh Kumari, Arumugam Meyyappan, Debkumar Nandia, Bikram Keshari Agrawalla, Avik Acharya Chowdhury, Palanisamy Selvamani, Subbaiah Latha, Venkatachalam Sesha Giri, Joydeep Mukherjee, Santu

Bandyopadhyay and Parasuraman Jaisankar**,** 2011**. "**Antioxidant and antibacterial activities of bark extracts from *Commiphora berryi* and *Commiphora caudata* **" .** *Nat Prod Res;* **25(15):** 1454–1462.

64. Rashad A Abdul-Ghani **,** Naguiba Loutfy **,** Azza Hassan **,** 2009 **. "** Myrrh and trematodoses in Egypt**, "**An overview of safety**,** efficacy and effectiveness profiles**" ,** *Parasitology Int ;* **58:** 210–214.
65. Ravikumar**,** 2015 **. "**Characterization of green synthesized silver nanoparticle of *Commiphora caudata* bark **". 3(1):** 64-65 .
66. River C **,** 2014**. "**Zebrafish: A Powerful alternative model for developmental toxicity testing**",** www.criver.com.
67. Sass, JE, 1940, "Elements of botanical microtechnique" McGraw Hill Book Co, New York, 222.
68. Selvamani P **,** Latha S **,** Dhivya P S **,** 2013**. "** Anti-inflammatory activity of extracts of *commiphora* species and its polyherbal formulation **" .** *Int J of Phytopharmacol;* **4(5):** 288-292.
69. Savithramma N**,** Linga Rao M and Ankanna S ***,*** 2011**. "**Phytochemical screening of traditional medicinal plants**" . .***J Pharm Res***, 4(10):** 3414-3416.
70. Shaik Azeem Taj **,** Balakumar B S **,** 2014**. "**Studies on Phytochemical and Antioxidant potential of certain medicinal plants from Udayagiri Hill Range**,** Andhra Pradesh **,** India**" .** *Sch. Acad. J. Biosci;* **2(7):** 432-436.
71. Shin. J.T, Pomerantsev. E.V, Mably J.D, and MacRae. C.A, 2010, "High-resolution cardiovascular function confirms functional orthology of myocardial contractility pathways in zebrafish", *Physiol Genomics* **42:** 300 – 309, doi: 10. 1152 / physiolgenomics.00206.2009.

72. Siddique M A , Mujeeb M , Najim A K and Akram M , 2010. "Evaluation of antioxidant activity quantitative estimation of phenol and flavonoid in different parts of aeglemarmelos **".** *Afr J Plant Sci;* **4(1):**1-5.

73. Singleto V L, Orthofer R, Raventos R M L, 1999. "Analysis of total phenols and other oxidation substrates and anti-oxidant by folin ciocalteu reagent **".** *Methods InEnzymology ;* **299:** 152-178.

74. Sivakumar T, Kannana K and Manavalan R , 2010. "**Blood** glucose lowering potential of *Commiphora caudata (Wight & Arn) Engl.* in alloxan induced diabetic rats" *. Int. J. Chem. Sci.:* **8(1):** 602-608.

75. Srinivasa Reddy C H , Ammani K , Rose Mary T and Nitya Jeeva Prada P, 2017 . "Efficacy of methanolic extract of *commiphora caudata (Wt & Arn.) Engl.* bark on various blood parameters in alloxan induced diabetic rats". **6(1):** 4652-4660.

76. Stéphane Berghmans , Paul Butler , Paul Goldsmith , Gareth Waldron , Iain Gardner , Zoe Golder , Frances M. Richards , Gillian Kimber , Alan Roach , Wendy Alderton , Angeleen Fleming , 2008. "Zebrafish based assays for the assessment of cardiac, visual and gut function — potential safety screens for early drug discovery". *J Pharmacol Tox Meth ;* **58:** 59–68.

77. Sundaram Dhivya, Palanisamy Selvamani , Subbiah Latha , 2017. "*Commiphora berryi* ameliorating effect on scopolamine-induced memory impairments in mice". .*Int. Res. J. Pharm ;* **8(5).**

78. Suter W, 2006 . "Predictive value of in vitro safety studies*". Curr opinion in chem. Bio;* **10:** 362 –366.

79. Syed Ghulam Musharraf, Naveed Iqbal , Umair Gulzar, Azhar Ali , Muhammad Iqbal Choudhary , Atta-ur-Rahman , 2011. **"**Effective separation and analysis of E and Z-guggulsterones in Commiphora mukul resin , guggulipid and their pharmaceutical product by high performance thin-layer chromatography- densitometric method**". ***J Pharm Biomed Ana.;* **56:** 240– 245.

80. Tao Shen , Guo-HuiLi , Xiao-NingWang , Hong-Xiang Lou , 2012. **"**The genus *Commiphora:* A review of its traditional uses, phytochemistry and pharmacology**"**, *J Ethnopharmacol;* **142 :** 319–330 .

81. Venkatachalapathi A., Tamilselvi S.S. and Paulsamy S , 2016. **"**Ethnobotanical knowledge of irula tribal community of walayar valley , southern western ghats , India**" .** *IJRAMR;* **3(4):** 1379-1392**.**

82. Venugobal.J , Anandalakshmi K , **"**Green Synthesis of Silver Nanoparticles Using *Commiphora caudata* Leaves Extract and the Study of Bactericidal Efficiency**". ***J Clust Sci;* DOI 10.1007/s10876-016-1032-9**.**

83. Verma. S, Singh.S.P,2008, "Current and future status of herbal medicines". *Veterinary World*, Vol.**1(11):** 347-350.

84. Vigneswaran , Nanthakumaran T , Shanthasubitha S and Shanmugapriya B, 2016.**"** Evaluation of tannin contents of some indigenous plant species in kolli hills, anchetty and sathyamangalam forests**",** *IAJMR;* **2(1):** *494 – 505*

85. Vikneshwaran D , Viji M and Raja Lakshmi K, 2008. **"**Ethnomedicinal plants survey and documentation related to paliyar community**".** *Ethnobot Leaflets;* **12:** *1108-1115.*

86. Wallis T E.1953, 'Practical Pharmacognosy', **6th edn,** J& A Churchill Ltd, London, *170-175.*

87. Wallis T E , 1965. Analytical Microscopy,**3rd edn,** J&A Churchill Ltd, London, *180, 183, 184.*

88. Yan Liu , Aarti Asnani , Lin Zou , Victoria L Bentley , Min Yu , You Wang ,