173922

PHARMACOGNOSTIC STUDIES AND ASSESSMENT OF ANTIINFLAMMATORY, ANTINOCICEPTIVE AND ANTIOXIDANT POTENTIAL OF 'ELLOOTI' (*Pterospermum rubiginosum* B. Heyne ex G. Don)

Submitted
by
SHAHASAD SALAM
(2011-09-120)

THESIS

Submitted in partial fulfilment of the
requirement for the degree of

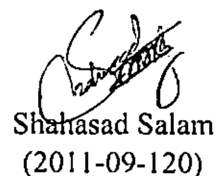
MASTER OF SCIENCE (INTEGRATED) IN BIOTECHNOLOGY

Faculty of Agriculture
Kerala Agricultural University, Thrissur

B.Sc. - M.Sc. (INTEGRATED) BIOTECHNOLOGY
DEPARTMENT OF PLANT BIOTECHNOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM - 695 522
KERALA, INDIA
2016

DECLARATION

I hereby declare that this thesis entitled **Pharmacognostic Studies and Assessment of Antiinflammatory, Antinociceptive, and Antioxidant Potential of 'Ellooti' (*Pterospermum rubiginosum* B. Heyne ex G. Don)** is a bona fide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any diploma, degree, associate ship, fellowship or other similar title, of any other university or society.



Vellayani
19-12-2016

Shahasad Salam
(2011-09-120)

JNTBGRI

Jawaharlal Nehru Tropical Botanic Garden and Research Institute
(An Institute of Kerala State Council for Science, Technology and Environment, Govt. of Kerala, National Centre of Excellence, Govt. of India)
Karimancode P. O., Palode, Thiruvananthapuram - 695 562, Kerala, India.

CERTIFICATE

Certified that this thesis entitled "**Pharmacognostic Studies and Assessment of Antiinflammatory, Antinociceptive, and Antioxidant Potential of 'Ellooti' (*Pterospermum rubiginosum* B. Heyne ex G. Don)**" is a record of research work done by Mr. Shahasad Salam (2011-09-120) under my guidance and supervision and this is not previously formed the basis for the award of any diploma, degree, fellowship or associateship to him.

Palode

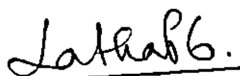
19-12-2016

Dr. P. G. Latha
Former Director
JNTBGRI, Palode
Thiruvananthapuram - 695 562

91(0)472 2869226; Fax : +91(0)472 2869646; e-mail : director_tbgri@rediffmail.com; web site : www.res.in

CERTIFICATE

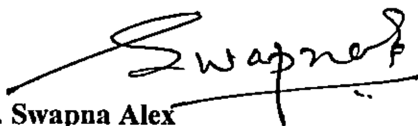
We, the undersigned members of the advisory committee of Mr. Shahasad Salam (2011-09-120), a candidate for the degree of **B.Sc.-M.Sc. (Integrated) Biotechnology**, agree that this thesis entitled **"Pharmacognostic Studies and Assessment of Antiinflammatory, Antinociceptive, and Antioxidant Potential of 'Ellooti' (Pterospermum rubiginosum B. Heyne ex G. Don)"** may be submitted by **Mr. Shahasad Salam** in partial fulfilment of the requirement for the degree.



Dr. P. G. Latha
Former Director
JNTBGRI, Palode
Thiruvananthapuram - 695 562

Dr. B. R. Reghunath
Former Dean, Professor and Head
Department of Plant Biotechnology
College of Agriculture, Vellayani,
Thiruvananthapuram – 695 522

Dr. Suja S. R.
Scientist C and Head in charge
Ethno medicine and
Ethno pharmacology Division
JNTBGRI, Palode
Thiruvananthapuram - 695 562



Dr. Swapna Alex
Professor and Head
Dept. of Plant Biotechnology
College of Agriculture, Vellayani
Thiruvananthapuram - 695 522

ACKNOWLEDGEMENT

First and foremost, I must acknowledge and thank the God Almighty for blessing, protecting and guiding me throughout this period. This thesis is the end of my journey in obtaining my M. Sc. degree. I have not travelled in a vacuum in this journey. This thesis has been kept on track and been seen through to completion with the support and encouragement of numerous people including my guide, my parents, and my friends. At the end of my thesis I would like to thank all those people who made this thesis possible and an unforgettable experience for me.

It is with my heartfelt feelings, I wish to express my deep sense of gratitude and sincere thanks to Dr. P. G. Latha, Director, Jawaharlal Nehru Tropical Botanical Garden and Research Institute, for her valuable guidance, sincere help, patience, support, encouragement throughout the course of study and for giving me an opportunity to work at JNTBGRI for my thesis work.

My sincere gratitude to Dr. S. R. Suja, Scientist C, and Head of department (I/C) Division of Ethnomedicine and Ethno pharmacology, Jawaharlal Nehru Tropical Botanical Garden and Research Institute. I express my indebtedness to ma'am for her guidance, incessant inspiration, untiring attention and patience towards my lab experiments while devoting her precious time in the midst of her busy schedule.

I am thankful to the members of the advisory committee, Dr. B. R. Reghunath, Professor and Head, Department of Plant Biotechnology, College of Agriculture, Vellayani, Dr. Swapna Alex, Course Director, Integrated Biotechnology Course, for their valuable counselling and constructive suggestions that were much helpful throughout my research progress.

I am indebted to all my teachers for giving me the confidence to walk ahead. I am thankful to Prof. Dr. Lekha Sreekandan, Prof Dr. K.B. Sony, Dr. Swapna Alex (Couse Director), Dr Deepa S. Nair (Asst. Prof) and Dr. Geetha Ramesh (Asst. Prof) for their professional advice and direction throughout my course and research.

I take immense pleasure to express my thanks to every member of EM and EP Division for being there for me always, rendering whatever help I needed during my all experiments and for providing me suggestions and unwavering encouragement throughout my research work.

I express my heartfelt thanks to Dr. L. Rajamony and also Dr. Sverup John former deans of College of Agriculture, Vellayani, for all the facilities provided.

I thankfully remember my friends particularly Meera Mohan who have rendered her helping hands at all times and support during my research work.

I thank all my beloved folks Nisthu, Manu, Krishnaraj, Vipin, Sooraj Geethu S. Nair and Irfa and my seniors Sreekuttan, Edwin, Roshan, Sudheep, Achuth, Anoop, Adil, Athul, Nandu, Arun, Abeesh, Pamitha and Pareek.

I owe this achievement to my dear Vapa, Umma, Chemban and Aeppu for their supports for completing this course and research work and my special thanks to them.

I acknowledge the favour of numerous persons who, though not been individually mentioned here, who have all directly or indirectly contributed to this thesis work.

Vellayani
14-12-2016

SHAHASAD SALAM

DEDICATED TO
ALMIGHTY AND MY
FAMILY

CONTENTS

Sl. No.	TITLE	Page No.
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	5
3	MATERIALS AND METHODS	30
4	RESULTS	46
5	DISCUSSION	64
6	SUMMARY	75
7	REFERENCES	77
8	APPENDICES	88
9	ABSTRACT	90

LIST OF TABLES

Sl. No	Title	Page no
1	Morphological characters of leaf of *Pterospermum rubiginosum*	46
2	Physio chemical parameters of powdered Leaves of *P. rubiginosum.*	49
3	Individual Extractive values of powdered leaves of *P. rubiginosum*	50
4	Fluorescence analysis of leaf powder of *Pterospermum rubiginosum*	51
5	Preliminary phytochemical screening of leaf extract of *P. rubiginosum*	53
6	Determination of total phenolic content in *P. rubiginosum* leaf extract	54
7	Determination of total flavonoid content in *P. rubiginosum* leaf extract	55
8	Effect of L-Ascorbic acid and ethanolic extract of leaves of *P. rubiginosum* on DPPH Radical Scavenging Activity.	56
9	Effect of L-Ascorbic acid and ethanolic leaf extract of *P. rubiginosum* on Nitric Oxide Scavenging Activity.	57
10	Effect of L-Ascorbate and ethanolic leaf extract of *P. rubiginosum* on Hydroxyl radical scavenging activity.	58
11	Cage side observation of acute oral toxicity studies of ethanolic extract of leaves of *P. rubiginosum.*	59

12	Effect of ethanolic leaf extract of P. rubiginosum and aspirin on acetic acid induced writhing in mice.	60
13	Effect of aspirin and ethanolic extract of leaves of P. rubiginosum on Eddy's Hot plate method in mice.	61
14	Effect of Indomethacin and ethanolic leaf extract of P. rubiginosum on carrageenan induced paw oedema in rats.	62
15	Effect of Indomethacin and ethanolic leaf extract of P. rubiginosum on formalin induced paw oedema in rats.	63

LIST OF FIGURES

Sl. No	Title	Between pages
1	The total phenolic content of ethanolic extract of leaf of *P. rubiginosum*	54-55
2	The total flavonoid content of ethanolic extract of leaf of *P. rubiginosum*	54-55
3	DPPH radical scavenging activity of *Pterospermum rubiginosum*	56-57
4	Nitric oxide radical scavenging activity of *Pterospermum rubiginosum*	56-57
5	Hydroxyl radical scavenging activity of *Pterospermum rubiginosum*	57-58
6	Effect of *Pterospermum rubiginosum* extract in acetic acid induced writhing in mice.	60-61
7	Effect of ethanolic extract of leaves of *Pterospermum rubiginosum* on Eddy's Hot plate method in mice.	60-61
8	Effect of ethanolic extract of leaves of *P. rubiginosum* on Carrageenan Induced Paw Oedema in Rats	62-63
9	Effect of ethanolic extract of leaves of *P. rubiginosum* on of Formalin Induced Paw Oedema in Rats	62-63

LIST OF PLATES

Sl. No.	Title	Between Pages
1	Leaves, branches and whole tree in aerial view of Ellotti (*Pterospermum rubiginosum* B. Heyne ex G. Don).	5-6
2	Global Distribution of *Pterospermum rubiginosum* B. Heyne ex G. Don.	6-7
3	Schematic representation of the development of drugs from medicinal plants	9
4	Different Nociceptors/ free nerve endings, and the fibres carrying pain sensation from the nociceptors to the spinal cord.	21-22
5	Tissue damage and the variety of the substances released from the injured site that activate the nociceptors.	22-23
6	Leaf pattern of the *Pterospermum rubiginosum*. A. Ventral view, B. Dorsal view, C. Stem and D. Aerial view of the tree.	45-46
7	Transverse section of midrib with lamina and petiole of *Pterospermum rubiginosum* leaf	46-47
8	Transverse section of petiole of *P. rubiginosum* leaf	47-48
9	Powder microscopy of *Pterospermum rubiginosum* leaf: (A) Fibers in bundle, (B) stellate covering trichome with thickened wall, (C) Trichome, (D) Fibre, (E)Annular	48-49

	vessels from vascular bundle, (F) Tissue fragment.	
10	Fluorescence analysis of leaf powder of *Pterospermum rubiginosum.*	50-51
11	A. Normal paw and B. Inflammation induced paw.	61-62

LIST OF APPENDICES

Sl. No.	Title	Appendix No.
1	Wagner`s reagent	I
2	Mayer's reagent	II
3	Hager's reagent	III
4	Reagents of DPPH Assay	IV
5	Reagents of Hydroxyl Scavenging Assay	V
6	Griess reagent	VI

LIST OF ABBREHIVATIONS

µg	Microgram
µl	Microlitre
µM	Micromolar
8-OHdG	8-Oxo-2'-Deoxyguanosine
AA	Arachidonic Acid
ACS	American Chemical Society
AGEs	Advanced Glycation End Product
CAT	Catalase
CNS	Centre Nervous System
Conc.	Concentration
COX	Cyclooxygenase
CXCL	Chemokine (C-X-C) Ligand 1 (CXCL1) Protein
DAMPs	Damage-Associated Molecular Patterns
DC	Dendritic Cell
DGLA	Dihomo- γ -Linolenic Acid
DNA	Deoxyribose nucleic acid
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
EFA	Essential Fatty Acid
EPR	Ethanolic Leaf Extract of *Pterospermum rubiginosum*
et al.	And others
Fas	Fas Cell Surface Death Receptor
GC	Gas Chromatography
GI	Gastro Intestinal

GPx	Glutathione Peroxidase
GR	Glutathione Reductase
GSH	Growth Stimulating Hormone
h	Hour
HPLC	High Pressure Liquid Chromatography
HPTLC	High Pressure Thin Layer Chromatography
IC_{50}	Inhibitory Concentration of 50%
IFN	Interferon
IFN γ	Interferon Gamma
IKK	Inhibitor of Kappa B Kinase
IL	Interleukin
IL-16	Interleukin-16
INFα	Interferon A
IR	Infrared
IUCN	International Union for Conservation Of Nature
IκB	Inhibitor of Kappa B
Kg	Kilograms
LD_{50}	Lethal Dose 50%
LP	Lipoprotein
MDA	Muscular Dystrophy Association
mg	Milligram
MHC	Major Histocompatibility Complex
MPs	Medicinal Plants
MyD88	Myeloid Differentiation Primary Response Gene 88

NFκβ	Necrosis Factor Kappa B
NLR's	Leucine Rich Repeats
nm	Nanometre
NO	Nitric Oxide
NOAEL	No-Observed-Adverse-Effect-Level
NSAIDS	Nonsteroidal Anti-Inflammatory Drug
°C	Degree Celsius
OD	Optical density
OECD	Organization for Economic Cooperation and Development
OH	Hydroxide
ORD	Oral Dose
PAMPs	Pathogen-Associated Molecular Pattern
PC	Paper Chromatography
PGs	Prostaglandins
PR	*Pterospermum rubiginosum*
RNA	American Chemical Society
RNS	Reactive Neutral Species
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
TGF β	Tumour Growth Factor
Th17	T Helper 17
TLC	Thin Layer Chromatography
TLR's	Toll-Like Receptors
TNF α	Tumour Necrosis Factor A

UNESCO	United Nations Educational, Scientific and Cultural Organization
UV	Ultraviolet
WHO	World Health Organisation

INTRODUCTION

1. INTRODUCTION

Since from the time of our ancestor itself, they had started the search for new medicine for curing various diseases. they were making new medicine from nature, especially from medicinal plants. The connection between man and his search for drugs in nature has started from long before and the evidence for this was obtained from numerous sources such as knowledge sharing, preserved monuments and written documents. In search of new drugs from medicinal plants, they were forced to eat the unknown plants to find the cure for their sufferings caused by acute or chronic illnesses, wound and injuries, physical discomforts and even terminal illnesses. These struggles against illnesses in man has learned how to track these drugs in root, leaves, barks, fruit bodies, seeds and other parts of the plants (Biljana, 2012).

Plants with therapeutic properties have a great important place in the healing practices and treatment of different diseases. The evidence about the preparation and usage of drugs from plants has been found written on an old Sumerian clay slab obtained from Nagpur, which is about 5000 years old. The clay slab contains twelve recipes for the preparation of drug referring to over 250 different plants such as poppy, henbane, and mandrake (Kelly, 2009).

Traditional medicine refers to the information, knowledge, skills and practices based on the theories, experiences and beliefs of different native cultural groups are used in the maintenance of health, diagnosis, prevention, and improvement or treatment of mental and physical illness among people. Traditional medicine covers a wide variety of therapies and practices which vary from group to group and region to region and it has got no or less side effect when compared to conventional medicine and side effects can be minimized by a dose-dependent manner.

Medicinal plants are the plants containing inherent active compounds which can be used to treat illness or get rid of nociception (Okigbo *et*

al., 2008) so, this has made medicinal plant as an important part of our natural wealth.

Nowadays traditional medicines are taken widely in the developing nations as therapeutic agents for wellbeing of good health has been widely witnessed (UNESCO, 1996). In modern pharmacopeia, 25% of its total drugs which is originated from plants and many other natural sources, synthetic analogs and fabricated prototype compounds from plants are isolated. Interest in therapeutic plants and phytochemicals is a re-emerging health support for maintaining well-being, fitness, and personal health and the bioprospecting of novel drugs from plant-derived (Lucy and Edgar, 1999).

In recent years there is a hike in the reliance on the use of medicinal plants and plant products by the industrialized societies has been drawn attention to the development of drugs, extraction and preparation of chemotherapeutics from traditionally used herbal medicines (UNESCO, 1998). These pieces of knowledge have widely helped in the development of concepts linked to usage of medicinal plants as well as, it has increased the responsiveness and ability of pharmacists, physicians, researchers and scientists to respond to challenges that have emerged with the spreading of professional services in the acceleration of quality of man's life.

Pharmacognosy is one of the important branches of pharmacology. It mainly deals with the study of structural, biological, chemical and physical characteristics of crude drugs and also includes history, cultivation, collection, storage and use of drugs. As well as the exploration for potential natural sources for making new drugs.

Inflammation is the body's instantaneous response to damage to its tissues and cells by harmful biological agents and harmful stimuli such as chemicals, or physical injury. Basically, it is a tissue destroying process, during this process there are many steps are involving such as employment of blood-derived products, such as fluid, leukocytes and plasma proteins into perturbed tissue. Relocation is assisted by the modifications in the native vasculature that lead to

increased rate of vascular penetrability, vasodilation and blood flow. Inflammation is categorized into two acute inflammation and chronic inflammation.

Nociception also known as pain is the processing and encoding of harmful response in the nervous system and it is an ability of a body to sense harm. Pain is categorized into acute and chronic. Anxiety and hyperactivity of the sympathetic nervous system (e.g. Diaphoresis, increased rate of respiration and blood pressure, dilated pupils, tachycardia etc.) are linked to Acute pain. Chronic pain does not involve sympathetic hyperactivity, but it may be associated with sign such as appetite, depressed mood, fatigue and loss of libido (Amir *et al.*, 2009).

An antioxidant is an agent that inhibits oxidation, some of the compounds obtained from natural sources has got the potential to neutralize the oxidant effect of free radicals and other harmful substances produced in our body. Examples of antioxidants are curcumin, alkaloids, ascorbic acid, uric acid, cucurbitacin, glutathione, lipoic acid, β-carotene, selenium etc.

Nonsteroidal anti-inflammatory drugs (NSAIDs) is a class of drugs which decreases pain and oedema by suppressing the formation of prostaglandins, by delaying the activity of cyclooxygenase (COX-1 and COX-2) enzyme. On the other hand, prostaglandins are key intermediaries of several constituents of gastrointestinal mucosal defence, so suppression of synthesis of prostaglandins by NSAIDs which reduces the resistance of the mucosal membrane to injury as well as interfering with repair mechanism. Selective COX-2 inhibitors were thought to be the solution to this problem as it is required that NSAIDs suppress prostaglandin synthesis at sites of inflammation, and not in the gastrointestinal tract. All drugs have a risk of side effects, NSAIDs has too. The selective inhibition of certain proinflammatory mediators significantly has adverse effects on renal and cardiovascular systems, it damages the stomach and intestine due to their acidic nature, as with naproxen, indomethacin, diclofenac, ibuprofen, aspirin, etc. (Cena *et al.*, 2003). Significant side effect profiles of steroidal and NSAID medications can be reduced by switching into drugs from natural sources. This has arisen greater interest in the use of natural compounds, such as phytochemicals, dietary

supplements and herbal medicines, which have been used by our descendants for many centuries to reduce pain and inflammation (Reynold *et al.*, 1995).

Pterospermum rubiginosum B. Heyne ex G. Don (Sterculiaceae) which is an average sized tree generally known as Ellootti and Edinjal in Malayalam and endemic to Western Ghats (Shalini *et al.*, 2013). The plants can be identified by its leaf colour. The upper side of the leaves is green and the bottom part is having a golden colour. It flowers during November to June. Stem bark and leaf of the *P. rubiginosum* is used traditionally for the treatment of bone fracture and inflammation by tribal peoples of Wayanad. The paste of the bark is applied on the fractured area just like plastering and its bark boiled in water is consumed to treat inflammation on the outside and inside respectively. The leaf is used to prepare oil for massaging for treating inflammation and pain relief.

However, no work has been reported till on the following aspects. So the current study is to assess various pharmacognostical parameters such as microscopic, macroscopic, fluorescence, physicochemical and phytochemical studies of the plant and pharmacological properties such as antioxidant, anti-nociceptive and anti-inflammatory properties.

2. REVIEW OF LITERATURE

2.3 *Pterospermum rubiginosum* B. Heyne ex G. Don

Pterospermum is belongs to *Sterculiaceae* family according to the Benthaman and Hooker Classification. The name *Pterospermum* is combination of two Greek words, "Pteron" and "Sperma," meaning "winged seed". Some species of this genus ornamental plants and while others are valued for their timber.

Basic information

Habit: Tree

Medicinal: Yes

Habitat: Evergreen and semi-evergreen forests

Flowering and Fruiting: April -November

Distribution: South and Central Western Ghats

Endemic to: South and Central Western Ghats

Localities: Chimmini, Kallar, Koruthode, Nadugani ghats, Kohala, Mukkali forests, Karamanayar region, Palakkad, Kollam, Idukki, Thiruvananthapuram, Thrissur, Wayanad, Kozhikode, and Kannur.

Morphological information about it features was given in the plate 1.

Systematic position (Bentham and Hooker's Classification)

Kingdom : Plantae

Subkingdom : Tracheobionta

Superdivision : Spermatophyta

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Dilleniidae

Order : Malvales

Family : Sterculiaceae

Genus : *Pterospermum*

Plate 1: Leaves, branches and whole tree in aerial view of Ellotti (*Pterospermum rubiginosum* B. Heyne ex G. Don).

Species : *rubiginosum*

2.3.1 Plant Description

It is an evergreen tree which grows up to 35-45 m high. The thickness of bark is range from 3-6 mm which is brown colour and bark will be flaking off in thin rectangular strips. Branchlets are tawny pubescent and the branches were drooping. Leaves are simple, alternate and the dimensions of the leaves range from 4-8.5 x 2-3 cm. Morphological description of leaves are falcate, apex acuminate, margin entire, base oblique, coriaceous, glabrous above and densely white tomentose beneath, palmate, ribs ranges from 3-5 numbers, lateral nerves ranges from 4-6 pairs, pinnate, slender, prominent, intercostal scalariform, prominent. The petiole is present, its length ranges from 4-6 mm and strong and thick pubescent hairs are seen. Stipules are lateral, filiform in nature, oblique, densely covered with pubescent hairs and caducous. Flowers are bisexual, solitary, axillary and white in colour. Calyx is tubular and cylindrical in shape, brown-hoary outside, splitting into 5 linear lobes and white silky within. Petals are 5, white in colour, slightly smaller than sepals and it is linear-oblong. Staminal column is adnate to the gynophore, bearing 5 groups of 3 stamens each between the staminodes, minutely tubercled towards the tip. The ovary is superior, cylindrical, ridged, tomentose, within the apex of the column, 5-celled and many ovules are present in each cell. The style is hairy at base. Stigma is simple. Fruit is capsule shaped, brown, sub clavate, acutely 5-angled and its dimension ranges from 40-50 x 6-9 mm. Seeds are winged at one end.

2.3.4 Global Distribution

Pterospermum rubiginosum is endemic to the western Ghats and south and central Sahyadris. It mainly found in the localities such as Chimmini, Kallar, Koruthode, Nadugani ghats, Kohala, Mukkali forests, Karamanayar region, Palakkad, Kollam, Idukki, Thiruvananthapuram, Thrissur, Wayanad, Kozhikode, and Kannur (Plate 2).

Plate 2: Global Distribution of *P. rubiginosum*.

2.1 MEDICINAL PLANTS

The plant which possesses therapeutic activity or exerts favourable pharmacological effects on the animals and humans are commonly selected as medicinal plants. It naturally synthesizes and accumulate secondary metabolites in different parts of the plant, like alkaloids, resins, terpenes, flavonoids, saponins, sterols, glycosides, tannins, volatile oils etc. and these secondary metabolites are produced for the survival of plant from environmental stressors. The medicinal plants have been used for the curing of illness and diseases, since from ancient times (IUCN, 2011).

Selection of these medicinal plants is done by humans, devoid of any preceding knowledge and it was largely established on intuition of guesswork or trial and error methods. The curing potential of these plants was often revealed accidently. A combination of these processes has emerged as a considerable body of knowledge of medicinal plants that was transferred from one generation to another through verbally and later, in written form as baked clay tablets,

manuscripts, papyri, parchments, and finally as printed books, pharmacopoeias and other workings. Meanwhile, from ancient times the plants with therapeutic properties have occupied an important niche in the disease treatment practices (Ghani, 1998).

Currently, herbal medicines are becoming new probable choice over the modern synthetic drugs available in the market and it is because of, they are showing show minimum or no side effects or it can be reduced through dose-depended manner was considered to be harmless. Usually, contains dried or fresh plant parts in the herbal preparations. When we using certain parts of the plants for preparing drugs, there are some important aspect that also looked during the preparation of herbal products is the safety and efficacy (Modi *et al.*, 2010).

Medicinal plants are having a great significance in the healthiness of individuals and populations. The therapeutic properties of these plants lies in some of the phytochemical constituents that can induce a definite functional action against illnesses in the animals and humans. Most of these native medicinal plants are used as spices and additives in our diet. Medicinal plants are considered to be a chemical factory because it contains a wide range of chemical compounds like resins, sesquiterpene, alkaloids, oleoresins, glycosides, saponins and oils (fixed and essential oils) (Rajalkshmi *et al.*, 2013).

In complex plants, they produce both primary and secondary metabolites that are accumulated in their body parts which is critically significant in the usual development and reproduction of plants. In contrast, secondary metabolites have an imperative role in the survival of the florae against adverse biotic and abiotic situations (Edriss *et al.*, 2012).

Extraction is a crucial step in the separation of therapeutically active compounds from plant parts using significant solvents with the help of standardized protocols. The products that are obtained from plants can be in state of semisolid or oily or dry powder form (after removing the solvent from the mixture) and they are given orally or externally during treatment (Tiwari *et al.*, 2011).

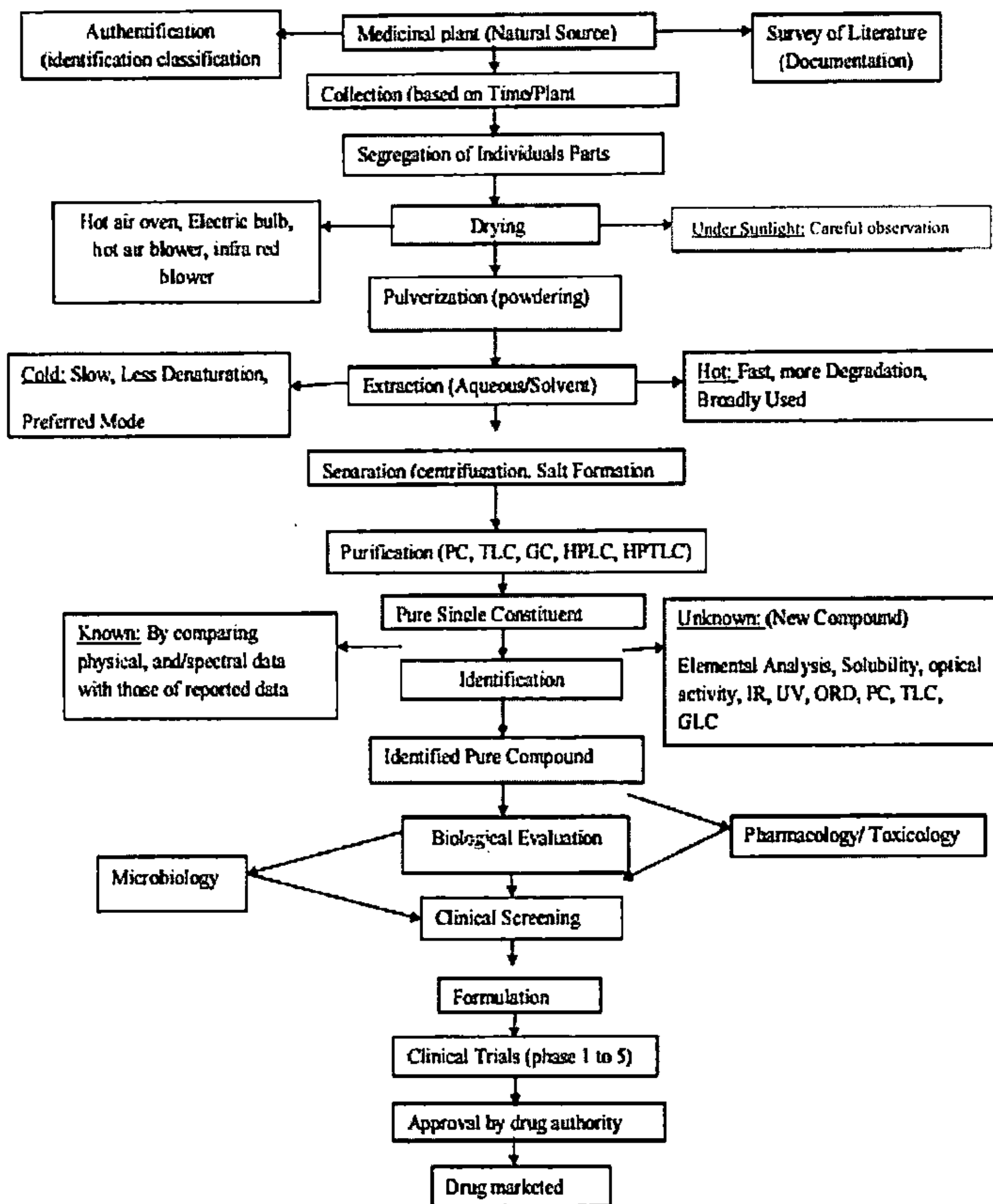


Figure 3: Schematic representation of the development of drugs from medicinal plants (Ashutosh, 2007).

2.1 ETHNOBOTANY, ETHNOMEDICINE AND ETHNO PHARMACOLOGY

Ethnomedicine is deals with the study of practices and preparations in traditional medicine, which is concerned with the traditional clarification about health condition, illness and diseases. It also gives an idea about the process of seeking of health care and healing practices. It is a complex multi-disciplinary health system of practice encompasses many areas such as perceptions and classifications of health problems, preventive measures, diagnosis, healing (supernatural, religiousness, scientific, systematic, therapeutic substances), and healers. The traditional knowledge in medicine has been used as the source of healing for people for over many ages.

Research activities and awareness in this field of ethnomedicine have been tremendously increased during in the last decade. Scientific and systematic researches in ethnomedicine has made crucial contribution for understanding information about the traditional existence of therapeutic knowledge and practice. Exploration of the collected works in ethnomedicine which was written in the past has been stimulated awareness about the significances of, recognition of indigenous health concepts of ethnic identities, acculturation of indigenous people, search for new medical treatments for diseases (Lowe *et al.*, 2000).

The use of medicinal plants was passed over by many biomedical physicians because of several reasons like the chemical constituents, dosages and toxicity of the medicinal plants used in traditional medicine are not clearly defined. Increased interest in the field of ethnomedicine boosted the use of medicinal plants became one of the most progressive measures taken by the pharmaceutical companies around the world for finding new pharmacologically active ingredients for the numerous areas of biomedicine (Cox and Balick, 1994).

There are outstanding therapeutic drugs which have been developed from the traditional knowledge about ethnomedicinally important plants includes reserpine which is obtained from *Rauwolfia serpentina* (Indian snake root) is used for treating hypertension, quinine from *Cinchona pubescens* (cinchona) used for treating malaria, aspirin from *Salix purpurea* (willow) used for treating inflammation, pain and thrombosis, and vinblastine and vincristine from *Catharanthus roseus* (the periwinkle) used for treating acute lymphoma, acute leukaemias etc.

Nowadays, a majority of the world's population are depending upon plants and plant extracts for improving the health condition (Setzer *et al.*, 2006) and among the top exclusively used drugs in the United States of America (USA), on that 57% of drugs have at least one key active compound which was obtained from various plants sources (Grifo and Rosenthal, 1997).

ETHNOPHARMACOLOGY

It is a branch of pharmacology which deals with the systematic study of the use of medicinal plants by specific cultural groups for treating diseases and it is an inter-disciplinary of various subjects such as botany, chemistry, history, pharmacology, biochemistry, anthropology, and archaeology. The main focus of ethnopharmacology is to deal with the study indigenous knowledge about the plants used for therapeutic purposes.

Ethnopharmacology also observes how indigenous people find medicines from fungi, animals and other natural sources. Still a large majority of the discussion involved in ethnopharmacology is focused primarily on plant usage.

2.4 PHARMACOGNOSY

World Health Assembly have emphasized that there is a need for resolution to ensure the quality and identify the therapeutic products obtained from plant by using existing appropriate standards and control methods (Radha *et al.*,

2008). The quantitative determination is done by some of pharmacognostical parameters which will be beneficial for giving values and standards for crude drugs accessible in the market (Calixto and Barz, 2000).

Pharmacognostical investigation becomes an extremely important effort taken towards the standardization of plant material to be used as a medicine. Thus, in recent years, there has been an emphasis on standardization of plants having therapeutic potential (Bhutani, 2006; Patel *et al.*, 2006). As stated in the World Health Organization, initial step in establishment of identity and purity of a medicinal plant is the investigation of macroscopic and microscopic description.

Pharmacognosy of *Coccinia grandis* used traditionally in folklore medicine for several purposes like jaundice, diabetes, wound healing, ulcers, stomach ache, skin disease, fever, asthma and cough are reported by (Tamilselvan *et al.*, 2011).

Pharmacognosy of *Cassia alata* Linn, which is a normally well-known as semaiagathi in Tamil and it has several therapeutic properties in the Indian systems of medicine which was reported by (Mohideen *et al.*, 2005). This plant was used as expectorant, purgative, astringent and vermicide and it also used to cure various skin infections. In his study, it deals with the quantitative microscopy, anatomy, fluorescence analysis and physical constants of the plant leaves.

The pharmacognostic parameters of leaves and stem of *Careya arborea* (*C. arborea*) Roxb. (Lecthyidaceae), is reported by (Prakash *et al.*, 2012). This is an imperative therapeutic plant used in Indian medicine. In his study, its leaves have some unique characteristics that is the leaves which turn red in winter and it has thick dark grey bark and large showy flowers. The bark of *C. arborea* was conventionally used for the treatment of skin disease, epileptic fits, tumours, ulcer, bronchitis, abscesses, an antidote to snake venom, and astringents. Flowers and leaves are made in to paste and to cure several skin diseases, diarrhoea, dysentery with bloody stools and ear pain. Leaf pulp is used as poultice and it helps to heals rapidly. The quantitative microscopy, anatomy, physico-chemical

parameters and fluorescence investigation of the plant leaves and stem were also carried out.

Microscopic, macroscopic and physiochemical analysis of *Dillenia indica* (*D. indica*) leaves evaluated by (Kumar *et al.*, 2011). The detailed microscopy study exposed the presence of calcium oxalate crystals, xylem fibres, vascular bundles, unicellular trichome, anomocytic stomata etc. Leaf constants such as vein-islet number, stomatal index, veinlet termination numbers and stomatal number and physico-chemical parameters were also determined. Preliminary phytochemical screening exposed the occurrence of flavonoids, terpenoids, steroids, fatty acids, glycosides, phenolic compounds and carbohydrates. These observed data of *D. indica* leaf will help to recognize the purity and quality of the sample.

Pharmacognostic parameters of *Kedrostis foetidissima* (Jacq.) Cogn. is an indigenous medicinal plant was evaluated by (Kavitha *et al.*, 2015). The evaluations were carried out for anatomical studies, organoleptic evaluation and powder microscopy of leaves and roots.

Pharmacognostical studies and phytochemical Screening of leaves of *Dodonaea viscosa* LINN. which are used for treating gout and rheumatism was assessed by (Javahar *et al.*, 2004). In this present work deals with pharmacognostical parameters such as extractive values, ash values, leaf constants and preliminary phytochemical screening of leaves of *D. Viscosa*. And these parameters of this leaf help to identify the purity and quality of the sample.

Commiphora caudata (Wight and Arn) is a potential medicinal plant used for its antispasmodic activity, cytotoxic activity and hypothermic activity. Owing to its medicinal importance, macroscopic and microscopic characters of leaves of *Commiphora caudate* were studied by (Latha *et al.*, 2006).

2.5 PHYTOCHEMISTRY AND PHYTOCHEMICALS

Phytochemicals are biologically active naturally occurring chemical compounds which are found in plants, it delivers health benefits and wellbeing for humans (Hasler and Blumberg, 1999). Their main role is to protect the plants from disease and damage caused by abiotic and biotic stresses and these chemicals also contribute to the plant's aroma, colour and flavour. These plant chemicals i.e. phytochemicals, are produced to protect plant cells from environmental hazards such as drought, stress, pollution, pathogenic attack and UV exposure (Mathai, 2000). Currently they have an imperative role in maintaining the well-being of humans without any diseases so it ultimately enhances the quality of life. According to the American chemical society recorded that there are more than 4,000 phytochemicals have been identified and are classified by chemical and physical characteristics and its protective function. Approximately 150 of these phytochemicals have been investigated in depth (Meagher and Thomson, 1999).

There is a wide-ranging dietary phytochemical that are found and obtained from edible fungi, vegetables, fruits, nuts, whole grains, seeds, herbs, spices and legumes. They are accumulated in various plant portions such as in the seeds, roots, stems, leaves, flowers, and fruits. In the external layers of the different plant tissues the pigment molecules are concentrated. Concentration of the phytocompounds will fluctuate from plant to plant depending upon different varieties of plant species and growing environments (Costa *et al.*, 1999).

The phytochemicals are classified into two: primary and secondary metabolites. Among these two, Secondary metabolites from plants have biological properties such as stimulation of the immune system, antimicrobial effect, antioxidant activity, modulation of detoxification enzymes, anticancer property and decrease of platelet aggregation and modulation of hormone metabolism. Plants produce these chemicals to protect themselves, but recent researches demonstrate that many of these phytochemicals can also protect human from illnesses (Narasinga, 2003).

Savithramma and Suhrulatha (2011) reported that traditional medicine involves the use of different plant extracts or the bioactive constituents. Qualitative phytochemical analysis of some medicinal plants are investigated and the presence of various phytochemicals like terpenoids, saponins, anthocyanins, steroids, coumarins, tannins, fatty acids, emodins and leucoanthocyanidins.

Chemical fingerprinting of several secondary metabolites from *Zanthoxylum rhetsa* (Roxb.) DC, were investigated by (Priya and Aparna, 2012). Preliminary phytochemical screening test was investigation for the identification of several secondary metabolites. HPTLC profiles for several individual secondary metabolites were developed for authentication. In the ethanolic fruit extracts showed the presence of essential oils, flavonoids, glycosides, anthraquinones, terpenoids and coumarins. Finally, result of this investigation will be useful in distinguishing the species, for checking the adulterant in the crude sample and also help to develop biomarkers for this plant for the Pharmaceutical industry.

Amritphale (*Psidium guajava* L.), belongs to Myrtaceae family and this plant was used conventionally for curing of several illnesses such as hypertension, inflammation, wounds, diabetes, fever and pain its phytochemical profile of leaves investigated by (Vikrant *et al.*, 2012). In their study, leaf powder was successively extracted with different solvents such as petroleum ether, chloroform, ethanol, hydro-ethanolic and water solvents. In Phytochemical analysis has shown the occurrence of several secondary metabolites such as saponins, flavonoids, triterpenoids, carbohydrates, tannins, sterols and alkaloids.

Phytochemical constituents in the bark of *Ficus racemosa* was investigated by (Poongothai *et al.*, 2011). Bark was successively extracted with various solvents such as ethyl acetate, benzene, ethanol, chloroform, petroleum ether and methanol. As a result of phytochemical screening confirms the occurrence alkaloids, sugars, proteins, glycosides, sterols and flavonoids in the different extracts.

Qualitative phytochemical analysis of root tubers of six species of *Dioscorea* found in Meghalaya was done by (Nilofer *et al.*, 2013). In their studies, they confirm the occurrence of various phytochemicals like terpenoids, flavonoids, saponins, cardiac glycosides and steroids in two aqueous extracts of methanol and ethyl acetate. Methanolic extract shows the occurrence of maximum number various phytochemical compounds than ethyl acetate extract during screening.

The screening of preliminary phytochemicals presents in the leaves of *Ziziphus oenoplia* belonging to Rhamnaceae family were investigated by (Lalitha *et al.*, 2013). In their investigation dried leaves of the plant were taken for successive soxhlet extraction by continuous hot percolation method using several solvents such as ethanol, ethyl acetate and petroleum ether. Cold maceration technique was used for preparing the aqueous extract. Qualitative phytochemical screening was done in all the extracts and it exposed the occurrence of active compounds such as flavonoid, alkaloid, triterpenoid and phenol. Quantitative determination of these compound was done by methods prescribed in the text book of Harborne.

Extractive yield in different solvents such as hexane, acetone, chloroform and methanol, qualitative and quantitative phytochemical screening, antioxidant and antimicrobial activity of the crude extracts of *Leucas indica* (L) var. was investigated by (Kamala *et al.*, 2014). In the studies it has revealed occurrence of wide range of bioactive compounds such as saponins, alkaloids, steroids, phenols, flavonoids, tannins and reducing sugars were confirmed in the plant extracts and total phenolic and flavonoid contents were also determined.

Antioxidant activity and screening of phytochemicals and also determined the total phenolic and flavonoid contents of Ephedra *alata* was evaluated by (Nidal *et al.*, 2015). Total flavonoid content of the plant was confirmed by using the rutin as reference standard compound and total phenols was determined by Folin-Ciocalteu method whereas, for antioxidant activity evaluated by DPPH assay. Screening test for phytochemicals showed the occurrence of

various phytochemical such as phenolic compounds, reducing sugars, cardiac glycosides, flavonoids and alkaloids.

2.6 OXIDANTS

Oxygen is an essential element which is essential for life. Presence of molecular oxygen is required for the survival of living systems. Oxidative activity of oxygen plays a vital role in diverse biological phenomena. Oxygen has a double-edged property, being essential for life and it can also aggravate the damage caused by oxidative events within the cell (Shinde *et al.*, 2006).

Free radicals and its antagonistic impacts were found in the most recent decade and they are risky substances delivered inside the body alongside poisons and waste items which are shaped amid the ordinary metabolic procedure of the body. At the point when the body acquired vitality through the oxidation of starches, proteins and fats by both high-impact and anaerobic process prompt the era of free radicals in the body.

Oxidative stress plays an imperative part in the advancement of chronic and degenerative infections, for example, aging, immune system issues, tumour growth, joint inflammation, cardiovascular and neurodegenerative diseases (Young and Woodside, 2001). Human body has various systems to balance oxidative stretch by generating antioxidant agents, which are either naturally formed in situ, or externally provided through diet and dietary supplements. Endogenous and exogenous antioxidants act as "free radical scavengers" by averting and repairing harms created by ROS and RNS and boost immune protection and lower the risk of cancer and other degenerative ailments (Singh, 2004). At low or moderate levels, ROS and RNS apply useful impacts on cellular responses and immune functions. At higher concentration, they create oxidative stretch, a pernicious procedure that can harm all cell structures. So Overproduction of free radicals can be in charge of the tissue damage (Valko *et al.*, 2007).

Biological molecules as well as DNA, RNA and protein catalysts are additionally helpless to oxidative damage. Agents presents in the environment, for example, pesticides, cadmium, harmfulness of ionizing radiation, tobacco smoke, liquor, lead, contamination and UV light are also initiate free radical generation and it different complication in the body.

2.7 ANTI-OXIDANTS

Antioxidant agents are the substances that have the ability to represses or postpone oxidative harm brought about by the free radicals produced in the body. One antioxidant molecule can respond with single free radicals at once and are equipped for neutralizing the free radicals by giving one of their own electrons, finishing the carbon-taking response. It avoids cell and tissue harm as they go about as a scavenger. Cell creates a protection against over the top free radicals by their antioxidant defences, physical defences, repair mechanisms and preventative mechanisms.

An assortment of segments acts against free radicals to kill them from both endogenous and exogenous in starting point. This include:

- Phytoconstituents and phytonutrients.
- Nonenzymatic, metabolic and supplemental antioxidants.
- Metal binding proteins like ferritin, albumin, ceruloplasmin and lactoferrin.
- Endogenous enzymatic antioxidants. (Jacob, 1995)

Our body generates several kinds of endogenous antioxidants to nullify the free radicals produced and defend the body from various ailment leads by the tissue damage. Exogenous antioxidants are supplied externally in to the body through diet and other natural sources such as especially from the medicinal plants also plays important role in neutralizing the surplus free radicals produced, to prevent diseases and to defend the cells in the body against their toxic effects of free radicals. Several endogenous antioxidant defence systems was developed in

our body and it is categorized into two sets such as non-enzymatic and enzymatic non-enzymatic. The enzymatic defence system includes different endogenous enzymes like glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) etc. and non-enzymatic defence system included glutathione (GSH), vitamin E and vitamin C (Harris, 1992).

2.7.1 Antioxidant Process

During an antioxidant process it extinguishes a free radical at a time after the reaction, antioxidant itself gets oxidized. So, antioxidant must be frequently restored in the body from internally as well as externally sources. Thus, an antioxidant in a particular system is effective against a single type of free radicals, in other systems the identical antioxidant could become incompetent or weak. Also, in definite conditions, an antioxidant may even act as a pro-oxidant for example, it can be able to produce toxic reactive oxygen species (ROS)/ reactive oxygen species (RNS).

The functioning of antioxidant process can be operating in one of two ways: chain-breaking or prevention. During the chain- breaking process, an electron will be releases or takes from a radical, a second radical is produced in this reaction. The last one applies similar activity on another atom and proceeds until either the free radical shaped is balanced out by a chain-breaking cell reinforcement. One of the classical case for chain breaking response is lipid peroxidation. For the preventive way, some antioxidant enzymes like glutathione peroxidase, superoxide dismutase and catalase can halt the oxidation by decreasing the rate of chain origination, e.g., either by stabilizing transition metal radicals such as iron and or copper by scavenging initiating free radicals (Young and Woodside, 2001).

2.8 TOXICITY

The degree of toxicity of a product or a substance that can initiate harm and damage to humans or other organisms. Toxicity is categorized into three, such as acute, sub chronic and chronic toxicity. Acute toxicity involves the destructive impacts to a living being through a single or short-term exposure. Sub chronic toxicity is the capacity of a dangerous substance to bring about impacts. Chronic toxicity is the ability of a substance or blend of substances to bring about hurtful impacts for a long period, usually upon a repetitive or steady exposure, sometime lasting for the whole life of the exposed individual (Fauci, 2008).

2.8.1 Objectives of toxicity testing of drugs originated from plants

The primary objective of toxicological assessment is carried for adverse effects of plant derived pharmaceutical and to determine dose limits of exposure level at which such potential effects occur. Two major factors which are taken into attention in the safety assessment of every plant derived drugs are the nature and significance of the antagonistic effect. Likewise, the greatest introduction level of the plant derived drugs and their effect are observed.

Toxicity investigation can reveal a portion of the threats that might be associated with the utilization of medicinal plants, exclusively insensitive populaces. Similarly, it is also an equally imperative objective for toxicity investigation is the detection of the toxic effect of plant extracts or compounds derived thereof in the pre-clinical and clinical phases throughout the drug discovery and development from various plant sources. These phases can assist in the identification of various toxicants which can be disposed or altered during the process and create an opportunity for extensive assessment of harmless, promising alternatives for the toxic substances (Gamaniel, 2000). Modifications like structural or chemical group adjustments and dosage reduction may improve their tolerability.

2.9 NOCICEPTION

Nociceptive is also known as pain and it is experienced by everyone due to suffering of any kind of injury or localized inflammatory process. This pain is due to initiation of small diameter afferent nerve fibres that are sometimes called "nociceptive" nerve fibres. Nociception is a sub modality of somatic sensation that has been defined as a "emotional, unpleasant sensory and complex constellation of cognitive experiences that provoked by real or perceived tissue damage and manifested by psychological, behavioural reactions and certain autonomic" (Terman and Bonica, 2003).

Advantage of these type of unpleasant sensations that helps us to recognize and diagnose different ailments depending up on the knowledge of different causes of pain to a great extent. Sensitivity to harmful provocations are important for the survival and well-being of individuals.

Nociceptive stimulus moves through redundant pathways; this stimulus make sure by informing the individual to "Get out from this condition instantly." Devoid of these characteristics, the organism has no means to prevent or reduce tissue damage. Individuals with congenital disorders were unresponsive to pain are easily get injured and most of them die at an early age. And in extreme cases, there are patients lacking the capability to recognize pain due to genetic neuropathic diseases are often maintain self-mutilate, unrealized infections and have shortened life spans (Axelrod and Hilz, 2003).

High threshold physical and noxious chemical stimuli are detected by specialized peripheral sensory neurons called nociceptors.

They are sensory receptors that identify signals from injured tissue or the risk of damage and indirectly it also reacts to chemicals discharged from the injured tissue. This sensory nerves are having free (bare) nerve endings found in the skin (Figure 5), joints, muscle, viscera and bone. In recent times, it was found that nerve endings contain channels called transient receptor potential (TRP) that

sense and identify injury. The TRP channels are much alike to nucleotide-gated channels or voltage-gated potassium channels and these channels are having six transmembrane domains with a pore between domains five and six. They transduce a variety of harmful stimuli into receptor potentials, which in turns and initiate the action potential in pain nerve fibres. This action potential is transmitted to the spinal cord and makes a synaptic connection in lamina I as well as II. The cell bodies of nociceptors are mainly in the dorsal root and trigeminal ganglia. Inside the Central Nervous System there is no nociceptors are found.

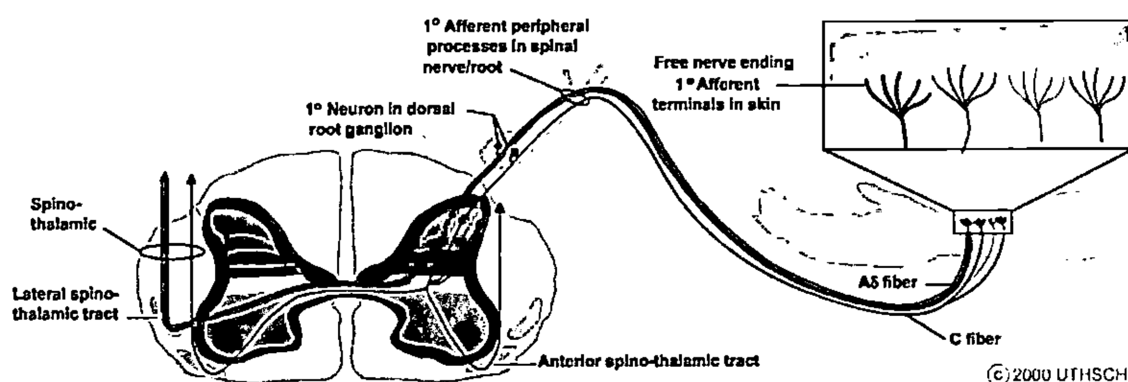


Figure 5: Different Nociceptors/ free nerve endings, and the fibres carrying pain sensation from the nociceptors to the spinal cord.

2.10.1 Classification of Pain

Pain has been classified into three major types:

1. ***Pricking pain***: Pain which was triggered by a skin cut, needle, pinprick, etc. and this pain are carried fast by A-delta fibre.

2. ***Soreness pain or Burning pain:*** Pain initiated by inflammation, burned skin, etc. and itis passed by the C fibres. This fibre is a slowly conducted pain nerve fibres.

3. ***Aching pain***: This pain emerges basically from the visceral and somatic deep structures. It is not distinctly localized and is an intolerable and

annoying pain. And this pain is also carried by the C fibres from the deep structures to the spinal cord.

2.10.2 Components that Activate Nociceptors

Nociceptors have become active when a stimulus causes tissue injury, for example, that resulting from extreme heat, strong cut, mechanical pressure, etc. The injury of tissue effects in the discharge of a variety of substances from lysed cells as well as from new substances that is synthesized at the site of the injury (plate 5). Some of these substances activate the TRP channels which in turn initiate action potentials. These includes substances such as arachidonic acid, globulin and protein kinases, nerve growth factor (NGF), acetylcholine (ach), muscle spasm calcitonin gene-related peptide (CGRP), histamine, substance p (SP) potassium - K^{+}, lactic acid and serotonin (5-HT).

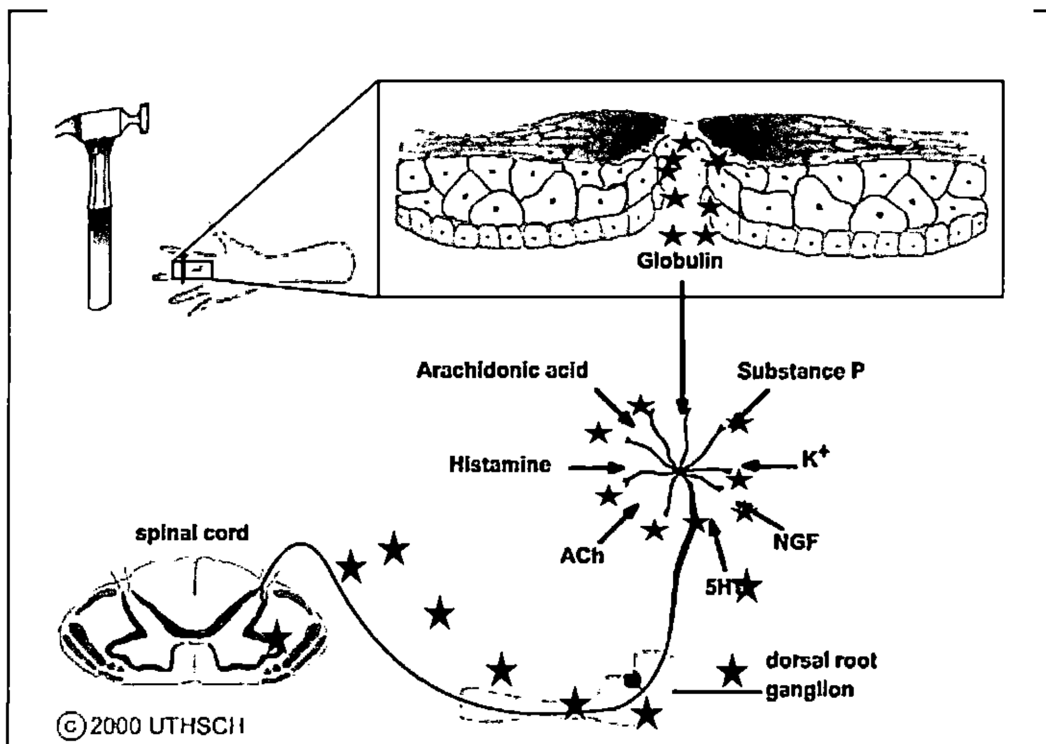


Figure 6: Tissue damage and the variety of the substances released from the injured site that activate the nociceptors.

2.10 INFLAMMATION

Inflammation is a physiological response of a body to a variety of stimuli for example, infection and tissue injury. Injuries may be triggered in many ways such as physically (temperature), mechanically (pressure or foreign bodies), microorganisms (bacteria, virus and parasites), chemically (acidity and alkalinity toxins) and by internal processes (uremia). It is a complicated process and the communication between cellular and humoral elements involved in this process was not fully understood. During Inflammation, it removes and frees foreign matter and injured cells from the body and promotes the initiation process of wound healing.

Mast cells are seen in close proximity to autonomic nerves that controls the process of inflammation. Mast cells are the components of connective tissues having large granules that contains heparin, serotonin, histamine and bradykinin. These components of mast cell are released into tissues in response to an infection, damage or injury etc. Mast controls almost all of the processes involved during inflammation and it also responsive to other situations such as, under the impact of oestrogen they discharge histamine and under the impact of progesterone they discharge serotonin.

Arachidonic acid cascade is an another chief pathway for activating inflammation and it is mostly controlled by eicosanoids. It is a native hormone that prepared from 20-carbon essential fatty acids (EFA, AA and DGLA), which is only lived for a short duration and it affect many aspects of the physiological function at the cellular level. Eicosanoids comprise all the prostaglandins, leukotrienes and thromboxanes. Depending on genetic as well as other factors that transform or controls the manufacture of these inflammatory mediators such as prostaglandins, thromboxanes, and leukotrienes in response of any stimulus. Eicosanoids has the ability to initiate, regulate, and terminate all native inflammatory responses (Wichers and Maes, 2004).

Aspirin, indomethacin and other non-steroidal anti-inflammatory drugs NSAIDs hinder the synthesis of prostaglandin and other inflammatory

mediator production. Steroidal hormones have been assumed that, they have a multitude of effects. Probably the most significant in this context is the stabilization of lysosomal membranes.

2.10.1 Types of Inflammation

i. Chronic Inflammation

This inflammation can be evoked specifically from acute inflammation or without occurring an acute phase. Histologically, the two primary features of chronic inflammation such as the presence of mononuclear predominance and granulation tissue. The combination of extracellular matrix, fibroblasts and new blood vessels is known as granulation tissue. In the last part of acute inflammation, mononuclear predominance can be seen as mononuclear phagocytes or macrophages.

ii. Acute Inflammation

Acute inflammation is the initial response of a tissue to a non-specific or to any type of damage. In its initial line of defence against a damage and is characterized by changes in the microcirculation such as emigration of leukocytes and exudation of fluid from blood vessels to the area of damage. This inflammation is characteristically of short period, happening before the immune response becomes recognized and it is targeted mainly at eliminating the damaging agent.

2.11 PHARMACOLOGICAL REVIEWS

The ethyl acetate fraction of leaves of *Pterospermum acerifolium* L. (Sterculiaceae) (EAF) reported by (Sannigrahi *et al.*, 2010) showed significant free radical scavenging activity and anti-inflammatory activity in both *in vivo* and *in-vitro* studies, which substantiate traditional use of *P. acerifolium* L.

Pterospermum acerifolium (L) Wild which belongs Sterculiaceae family and it is traditionally used for curing of inflammatory diseased conditions in

the Indian traditional medicine. (Rasika and Subodh, 2013) investigated this plant for anti-inflammatory and antinociceptive effects of unsaponified petroleum ether extract of leaves of *Pterospermum acerifolium* and USPEL of 100 mg/kg and 200 mg/kg and isolated a pure compound from the leaves known as β-sitosterol of 10 and 20 mg/kg were given orally to animal models are assessed for its anti-inflammatory potential by carrageenan-induced paw oedema model in rats and analgesic potential by acetic acid-induced writhing, formalin induced paw licking and hot plate models in mice.

Anbalagan *et al.* (2003) conducted experiments in Vacuum dried chloroform and methanol extracts of *Pisonia grandis* leaves and evaluated the analgesic and anti-inflammatory (acute and chronic) and diuretic activity of the extracts at 2 dose levels (250 and 500mg/kg) in Wistar albino rats and Swiss albino mice. Analgesic and anti-inflammatory activities of chloroform and methanol extracts were tested. In chloroform extracts, at dose level 500mg/kg shown a significant increase in activity were observed.

Withania somnifera is an herb used extensively used in the traditional Ayurvedic and indigenous medical system in Bangladesh and this plant belongs Solanaceae family. (Mohammad *et al.*, 2013) stated that this plant is used for the prevention of various disorders related to central nervous system (CNS), especially for the disorders such as stress and neurodegenerative diseases. In this investigation they used different root extracts of *W. somnifera* were inspected to reveal the antioxidant potential. They measured its antioxidant potential by its capability for scavenging 2, 2-diphenyl-1-picrylhydrazyl (DPPH.) radical, hydrogen peroxide radical, nitric oxide radicals (NO.), as well as its ability in Reducing power capacity assessment, Cupric Reducing Antioxidant Capacity, using appropriate assay systems compared to natural and synthetic antioxidants. Total antioxidant, phenolic and flavonoid contents were determined by spectrophotometrically.

Santos *et al.* (2011) stated that methanol stem bark extract of *Cariniana rubra* can reduce the leukocyte migration and volume on the vascular permeability induced by acetic acid and on the carrageenan-induced pleurisy. It can also inhibit pain on the acetic acid induced writhing and in the second phase of formalin test which supports the traditional use of *Cariniana rubra* preparations to treat inflammation.

The ethanolic extract of rhizome of *Drynaria quercifolia* phytochemical profile and Anti-inflammatory and analgesic properties of was evaluated by (Anuja *et al.*, 2010). The drug *Drynaria quercifolia* was orally administrated and it significantly inhibited granuloma formation and carrageenan-induced paw oedema in rats, nearly comparable inhibition that of indomethacin. *Drynaria quercifolia* significantly delayed phases of acetic acid-induced writhing and formalin-induced pain experienced in mice. The analgesia was similar to that produced by sodium salicylate and aspirin respectively. Tests for catechin, coumarins, flavonoids, phenolics, saponin, steroids, tannins, and triterpenes were done and it gave positive result in phytochemical analysis. In *Drynaria quercifolia* the total phenolics was 244 mg/g and naringin content was 0.048%. The overall results presence of potent anti-inflammatory and analgesic properties in *Drynaria quercifolia* that justifies its use for alleviating painful inflammatory conditions.

Chakraborty *et al.* (2012) reported that the methanol extract of *Phyllanthus acidus* leaves exhibit significant anti-inflammatory and analgesic property in a dose dependent manner. The presence of flavonoids and phenolic compounds could be correlated with the antioxidant, anti-inflammatory and analgesic property of the methanolic leaf extract of *Phyllanthus acidus*.

Anti-inflammatory and analgesic potential of ethanolic root extract of *Adhatoda vasica* Linn. (Acanthaceae) was evaluated by (Wahid *et al.*, 2010). The anti-inflammatory potential of ethanolic extract has been determined by using formalin-induced paw oedema and carrageenan-induced paw oedema assay in Wistar albino rats. The analgesic activity was tested by using tail flick method in

albino rats hot plate method and acetic acid-induced writhing response. The administration of extract at doses of 200 and 400 mg/kg, by orally it has significantly inhibited inflammation on both carrageenan and formalin-induced inflammation and also significantly inhibited pain effect in the acetic acid-induced writhing, formalin-induced pain licking and hot-plate-induced pain.

Anti-inflammatory activity of *Ficus dalhousiae* Miq roots ethanolic extract (FDREE) in Wistar albino rats using carrageenan and formalin-induced paw oedema model was investigated by (Sared *et al.*, 2015). Phytochemical screening was carried out for the detection of the phytoconstituents by simple qualitative methods. The dosing was designed as per the acute toxicity study reported earlier. The anti-inflammatory activity was evaluated by carrageenan and formalin-induced paw oedema model at three different doses, 150 mg/kg, 300 mg/ kg and 600 mg/kg. Wistar rats of 130-150 g weight of either sex were used for the investigation. This study has shown significant reduction of elevated paw volume in the test groups observed in both carrageenan and formalin-induced paw oedema models. The percentage inhibition of inflammation was also high in the test groups compared with the negative control. Therefore, FDREE exhibited anti-inflammatory activity in both acute and subacute experimental models which provide the evidence for effective drug for treating inflammation.

Anti-inflammatory and analgesic potential of methanolic extract of *Vallaris solanacea* leave belongs to the family Apocynacea was evaluated by (Poonam *et al.*, 2014). Methanolic extract of *Vallaris solanacea* at doses of 250mg/kg and 500mg/kg were investigated for their anti-inflammatory activity against carrageenan induced paw oedema and formalin-induced arthritis models in rats and analgesic activity was investigated against acetic acid induced writhing in mice and the two phases of formalin-induced paw licking models of pain. The methanolic extracts at the doses of 250 and 500 mg/kg body weight significantly inhibited both inflammation and pain in the animal models

Pharmacological potential such as antioxidant, anti-Inflammatory, antimicrobial and cytotoxicity of fungal endophytes isolated from Garcinia Species are investigated by (Karmakar *et al.*, 2013). From four species of Garcinia they isolated endophytes and then endophytes were subjected to fermentation in still culture and extracted using ethyl acetate. Obtained crude extracts were screened for the different biological activity through assays such as cytotoxicity, antioxidant activity, antimicrobial activity, 15-lipoxygenase/ human cyclooxygenase-2 inhibition and DNA nicking assay. Among these isolated and screened endophytes, the most promising *Garcinia* spp was *Aspergillus fumigatus* and *Fusarium* sp. The HPLC and MS profiling estimated the existence of the compound phloroglucinol, which could be involved in the biological activities of this extract. The endophyte extract inhibited LOX and COX enzymes which are produced during inflammation so, this can be used for efficient management of inflammatory disorders.

Anti-inflammatory activity of telmisartan was studied in a dose-dependent manner for chronic inflammation in rat models was investigated by (Waleed *et al.*, 2011). In combined dose with telmisartan and dexamethasone (1.5 mg/kg body weight) significantly inhibited inflammation in both models, which is significantly higher than all of the effects produced by other approaches of treatment when used telmisartan alone. Thus, telmisartan decreased cotton pellet induced granuloma and formaldehyde-induced chronic inflammation in rats in a dose-dependent pattern.

Anti-inflammatory effect of hydroethanolic extract of rhizome of *Nardostachys jatamansi* against acute, subacute and chronic models of inflammation in animal models was evaluated by (Rajnish *et al.*, 2014). *Nardostachys jatamansi* rhizome extract (150 and 300 mg/kg, p.o.) and the reference drugs phenylbutazone (100 mg/kg, p.o.) and acetylsalicylic acid (300 mg/kg, p.o.) were evaluated using models for inflammation such as cotton pellet granuloma formaldehyde-induced hind paw oedema, carrageenin-induced paw oedema, subcutaneous air pouch model and autacoids induced hind paw oedema. Final result is strongly indicating the protective role of *Nardostachys jatamansi*

rhizome extract against acute, subacute and chronic models of inflammation, which attributed to its anti-inflammatory potential.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study entitled "Pharmacognostic studies and assessment of the antiinflammatory, antinociceptive, and antioxidant potential of 'ellooti' (*Pterospermum rubiginosum* B. Heyne ex G. Don)" was carried out at Ethnomedicine and ethnopharmacology division of Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, during 2015-2016 academic year.

3.1 MATERIALS

3.1.1 Sample collection

The leaves of *Pterospermum rubiginosum* B. Heyne ex G. Don were collected from various other localities of Thiruvananthapuram and Wayanad district and maintained at JNTBGRI, Palode. The specimens of the plant material were deposited in the herbarium collection of the JNTBGRI (TBGT 86888/dated 12/04/2016).

3.1.2 Preparation of extract

The fresh leaves were taken for macroscopic and microscopic studies. Dried leaf powder was used to study microscopical, physicochemical parameters and fluorescence analysis. For phytochemical analysis, the leaves of *P. rubiginosum* were cleaned with water and shade dried until they become breakable and pulverised using an electric blender (Preeti Mixer Grinder). The pulverised leaves of *P. rubiginosum* were kept in a sealed polyethylene container until use. For the extraction, powdered leaves of *P. rubiginosum* (10 g each) was successively extracted with different solvents n-hexane, chloroform, ethanol, ethyl acetate, acetone hydro-ethanol and water are incubated for a period of 72h in a shaker at a uniform speed and then it filtered out. Each filtrate was collected, concentrated and dried under vacuum at 45^0C using a rotary evaporator (Buchi, rotavapor R – 215) and kept in a desiccator at room temperature until use.

3.1.2 Experimental animals

Swiss Albino Mice (20-25 g) and Wistar rats (175 to 220g), of either sex, were attained from animal house of Jawaharlal Nehru Tropical Botanical Garden Research Institute, Palode. The experimental animals were divided into different groups and caged in polyacrylic cages and maintained under standard laboratory environment (temperature 24° C, the relative moisture of 55-65% and 12-hour dark- light cycles). The animals were fed thrice in a day with commercial rodent feed (Hindustan Animal Feeds, Gujrat, India) and water. All animal experiments were carried out according to the guidelines of the NIH and only after getting the approval from the Institute Animal Ethics Committee (B/13/02/2015/EM and EP-27-31)

3.1.3 Chemicals and Equipment's

All chemicals were of analytical grade. Magnesium turnings, Sodium hydroxide, Ethanol, Sulphuric acid, Chloroform, Olive oil, Ferric chloride, Ethyl acetate, Lead acetate, HCl, Acetic acid, Ammonia, Copper sulphate, Sodium Nitro Prusside, Acetone, n-Hexane, Fehling's solution A and B, Ammonium sulphate, toluene, Gallic acid, Sodium carbonate, Folins ciocalteus reagent, Quercetin, Methanol, Aluminium chloride, Potassium acetate, Carrageenan, Indomethacin, Saline, Formalin, Indomethacin, DPPH (1-diphenil-2-picrylhydrazyl), Methanol, Ascorbic acid, Acetic Acid, Acetylsalicylic acid, Sodium Nitroprusside, Griess reagent.

3.2 PHARMACOGNOSY

The fresh leaves were taken for macroscopic and microscopic studies. Dried leaf powder was used for the study of microscopical, microscopical, physicochemical parameters and fluorescence analysis. For phytochemical analysis and pharmacological studies, the leaves of Pterospermum rubiginosum B. Heyne ex G. Don was cleaned with running water from the tap to remove dirt and other foreign materials attached to leaves. They were shade dried for two weeks until they

become brittle and pulverized, using an electric blender (Preethi Mixer Grinder). The powdered samples were stored in tight polyethylene cover until use. Later extracts were extracted from leaf powder.

3.2.1 Macroscopic characterization of plants:

The following macroscopic characters of the fresh leaves were noted: plant descriptions, size and shape, colour, surfaces, tip, arrangement, odour and taste.

3.2.2 Microscopical Characters:

In microscopical studies, a thin cross-section of midrib and petiole was cut and put that in watch glass containing water to prevent drying of the section. The sections of leaf were taken by the free hand by using a fine blade. Then the sections were stained by using safranin (0.5% w/v) and sections were placed on the slide and a few drops of glycerine poured above the section, a coverslip was placed above it without allowing any air bubbles to be formed in between them and finally, the slide was examined under the microscope (Binocular Microscope, Zeiss).

The microscopic analysis of the powdered leaves of *Pterospermum rubiginosum*, the firstly clean glass slide was taken. Secondly, a small pinch of powder was sparkled on it and added a drop pf glycerine above It without allowing the air bubble to be formed in between glass slide and cover slip. Finally, the slide was examined under the microscope (Binocular Microscope, Zeiss).

3.2.3 Physico-chemical evaluation

Physico-chemical evaluation of powdered leaves of *Pterospermum rubiginosum* was investigated by different parameters such as foreign organic matter, total moisture content, total ash, acid insoluble ash, water soluble ash and n-hexane, chloroform, ethanol, ethyl acetate, acetone hydro-ethanol and water soluble extractive values.

i. Foreign Organic Matter

This test is performed to the leafy part of the plant material of 150g of the leaf. The sample was spread in a thin layer on a clean surface and inspect it with the naked eye. Separated, weighed and calculated the percentage of foreign material obtained.

ii. Moisture content

10g of fresh leaves were taken and kept in the oven to remove moisture from the leaves and set the temperature at 60°C. And simultaneously take weight until the weight become constant. Compare the dry weight with fresh to get the total moisture content.

iii. Total Ash Content

3g of powdered leaves were taken in a tared silica crucible and incinerated by a Bunsen burner for 2-4 hours and kept the temperature does not exceed at 450°C, to obtain carbon-free ashes. Record the total weight of the ash obtained from it.

iv. Acid insoluble ash

100mL of 2% Conc. HCl was prepared. Washed the silica crucible containing the ash with 2% HCl, washout is collected is again poured into a beaker containing acid and boiled for 10 minutes. Filter it by using Gooch crucible (Borosil) and kept in the oven to dry. Record the weight of acid insoluble ash.

v. Water soluble ash

Washed silica crucible containing the ash were mixed with water, washout is collected. Filter it by using Gooch crucible and kept in the oven to dry. Record the weight of acid insoluble ash.

vi. Crude Fibre Content

Three grams of the drug powder were transferred to the thimble and to a soxhlet and extracted with petroleum ether. After repeated soxhlation, the residue was transferred to a dry 500mL beaker and added 200mL of 0.25 N H_2SO_4, covered with a watch glass and gently boiled for 30 minutes. After boiling, the mixture was filtrated using Whatman No.1 filter paper into a 500mL conical flask, followed by washing in hot water to remove sulphate (using $BaCl_2$). Then the residues were transferred into a 500mL beaker, then added 200 mL of NaOH (0.31 N) solution and covered with a watch glass and put a glass rod, boil it for a period of 30 minutes. The content was then filtered through a Buchner funnel with suction by using weighed Whatman No. 41 filter paper and then washed with hot water until it is free from alkali (using phenolphthalein).

The residue (substance weight = (total weight of residue - filter paper residue) – ash weight) was dehydrated at 100°C to get a persistent weight.

Percentage of fiber content = (weight of residue/3) *100

vii. Swelling Index

Take a 50 ml measuring cylinder containing 1g of leaf powder, add 25 ml of water into it and mix well. Then periodically shake the mixture thoroughly in every 10 minutes up to 1 hour. Then allow the mixture in the cylinder to stand undisturbed at room temperature for 3 hours. Measure the volume in mL of the herbal material occupied in the cylinder, including sticky mucilage. The mean value was calculated from the individual determinations, for 1 g of herbal material.

3.2.4. Extractive values

10 g of the shade dried leaves of *Pterospermum rubiginosum* was macerated with 120 mL of the solvents (n-hexane, chloroform, ethanol, ethyl acetate, acetone, hydro-ethanol and water). And it was filtrated, dried and the percentage of extractives were calculated with reference to the weight of dried leaf powder.

3.2.5 Fluorescence Study

The fluorescence properties of plant powder were studied under visible light, short and long ultraviolet (UV) light. A small quantity of powdered leaves of *Pterospermum rubiginosum* was put in a glass test tube and 5 mL of freshly prepared fluorescence reagents viz. acetone, 1M NaOH, 1M NaOH + methanol, 1M NaOH + water, HCl, dil. HNO_3, 5% Iodine, 5% $FeCl_2$, dil. Ammonia, methanol, dil. HCl, 1M H_2SO_4, Conc. HNO_3, KCr_2O_7, 95% ethanol, toluene and chloroform were added into the numbered test tube, followed by mild mixing of reagent and powder. After the shaking, all the test tubes were allowed to stand undisturbed for about 25-30 min. The fluorescence behaviour of the leaf powder with different chemical reagents was observed under visible light and ultraviolet light at 254nm and 365nm and fluorescence characters and colours were compared with a standard colour chart (RAL Colour Chart) were recorded.

3.3 PHYTOCHEMICAL TESTS

3.3.1 Preliminary Phytochemical Screening

Preliminary phytochemical screening tests were determined by the method described by Harborne (Harborne, 1998) and these tests were conducted in drug (leaf extract of *Pterospermum rubiginosum*) concentration of 5 mg/mL.

1. **Detection of Alkaloids**

A) Wagner's Test

To a clean test tube containing 1mL of an extract, a few drops of Wagner's reagent were added. The occurrence of alkaloids was indicated by the formation of a reddish brown precipitate.

B) Hager's Test

To a clean test tube containing 1mL of an extract, a few drops of Hager's reagent were added. The occurrence of alkaloids was indicated by the formation of yellow colour.

C) Meyer's Test

To a clean test tube containing 1mL of an extract, a few drops of Meyer's reagent were added. The formation of yellow or cream precipitate indicates the occurrence of alkaloids.

2. Detection of Flavonoids

Shinoda Test

To a clean test tube containing 1mL of an extract, a pinch of magnesium turnings and 1-2 drops of concentrated HCl were added into it. The occurrence of flavonoids was indicated by the formation of pink colour.

Lead Acetate Test

To a clean test tube containing 1mL of an extract, a few drops of 10% 1M lead acetate solution was added. The formation of yellow precipitate indicates the occurrence of flavonoids.

Alkaline Test

To a clean test tube containing 1mL of extract, a few drops diluted HCl was added. Observe the yellow colour which turns colourless of adding few drops of diluted HCl.

3. Detection of Phenols

Lead Acetate Test

To a clean test tube containing 1mL of extract, 3 mL of distilled water and few drops of 1% lead acetate was added. Observed for the formation yellowish cream precipitate.

4. **Detection of Tannins**

Braymer's Test

To a clean test tube containing 1mL of an extract, 1mL of water and 2-3 drop ferric chloride was added. The occurrence of tannins was indicated by the formation of a green precipitate.

5. **Detection of Saponins**

Froth Test

1g sample was added to the test tube containing 5 mL water and heated. Froth appearance indicates the positive result for saponins.

Foam Test

1g sample was added to the test tube containing 5 mL water and shakes it well. Foamy appearance indicates the positive result for saponins.

6. **Detection of Steroids**

Salkowski Test

To a clean test tube containing 1mL of an extract, 1mL of chloroform and sulphuric acid was added along the sides of the test tube. The occurrence of steroids was indicated by the formation of the reddish brown ring at the junction.

7. **Detection of Protein**

Xanthoproteic Test

To a clean test tube containing 1mL of extract, 1mL sulphuric acid was added. The formation of white precipitate shows the presence of protein.

Biuret Test

To a clean test tube containing 1mL of extract, 10% sodium hydroxide was added and heated. 0.7% of copper sulphate was also added after

heating. Formation of purplish violet colour was observed which indicates the occurrence of protein.

8. **Detection of Anthocyanin**

To a clean test tube containing 1mL of extract, 1 mL 2N HCl and a few drops of Ammonia was added. Observe the colour change from pinkish red to bluish violet, indicating the presence of anthocyanin.

9. **Detection of Carbohydrates**

Fehling's Test

To a clean test tube containing 1mL of extract, equal parts of solution A and B was added and kept it for a few minutes in a water bath *(Beston Industries, Cochin)*. The occurrence of reducing sugars indicated by the formation of brick red colour.

10. **Detection of Glycosides**

To a clean test tube containing 1mL of the extract, 1mL of chloroform and acetic acid was added into it. The formation of violet colour which changes to blue and then blue indicate the presence of glycosides.

Killer Killiani Test

To a clean test tube containing 1mL of extract, 1mL of distilled water and sodium hydroxide solution was added into it. Yellow coloration was observed, indicating the presence of glycosides.

11. **Detection of Phlobatanins**

To a clean test tube containing 1mL of extract, 1mL of dil. HCl was added and heated. Formation of a red precipitate indicates the presence of Phlobatanins.

3.4 ANTI-OXIDANT STUDIES

3.4.1 Total Phenolic Content

The total contents of phenolics were determined by the method of Singleton *et al.* (1965) with slight modification. leaf extract of *Pterospermum rubiginosum* (0.2mL) was taken and 2.5mLof Folin–Ciocalteu phenol reagent (1:50 v/v) was added and vigorously shaken. After 10 minutes of incubation, 2 mL of 7.5% w/v Sodium carbonate was added and the mixture was shaken thoroughly. After 30 min of incubation, the absorbance of the mixture was measured at 743 nm using a spectrophotometer. The concentration of polyphenols in the samples derived from a standard curve of Gallic acid ranging from 5 to 50μg/mL. The contents of total phenolics were expressed as milligrams of Gallic acid equivalents per gram of dry extract.

3.4.2 Total Flavonoid Content

The total contents of flavonoids were determined by the modified method done by (Zhishen *et al.*, 1991). 0.5mL leaf extract of *Pterospermum rubiginosum* was taken in a test tube and 1.5mL of 95% methanol, 0.1mL 10% Aluminium chloride (w/v), 0.1mL 1M potassium acetate and 2.8mL DD H_2O was added, mixed and vigorously shaken for 30 min. Then the absorbance of the mixture was measured at 415 nm using a spectrophotometer. The total flavonoids content of leaf extract of *Pterospermum rubiginosum* were expressed as milligrams of Quercetin equivalent (QE)/ g dry extract.

3.4.3 DPPH free radical scavenging assay

The DPPH radical (1, 1-diphenil-2- picrylhydrazyl) was determined by the method described by (Blois, 1958). A stock solution of ethanolic leaf extract of PR and L-Ascorbic acid as standard was prepared in the concentration of 1 mg/mL. Varying concentration from 50 to 250 μg/mL of leaf extract of *Pterospermum rubiginosum* and L-Ascorbic acid was taken respectively and

transferred into different tubes. Add 200 µL by methanol and 2mL DPPH (0.02mM) and incubated for 20 minutes at dark at room temperature and absorbance was measured at 517 nm by a spectrophotometer (Agilent Technologies). The annihilation activity of free radicals was calculated in percentage of scavenging according to the formula given below:

Percentage of scavenging = (Absorbance of control - Absorbance of Test)/ Absorbance of control * 100.

3.4.4 Nitric Oxide Scavenging Activity

Nitric oxide radical scavenging activity of ethanolic leaf extract of *Pterospermum rubiginosum* was investigated by the method described by (Marconii *et al.*, 1994). L- ascorbic acid was used as a standard. A stock solution of ethanolic leaf extract of *Pterospermum rubiginosum* (PR) and L-Ascorbic acid as standard was prepared in the concentration of 1 mg/mL. 1mL of leaf extract of PR and L-Ascorbic acid solution at different concentrations (50 to 250 µg/mL) was added into the different test tubes. 1 mL of SNP solution (10mM) was added and the tubes were incubated at 29°C for 2.5 hrs. 1mL of the incubated solution was taken and diluted with 1mL Griess reagent. Then the absorbance was measured at 532nm by a spectrophotometer (Agilent Technologies). Percentage inhibition is calculated according to the formula given below:

Percentage of scavenging = (Absorbance of control - Absorbance of Test)/ Absorbance of control * 100. Ascorbic acid was used as standard controls.

3.4.5 Hydroxyl Radical Scavenging Activity

Hydroxyl Radical Scavenging Activity was investigated by using the colorimetric deoxyribose method (Elizabeth *et al.*, 1990). The final volume of the reaction mixture is 5mL and it contains, 100µL 2-deoxyribose (2.8mM), KH_2PO_4.KOH buffer (20mM pH 7.4), 100µL $FeCl_3$ (100µM), 100µL EDTA (100µM), 100µL H_2O_2 (1.0mM), 100µL Ascorbic acid (100µM), and concentrations (50 to 250µg/mL) of the ethanolic leaf extract of *Pterospermum*

rubiginosum (PR) and L-Ascorbic acid as standard compound. After the incubation for one hour at 37^{0}C, 0.5mL of the reaction mixture was take in a test tube and 1.5mL of 2.8% of TCA and 1.5mL of 1% aqueous TBA was added and then the mixture was incubated at 100^{0}C for 15 minutes to develop the colour. After the incubation, the absorbance was measured at 530 nm by using a spectrophotometer. Quercetin was used as a positive control. Percentage of scavenging was evaluated by comparing the test and the blank solution.

Percentage of scavenging = (Absorbance of control - Absorbance of Test)/ Absorbance of control * 100.

3.5 Oral Acute Toxicity Study

Acute toxic effects of EPR was evaluated in Swiss albino mice using the method described by (Suja *et al.*, 2004). Before starting the study all groups were fasted for 12 h and weighed. The crude extracts were suspended in distilled water before administration. Five groups (A- E), consisting of four animals each. Group A-D animals were administrated orally with the help of gavage No. 16 with 25, 100, 400, 1600 mg/kg of body weight respectively. Group E received with vehicle only and it served as positive control. 0.5 mL of the test sample was orally given to the mice. After an hour of administration of the test samples, the feed was given to the mice. The mice were observed for 30 mins after administration of the test sample, followed by hourly observation for 6h and once a day for the next 14 days. Every observation was scientifically recorded and all records for individual mice are maintained for each test group. Survived mice were weighed and visually observed for pain and signs of illness, changes in physical appearance, injury, mortality, behavioural pattern etc.

3.6 ANTINOCICEPTIVE STUDIES

3.6.1 Acetic acid-induced writhing test

The writhing behaviour in mice was investigated based on the method described by (Koster *et al.*, 1959). Swiss albino mice of either sex were divided and categorized into five groups containing six animals each. Group A, the control group receives 0.5 mL Dist. H2O, Group B receive 0.5 mL Aspirin (10 mg/kg, in D. H2O, p. o.), Groups C, D and E receive various concentrations of the 0.5 mL of EPR (50, 150 and 450 mg/kg, p. o.). 1.0% Acetic acid solution (0.1 mL/10g body weight) was injected intraperitoneally in mice and the number of writhing's and stretchings was counted over a 15-min period. The plant extract and reference or co-solvent (control) were orally administered 30 min before Acetic acid. Percent reduction indicates the percentage protection against abdominal constriction which was taken as an index of analgesia. The percentage of inhibition of writhing count of the treated group was calculated from the mean writhing count of the control group.

$$\text{Percentage of Inhibition (\%)} = [(\text{No. of control}_{\text{writhing}} - \text{No. of the test group}_{\text{writhing}}) / \text{No. of control}_{\text{writhing}}] * 100$$

3.6.2 Eddy's Hot plate test

The thermal pain sensitivity was investigated to measure latency to paw licking by the method of (Eddy and Leimbach, 1953) hot plate. Swiss Albino mice of either sex were divided and categorized into 5 groups containing six animals each. Group A, the control group receive 0.5 mL Dist. H_2O, Group B receive 0.5 mL Aspirin (10 mg/kg, in D. H_2O, p. o.), Groups C, D and E receive various concentrations of the 0.5 mL of EPR (50, 150 and 450 mg/kg, p. o.). The temperature of the plate (Lyzer, Analgesometer type III) was fixed at 55 ± 10°C. The latency of nociceptive response such as licking of any of the hind legs or forelegs, jumping or tapping was recorded at 0, 15, 30, 60, 90, 120 min after drug administrations. The cut off time was put at 20 seconds to avoid tissue damage. When 30 sec elapsed without the animal presenting any of the response, it was

removed from the plate. The prolongation of the latency times comparing the values before and after administration of the test compounds.

3.7 ANTIINFLAMMATORY STUDIES

3.7.1 Carrageenan induces paw oedema

Antiinflammatory activity of EPR was investigated by the Carrageenan-induced paw oedema method described by (Winter *et al.*, 1962). Wistar rats of either sex were separated and categorized into five groups containing six animals each. Group A, the control group receive 1 mL Dist. H2O, Group B receives 1 mL Indomethacin (10 mg/kg, in D. H2O, p. o.), Groups C, D and E receive various concentrations of the 1 mL of EPR (50, 150 and 450 mg/kg, p. o.). After 30 mins of test sample administration, all animals of each group 0.1 mL of 1% carrageenan in saline were injected in the right hind limb under the plantar aponeurosis. The volume of the hind paw was measured using plethysmometer just before and after 3h carrageenan injection. The difference in the paw volumes will be recorded, which indicates the degree of inflammation. Percent inhibition of carrageenan-induced paw oedema was calculated using the formula given below:

Oedema volume = (oedema volume of control animals - oedema volume of EPR treated animals)

Percent inhibition = (oedema weight / oedema weight of control animals) * 100.

3.7.2 Formalin-induced paw oedema

The anti-inflammatory potential of EPR was studied by the formalin-induced paw oedema method described by (Chau, 1989) with slight modification. Wistar rats were categorized into five groups of six animals each. Group A, the control group receive 1 mL Dist. H2O, Group B receive 1 mL Indomethacin (10 mg/kg, in D. H2O, p. o.), Groups C, D and E receive various concentrations of the 1 mL of EPR (50, 150 and 450 mg/kg, p. o.). After 30 min of test sample administration, all animals of each group were injected with 0.1mL of 2% of formalin in saline were injected in the right hind paw under the plantar aponeurosis.

The drug was administered once in a day for 7 days. On the first and seventh day, after 1hr of drug administration paw volume was measured using a plethysmometer. The difference in paw thickness before and after induction of inflammation was calculated and presented as a mean increase in paw volume (mm). The difference in the paw volumes will be recorded, which indicates the degree of inflammation. Percent inhibition of paw oedema was calculated using the formula given below:

Oedema weight = (Oedema weight of control animals - Oedema weight of EPR treated animals)

Percent inhibition = (Oedema weight / Oedema weight of control animals) * 100.

3.8 Statistical analysis

Results were expressed as mean ± standard error of mean and presented as graphs and tables. Data were analysed by using a statistical software called IBM SPSS/PC+, Version 20.0 (SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) utilised for investigating the comparison of significant differences between groups. Pair-fed comparisons between groups were made by Duncan's. The results were considered significant at $p<0.05$.

RESULTS

Plate 6: Leaf pattern of the *Pterospermum rubiginosum*. a. Ventral view, b. Dorsal view, c. Stem and d. Aerial view of the tree.

4. RESULTS

4.1 PHARMACOGNOSTICAL STUDIES

4.1.1 Morphological / Macroscopic Features

The plant grows up to 35-40 m height, bark 3-6 mm thick and flowers during November to April. The morphological character of the tree (Plate 6) and parameters of fresh leaves were studied and observations were tabulated (Table 1).

Table 1: Morphological characters of leaf of *Pterospermum rubiginosum*

Sl. No	Morphological characters	Observation
1	Shape	Falcate
2	Margin type	Entire
3	Tip	Caudata
4	Arrangement	Alternate simple
5	Venation	Reticulate
6	Base	Cordata in one side and acute on other side
7	Texture	Pubescent beneath and leathery above
8	Stipules	Present
9	Petiole	Present

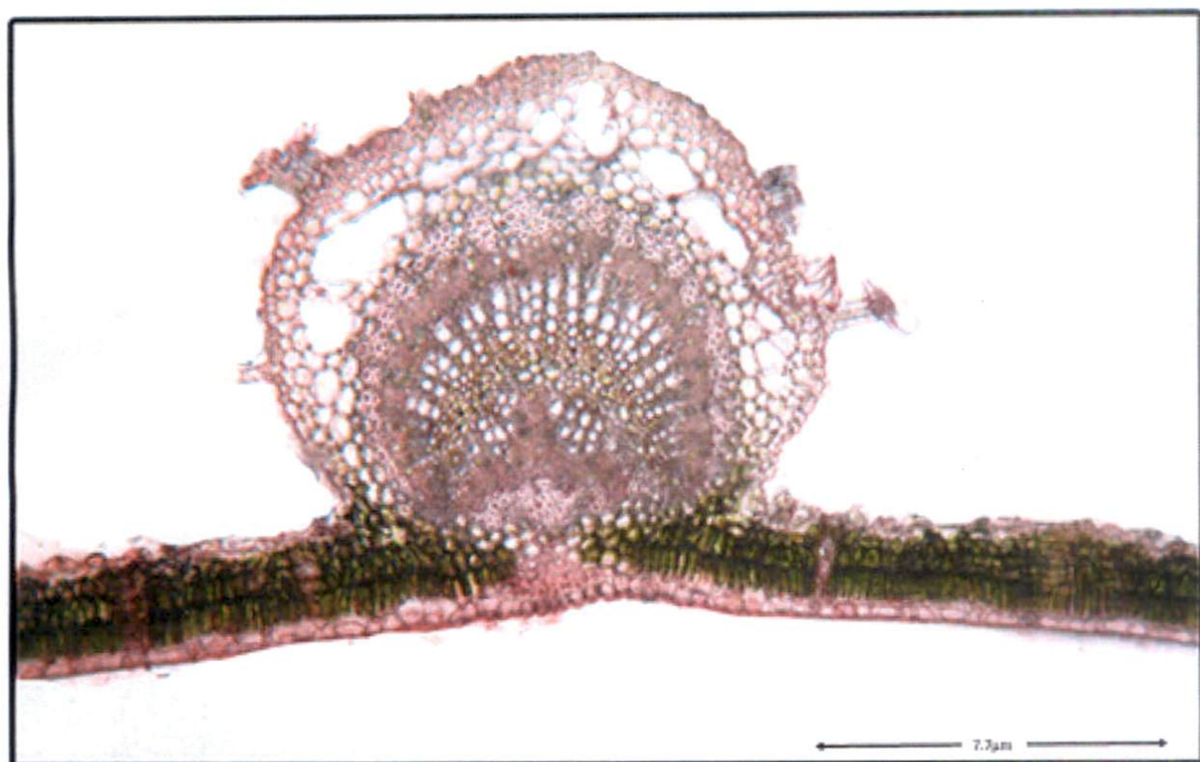


Plate 7: Transverse section of midrib and lamina of *P. rubiginosum* leaf

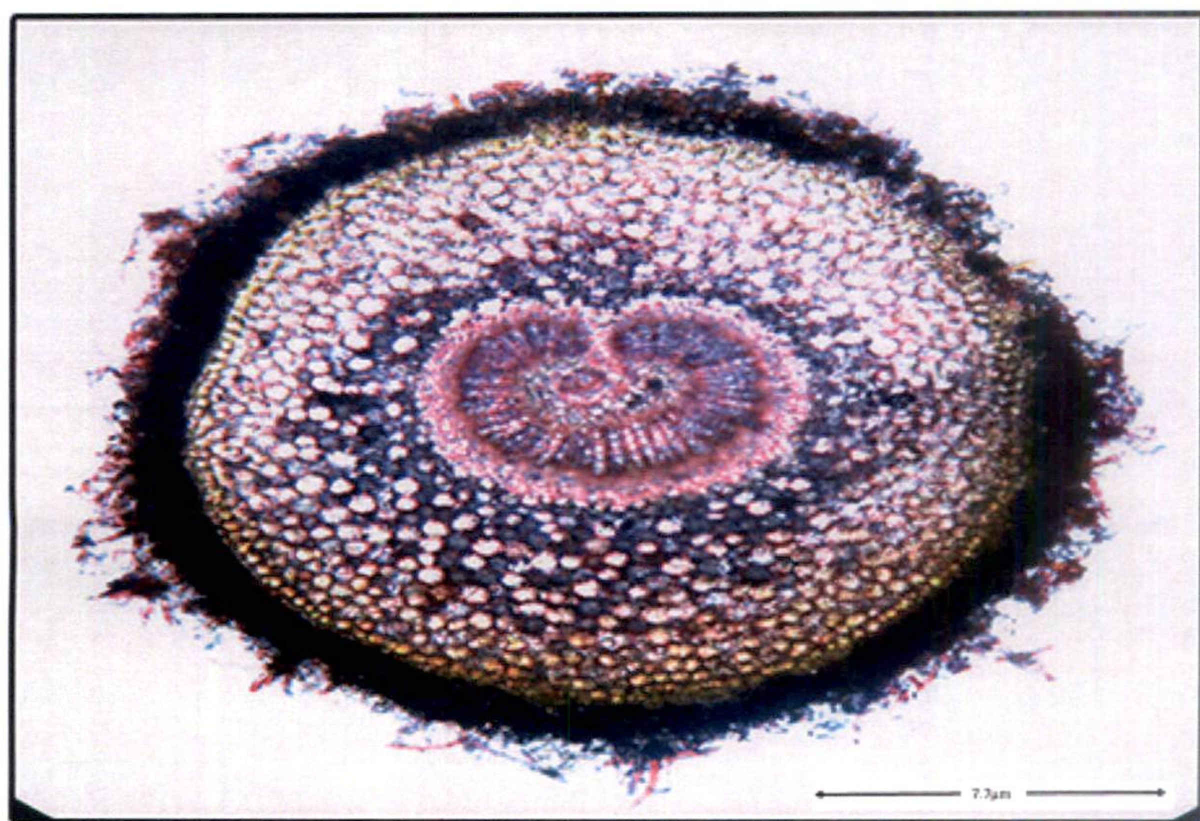


Plate 8: Transverse section of petiole of *P. rubiginosum* leaf

10	Colour	Green on dorsal and Golden brown on ventral
11	Lateral nerves	4-6 pairs
12	Leaf length	Ranges from 4-13 cm
13	Leaf width	Ranges from 2-4 cm
14	L / W of leaf	2.97
15	Odour	Tea leaf aroma
16	Taste	Bitter

Each Values is the mean, for n = 10

4.1.2 Microscopic characters

Transverse section of *P. rubiginosum* leaf showed the following tissue pattern in the midrib and petiole (Plates 6 and 7).

Midrib: Single layer of epidermis followed by two layer of palisade cells. Lower layer of epidermis with multicellular hairs. In the mid rib region single layer of epidermis followed by small patches of sclerenchyma cells. Small patches of phloem followed by xylem, Protoxylem towards the centre Xylem cells are surrounded by sclerenchyma cells. Followed by parenchyma cells an air cavities are present. Parenchyma cells with intercellular space are arranged around the air cavities. Outer layer of epidermis (Barrel shaped) with multicellular hairs. Dorsi ventral, single layered epidermis with lysogenous mucilage cavities, abaxial epidermis contains more stomata than adaxial epidermis, stomata's were anomocytic, numerous hairs are present, lamina contains secretory cavities, adaxial

hypodermis present, Hypodermis is chlorenchymatous. mesophyll contains both palisade and spongy tissues.

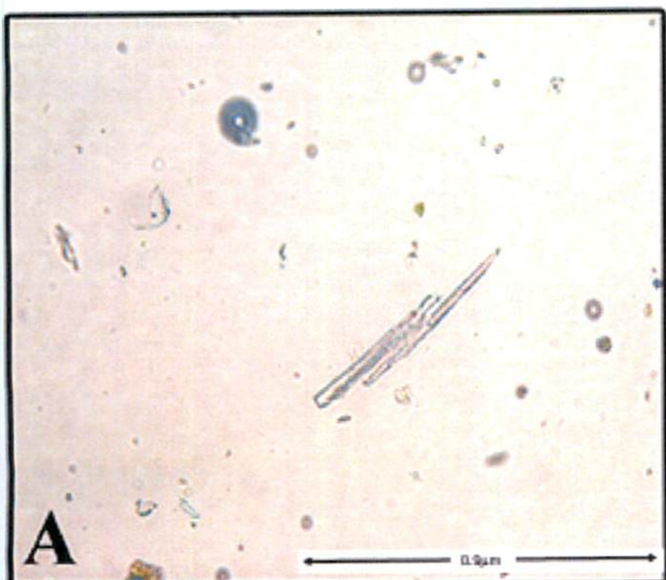
Petiole: Epidermis of petiole is single layered of barrel shape, elongated compact cells having no intercellular spaces between them. Hypodermis is multi-layered of collenchyma and sclerenchyma. Ground tissue is thin walled parenchyma cells and have definite intercellular space between them. The vascular bundle is nearly complete ring. Vascular bundles are of various size. The biggest vascular bundle is towards the lower surface and much smaller one on the lateral bundles, consists of both xylem and phloem. Xylem is found on the upper side whereas phloem towards the inner.

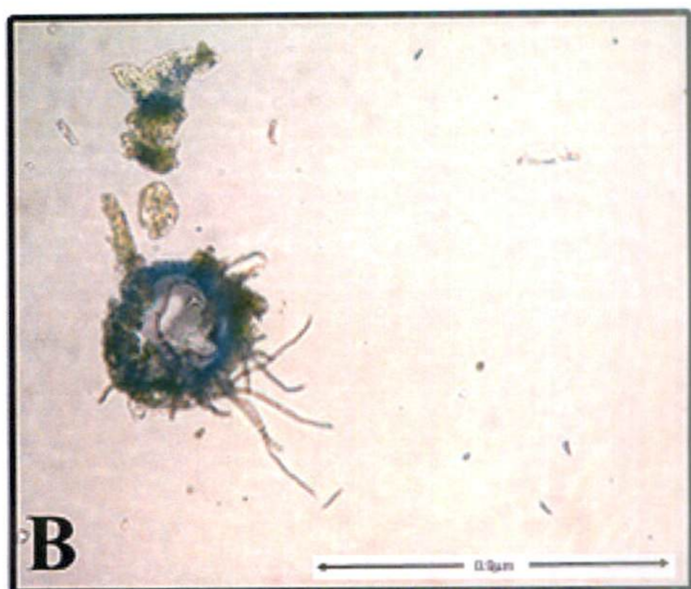
4.1.3 Organoleptic evaluation of leaf powder

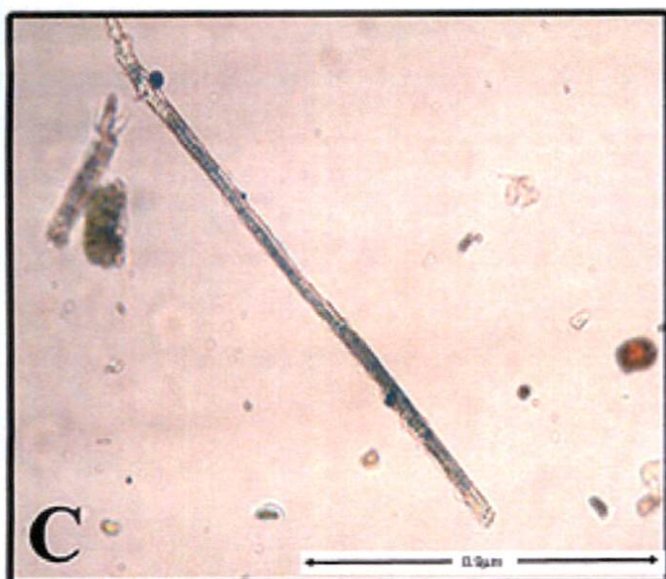
The leaf powder has greenish brown colour, a characteristic odour (tea leaves) and bitter taste. The powder is coarse in texture and not much free flowing. The microscopic observation of the leaf powder showed the following structures such as fibres in bundle, trichome, fibre were seen (Plate 9).

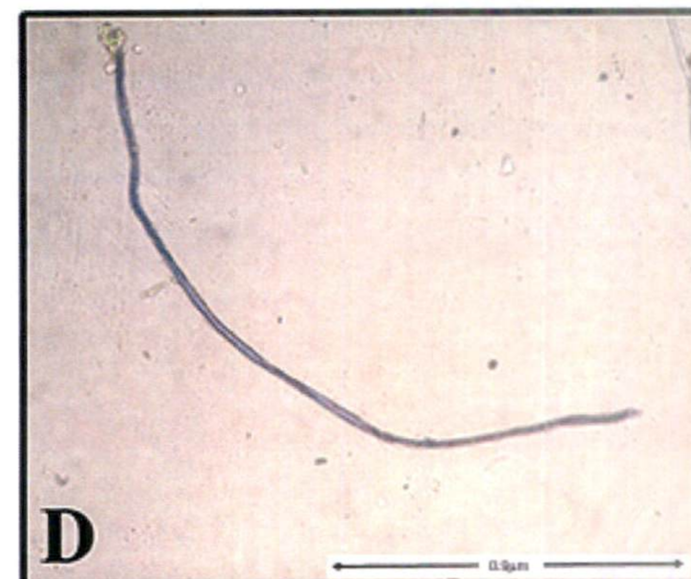
4.1.4 Physico-chemical standardization

The physico-chemical characters of the leaf powder were analysed. The percentage of total ash value, acid insoluble ash, water soluble ash, crude fibre content, swelling index and moisture loss on drying at 105°C were determined (Table 2). The percentage of individual and successive extractive values, consistency and colour were noted and tabulated (Table 3).









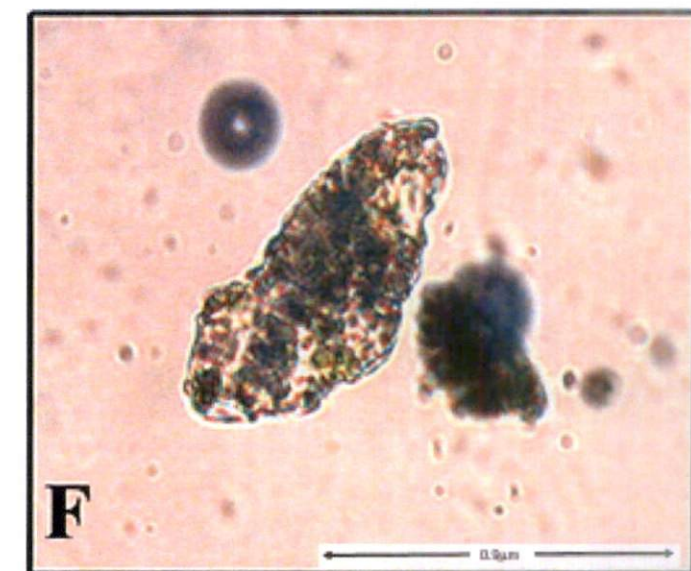


Plate 9: Powder microscopy of *Pterospermum rubiginosum* leaf: (A) Fibers in bundle, (B) tellate covering trichome with thickened wall, (C) Pitted Vessels, (D) Fibre, (E)Annular vessels from vascular bundle , (F) Tissue fragment.

Table 2: Physio chemical parameters of powdered Leaves of *P. rubiginosum*.

Sl. No	Physio chemical parameters	Values (% w/w)
1	Foreign matters	Nil
2	Total Moisture Content	52.57 ± 0.20
3	Total Fibre Content	7.86 ± 0.37
4	Total Ash	12.84 ± 0.07
5	Acid insoluble ash	3.65 ± 0.07
6	Water insoluble ash	9.12 ± 0.13
7	Swelling Index	Expressed in mL
	Initial Volume	3.7 ± 0.15
	Final Volume	9.8 ± 0.15

Values are the mean ± SD, n = 3.

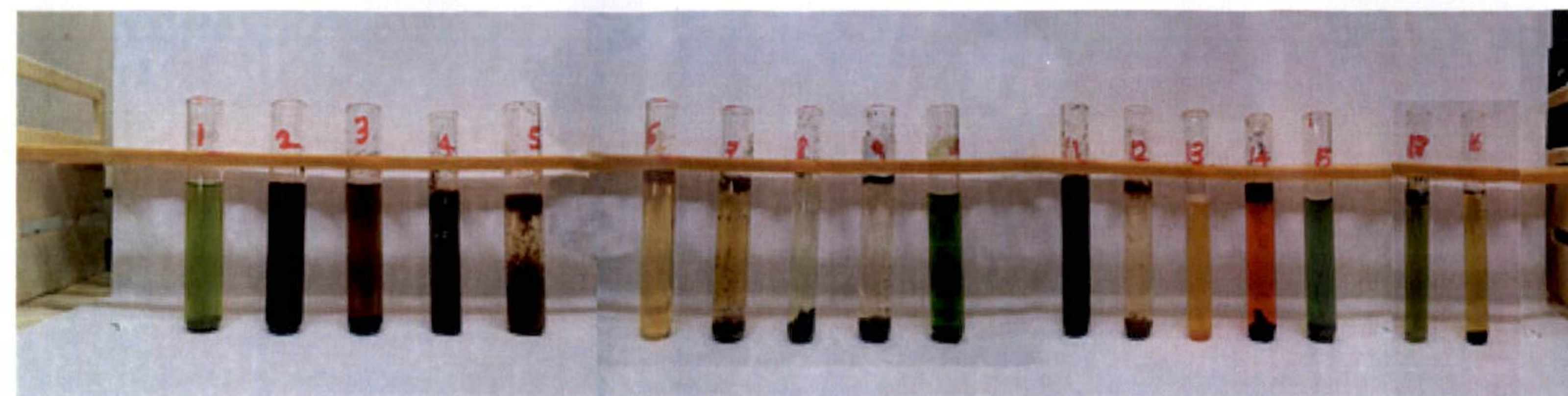
Table 3: Individual Extractive values of powdered leaves of *P. rubiginosum*

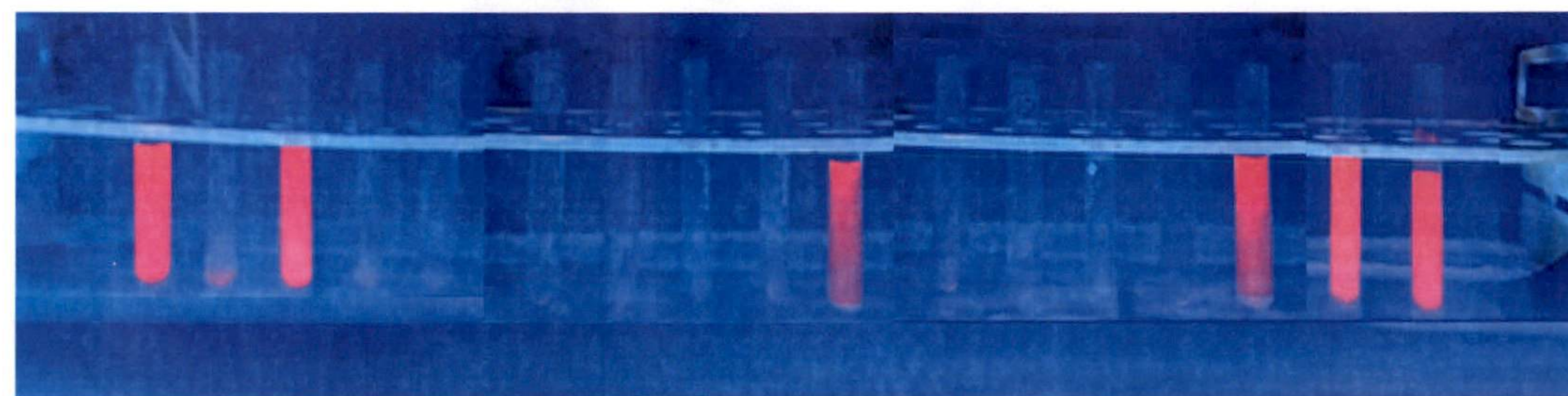
Sl. No.	Solvent	Yield (%w/w)	Colour	Consistency
1.	n Hexane	2.35 ±0.11	Light green	Sticky
2.	Ethyl acetate	2.68 ± 0.04	Green	Sticky
3.	Acetone	1.75 ± 0.06	Green	Sticky
4.	Chloroform	2.99 ± 0.08	Green	Sticky
5.	Ethanol	2.43 ± 0.09	Green	Sticky
6.	Hydro alcohol	8.57 ± 0.05	Reddish brown	Powdery
7.	Water	6.48 ± 0.16	Light red	Powdery

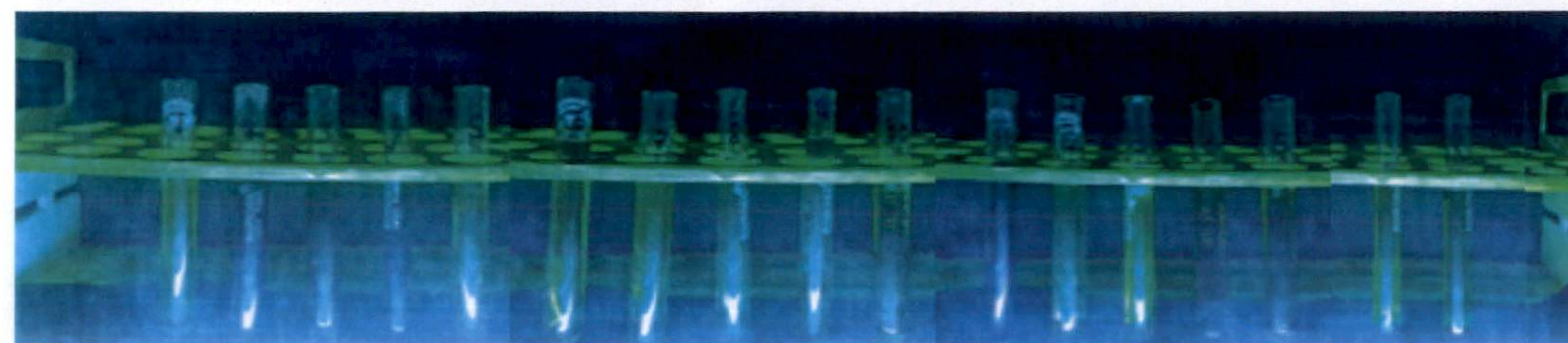
Values are the mean ± SD, n = 3.

4.1.5 Fluorescence property

The fluorescence behaviour of the powdered leaf of *P. rubiginosum* with different chemical reagent determined at both visible light and UV (Short UV 254nm and Long UV 365nm) (Table 4 and Plate10).







Sl. No	Treatment
1	Powder +acetone
2	Powder +1M NaOH
3	Powder +1M NaOH +methanol
4	Powder +1M NaOH +water
5	Powder +1M HCl
6	Powder +dil. HNO_3
7	Powder +5% Iodine
8	Powder +5% $FeCl_2$
9	Powder +dil. Ammonia
10	Powder +methanol
11	Powder +HCl
12	Powder +1M H_2SO_4
13	Powder +HNO_3
14	Powder +KCr_2O_7
15	Powder +95% ethanol
16	Powder +toluene
17	Powder +chloroform

Plate 10: Fluorescence analysis of leaf powder of *P. rubiginosum*.

173922

Table 4: Fluorescence analysis of leaf powder of *Pterospermum rubiginosum*

Sl. No	Treatment	Visible light*	Short UV (254nm)	Long UV (360nm)
1	Powder +acetone	Yellow green	Yellow green	Heather violet
2	Powder +1M NaOH	Chest nut brown	Black Blue	Light Pink
3	Powder +1M NaOH +methanol	Nut brown	Fir green	Traffic Violet
4	Powder +1M NaOH +water	Chocolate brown	Black green	Light Pink
5	Powder +1M HCl	honey brown	Yellow Green	Light Pink
6	Powder +dil. HNO_3	Broom yellow	Light Green	Water Blue
7	Powder +5% Iodine	Dark Golden yellow	Yellow Green	Traffic Blue
8	Powder +5% $FeCl_2$	Pale green	Grass Green	Traffic Blue
9	Powder +dil. Ammonia	Pale green	Water Blue	Water Blue

10	Powder +methanol	Leaf Green	Light green	Heather Violet
11	Powder +HCl	Chocolate brown	Steel Blue	Purple Violet
12	Powder +1M H_2SO_4	Green brown	Grass Green	Purple Violet
13	Powder +HNO_3	Mellon green	Yellow Green	Purple Violet
14	Powder +KCr_2O_7	Traffic red	Fern Green	Purple Violet
15	Powder +95% ethanol	Grass green	Pigeon Blue	Traffic Purple
16	Powder +toluene	Grass green	Yellow green	Heather Violet
17	Powder +chloroform	Curry yellow	Green Yellow	Heather Violet
18	Powder alone	Green	Greenish Brown	Greenish Brown

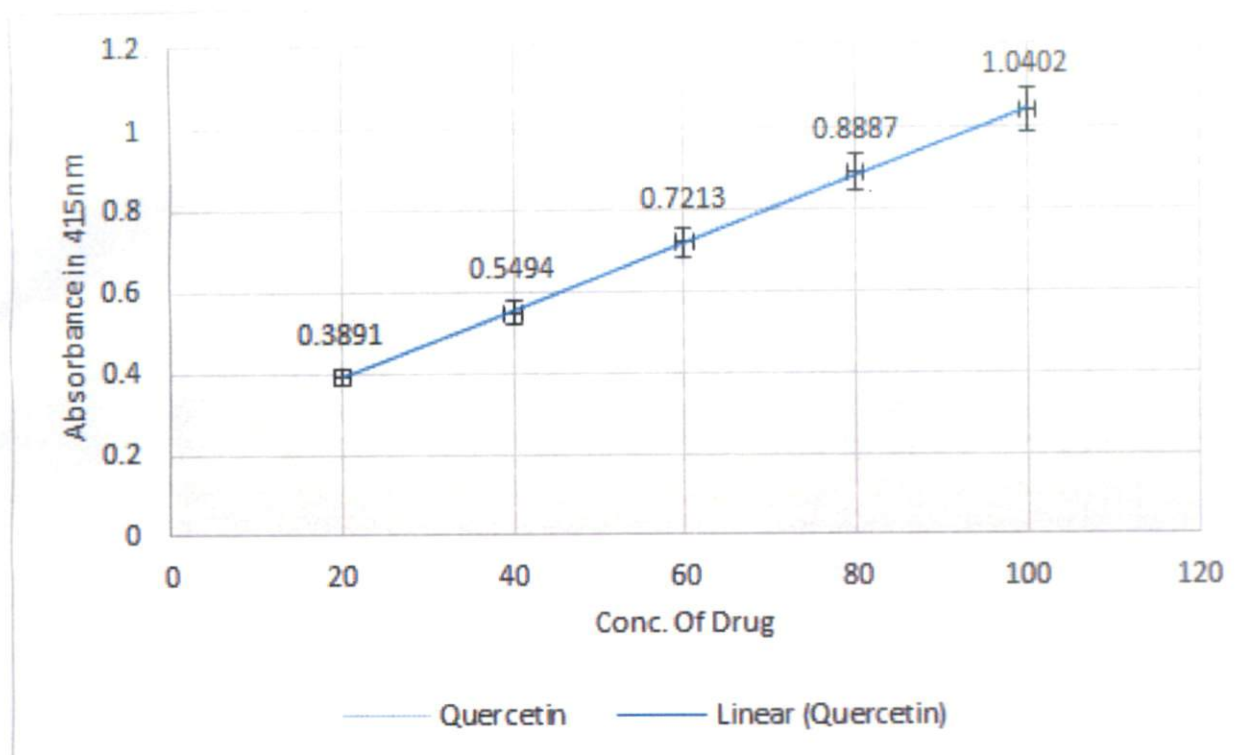
4.2 PRELIMINARY PHYTOCHEMICAL SCREENING

The various solvent extracts have showed the presence of different compounds in (Table 5).

Table 5: Preliminary phytochemical screening of ethanolic leaf extract of *P. rubiginosum*

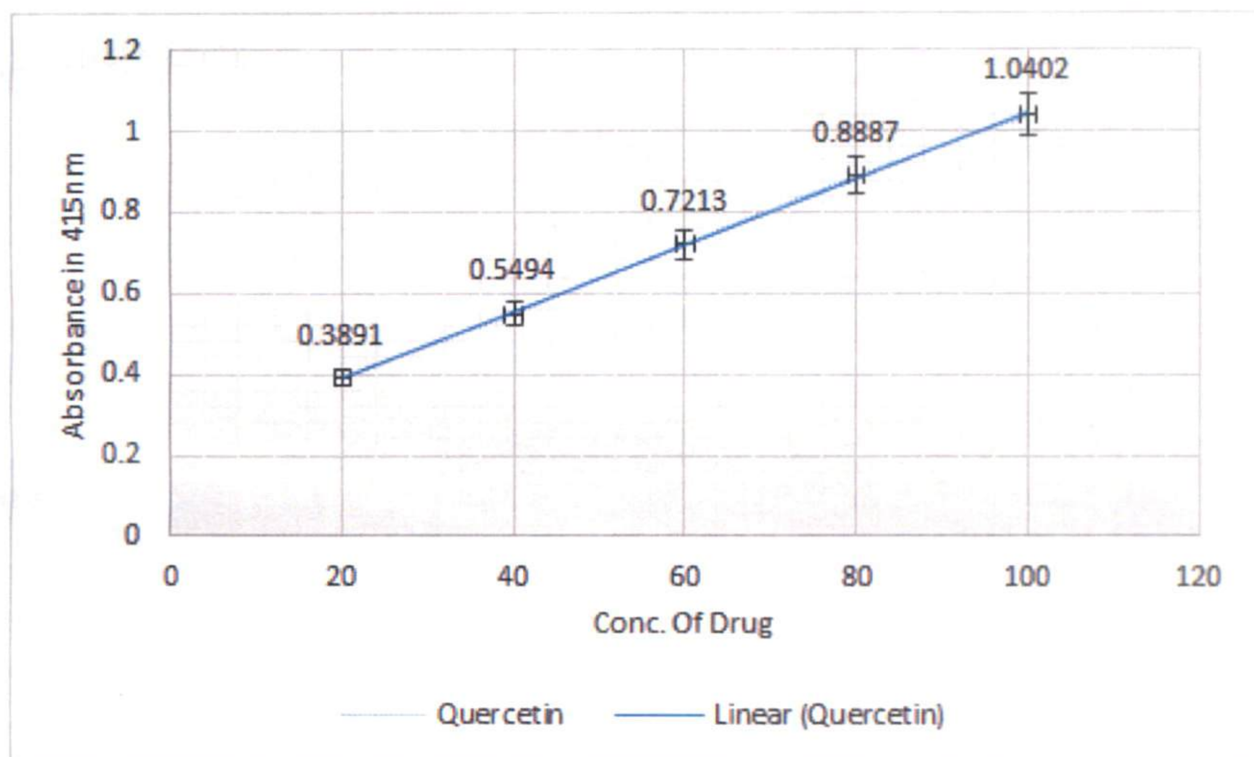
Si. No	Compounds	Acetone	Chlor-oform	Ethanol	Ethyl Acetate	Hexane	Hydro-ethanol	H_2O
1	Phenols	+	+	+	+	+	+	-
2	Flavonoids	+	+	+	-	-	+	+
3	Steroids	-	-	-	-	-	+	-
4	Alkaloids	+	-	+	-	-	-	+
5	Coumarin	-	-	+	-	-	+	+
6	Carbohydrates	+	+	+	+	-	+	+
7	Tannins	+	+	-	+	-	-	-
8	Saponins	+	+	+	+	+	+	+
9	Glycosides	+	+	+	+	-	+	+
10	Phlobatannin	–	-	-	+	-	-	+
11	Proteins	-	-	-	-	-	-	-

+ present and – absent



The TPC of ethanol extract = 22.68 µg GAE / 0.5 mg mass of extract

Figure 1: The total phenolic content of ethanolic extract of leaf of *P. rubiginosum*



The TFC of ethanol extract = 11.341 µg QE / 0.5 mg mass of extract

Figure 2: The total flavonoid content of ethanolic extract of leaf of *P. rubiginosum*

4.3 ANTIOXIDANT STUDIES

4.3.1 Total phenolic content

The values are expressed as Gallic acid equivalents (GA). The total phenolic content of ethanolic extract of leaves of *P. rubiginosum* was found to be 22.68 µg GA / 0.5 mg mass of extract (Table 6 and Figure 1).

Table 6: The total phenolic content of leaf extract of *P. rubiginosum*.

Conc. of Gallic acid (µg/mL)	Mean Absorbance (743 nm)
10	0.040 ± 0.004
30	0.233 ± 0.008
50	0.419 ± 0.004
70	0.648 ± 0.006
90	0.912 ± 0.003
Sample:	
Ethanolic extract EPR (0.5 mg/mL)	0.245 ± 0.013

The TPC of ethanol extract = 22.68 µg GA / 0.5 mg mass of extract

4.3.2 Total flavonoid Content

The values are expressed as Quercetin equivalents (QE). The total flavonoid content of the plant ethanolic leaf extract of *P. rubiginosum* was found that in 500µl sample contains 11.341 µg QE / 0.5 mg mass of extract. (Table 7 and Figure 2).

Table 7: Determination of total flavonoid content in *P. rubiginosum* leaf extract.

Conc. of Quercetin (μg/mL)	Mean Absorbance (415 nm)
20	0.3891 ± 0.01
40	0.5494 ± 0.002
60	0.7213 ± 0.001732
80	0.889033± 0.001528
100	1.042 ± 0.00347
Sample:	
Ethanolic Extract EPR (0.5 mg/mL)	0.3183 ± 0.003602

The TFC of ethanol extract = 11.341 μg QE / 0.5 mg mass of extract

4.3.3 DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of the ethanolic leaf extract of *P. rubiginosum* was shown in table 8. The results were compared with that of L-Ascorbate. /IC_{50} value was determined from the calibration curve (Table 8 and Figure 3). The lower IC_{50} value reflects to higher antioxidant activity of plant extracts.

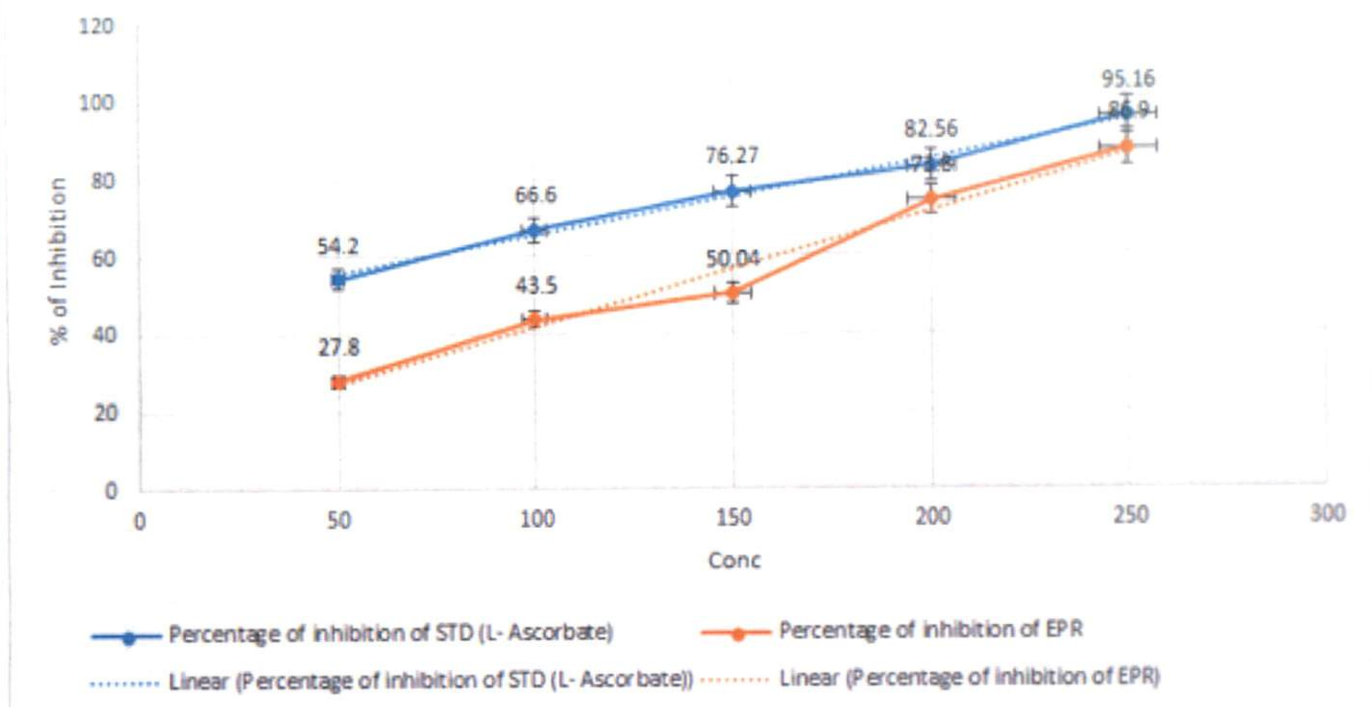
Table 8: Effect of L-Ascorbic acid and ethanolic extract of leaves *of P. rubiginosum* on DPPH Radical Scavenging Activity.

Sl. No	Conc. Of Standard (L-Ascorbic acid AA)	Percentage of Scavenging	IC_{50} Value	Conc. of EPR	Percentage of Scavenging	IC_{50} Value
1	AA 50 µg/mL	54.20 %	22.5 µg/mL	EPR50 µg/mL	27.80 %	128.42 µg/mL
2	AA 100 µg/mL	66.60 %		EPR 100 µg/mL	43.50 %	
3	AA 150 µg/mL	76.27%		EPR 150 µg/mL	50.04 %	
4	AA 200 µg/mL	82.56 %		EPR 200 µg/mL	73.80 %	
5	AA 250 µg/mL	95.16 %		EPR 250 µg/mL	86.90 %	

Values are the mean ± SD, n = 6.

4.3.4 Nitric oxide scavenging assay

The Nitric oxide radical scavenging activity of the ethanolic extract of leaves of *P. rubiginosum* was shown in table 9. The results were compared with that of L-Ascorbate. IC_{50} value was determined from the calibration curve (Table 9 and Figure 4).



Values are expressed as mean ± SD (n =6)

Figure 3: DPPH radical scavenging activity of *Pterospermum rubiginosum*

120
100
80
60
40
20
0
% of inhibition
52.39
69.2
84.39
92.08
94.35
71.4
56.86
51.09
41.99
31.61
0
50
100
150
200
250
300
Conc.
Percentage of inhibition of STD
Percentage of inhibition of EPR
Linear (Percentage of inhibition of STD)
Linear (Percentage of inhibition of EPR)

Values are expressed as mean ± SD (n =6)

Figure 4: Nitric oxide radical scavenging activity of *Pterospermum rubiginosum*

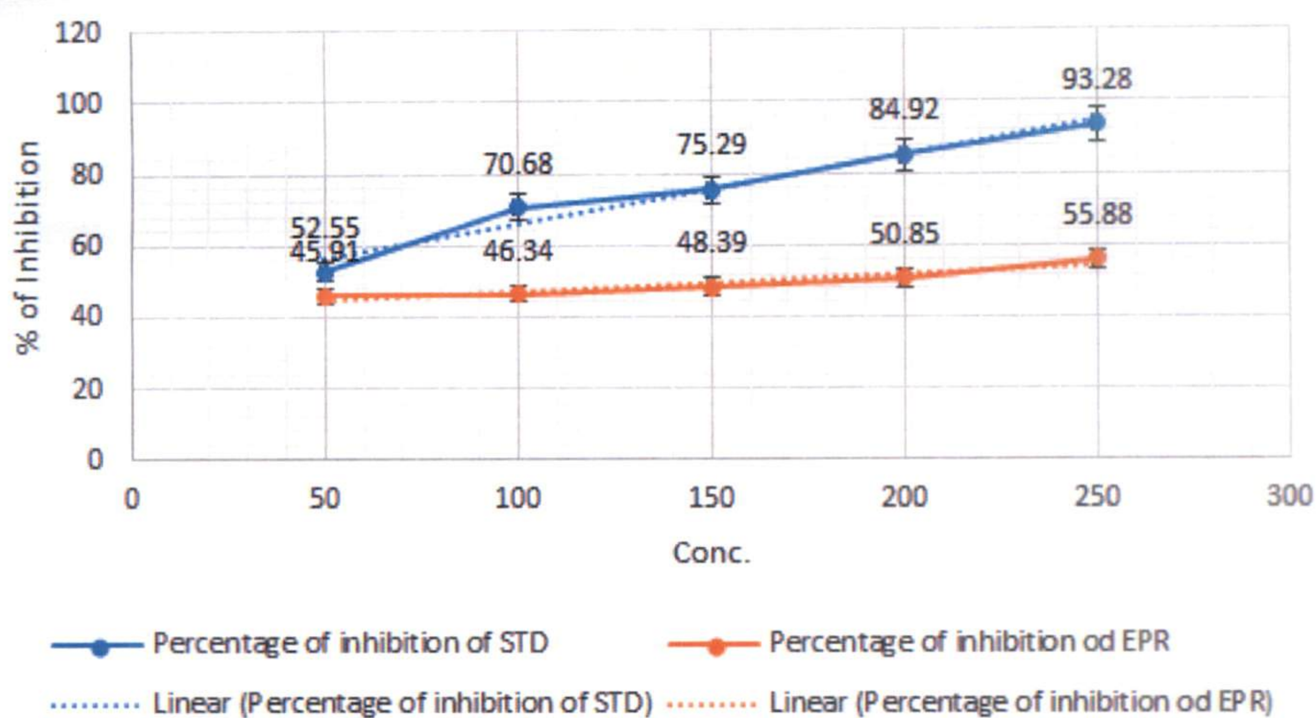
Table 9: Effect of L-Ascorbic acid and ethanolic leaf extract of *P. rubiginosum* on Nitric Oxide Scavenging Activity.

SI. No	Conc. of Standard (L-Ascorbic acid AA)	Percentage of Scavenging	IC_{50} Value	Conc. of EPR	Percentage of Scavenging	IC_{50} Value
1	AA 50 µg/mL	52.39 %	16.56 µg/mL	EPR 50 µg/mL	31.61%	146.87 µg/mL
2	AA 100 µg/mL	69.20 %		EPR 100 µg/mL	41.99%	
3	AA 150 µg/mL	84.39 %		EPR 150 µg/mL	51.09%	
4	AA 200 µg/mL	92.08 %		EPR 200 µg/mL	56.86%	
5	AA 250 µg/mL	94.35 %		EPR 250 µg/mL	71.40%	

Values are the mean ± SD, n = 6.

4.3.5 Hydroxyl radical scavenging assay

The Hydroxyl radical scavenging activity of the ethanolic extract of leaves of *P. rubiginosum* was shown in table 10. The results were compared with that of L-Ascorbate. IC_{50} value was determined from the calibration curve (Table 10 and Figure 5).



Values are expressed as mean ± SD (n =6)

Figure 5: Hydroxyl radical scavenging activity of *Pterospermum rubiginosum*

Table 10: Effect of L-Ascorbate and ethanolic leaf extract of *P. rubiginosum* on Hydroxyl radical scavenging activity.

Sl. No	Conc. Of Standard (L-Ascorbic acid AA)	Percentage of Scavenging (517nm)	IC_{50} Value	Conc. of EPR	Percentage of Scavenging (517nm)	IC_{50} Value
1	AA 50 µg/mL	52.55 %	17.91 µg/mL	EPR 50 µg/mL	45.91%	163.23 µg/mL
2	AA 100 µg/mL	70.68 %		EPR 100 µg/mL	46.34%	
3	AA 150 µg/mL	75.29 %		EPR 150 µg/mL	48.39%	
4	AA 200 µg/mL	84.92%		EPR 200 µg/mL	50.85%	
5	AA 250 µg/mL	93.28 %		EPR 250 µg/mL	55.88%	

Values are the mean ± SD, n = 6.

4.4 Oral acute toxicity study

Acute toxicity study of the aqueous extract of *Pterospermum rubiginosum* B. Heyne ex G. Don administered in mice orally. It doesn't show any mortality at the highest dose of 1500 mg / kg weight of the animal. And other parameters indicating toxicity of the drug such as Digestion, food intake, fatigue, behavioural pattern etc. of test animals observed and the result tabulated on the (table 11).

Table 11: Cage side observation of acute oral toxicity studies of ethanolic extract of leaves of *P. rubiginosum*.

Sl. No.	Parameters	EPR 50 mg/kg	EPR 100 mg/kg	EPR 400 mg/kg	EPR 1600 mg/kg
1	Respiration	Normal	Normal	Normal	Normal
1	Condition of the fur	Normal	Normal	Normal	Normal
2	Skin	Normal	Normal	Normal	Normal
3	Subcutaneous swellings	Nil	Nil	Nil	Nil
4	Abdominal distension	Nil	Nil	Nil	Nil
7	Pupil diameter	Normal	Normal	Normal	Normal
8	Ptosis	Nil	Nil	Nil	Nil
9	Colour and consistency of the faeces	Normal	Normal	Normal	Normal
10	Wetness or soiling of the perineum	Nil	Nil	Nil	Nil
11	Condition of teeth	Normal	Normal	Normal	Normal
12	Breathing abnormalities	Nil	Nil	Nil	Nil

13	Gait	Normal	Normal	Normal	Normal

*Four animals per group (Groups of four)

4.6 ANTI NOCICEPTIVE STUDIES

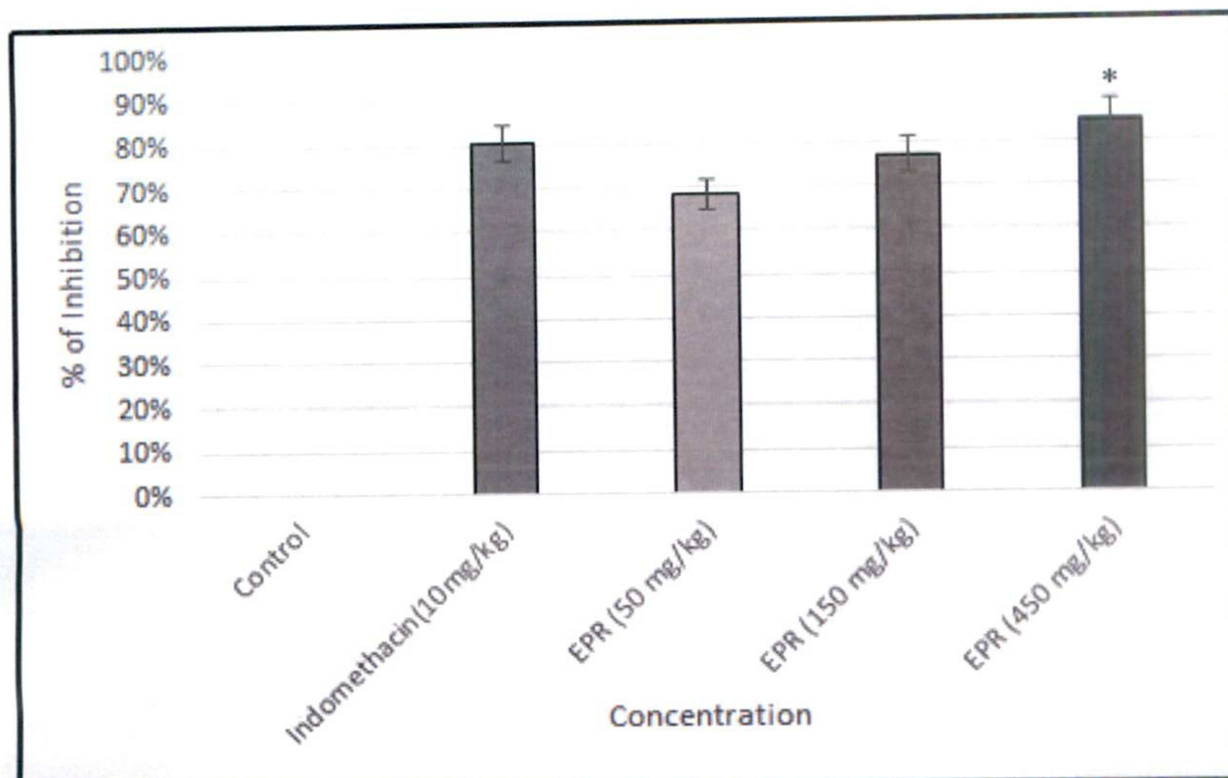
4.6.1 Acetic Acid Induced Writhing

Effect of ethanolic extract of leaves *of P. rubiginosum* on acetic acid induced writhing investigated in mice and the value are presented in the table 12 and graphically in figure 6. At a dose of EPR 450 mg/kg showed the maximum percentage of inhibition in mice around obtained was 85.24% and the mean number of writhes caused by the acetic acid was significantly lower than the that of control group. Acetyl salicylic acid used as standard drug and it also showed a promising protective role by inducing a protection of 80.32% of inhibition at a dose of 10mg/kg body weight against the pain.

Table 12: Effect of ethanolic leaf extract *of P. rubiginosum* and aspirin *on* acetic acid induced writhing in mice.

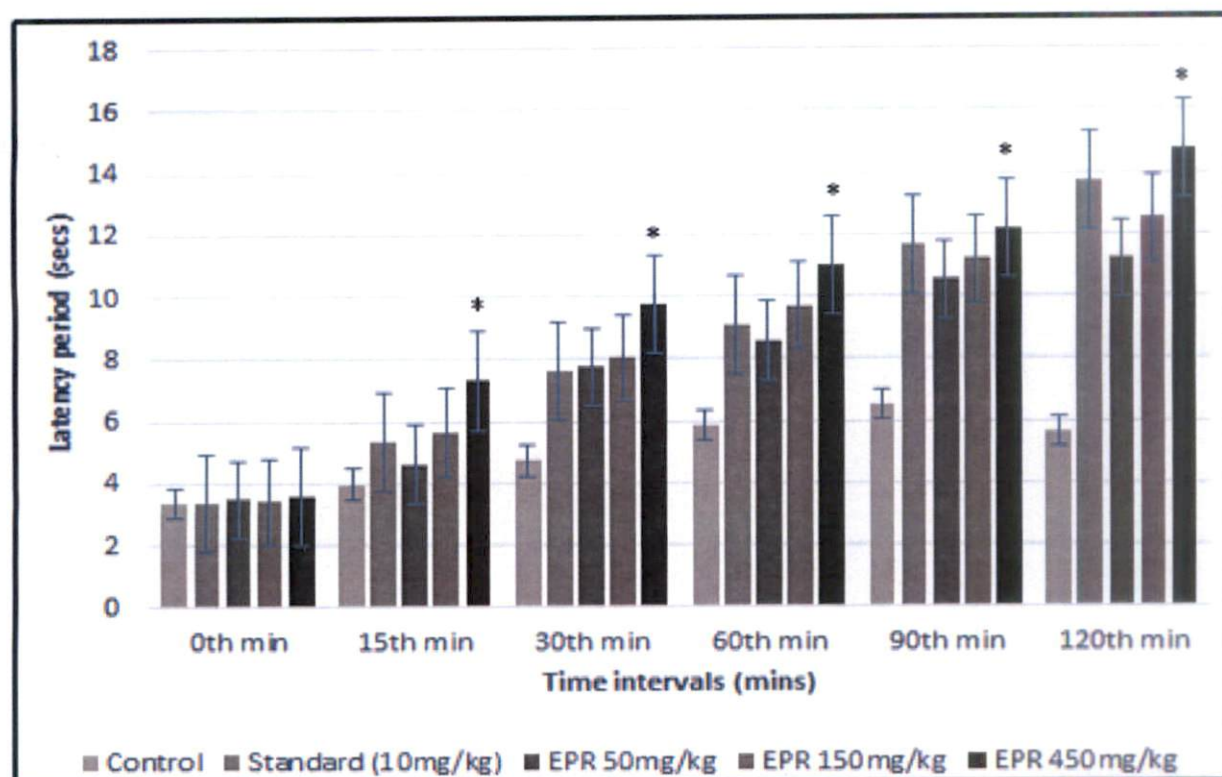
Si No.	Drug Concentration	Mean number of writhes in 20 min	Percentage of Inhibition
1	Control	61 ± 1.00	----
2	Aspirin 10mg/kg	12 ± 1.32	80.32%
3	EPR 50 mg/kg	19 ± 1.04	68.85%
4	EPR 150 mg/kg	14 ± 1.00	77.04%
5	EPR 450 mg/kg	9 ± 1.04	85.24%

Values are the mean ± SD, n = 6.



Each Values is the mean ± SD, for n = 6 mice.
*P<0.05 compared with standard aspirin treated group .
Data were analysed by using one way ANOVA followed by Duncan's Test.

Figure 6: Effect of *Pterospermum rubiginosum* extract in acetic acid induced writhing in mice.



Each Values is the mean ± SD, for n = 6 mice.
*P<0.05 compared with standard aspirin treated group .
Data were analysed by using one way ANOVA followed by Duncan's Test.

Figure 7: Effect of ethanolic extract of leaves of *Pterospermum rubiginosum* on Eddy's Hot plate method in mice.

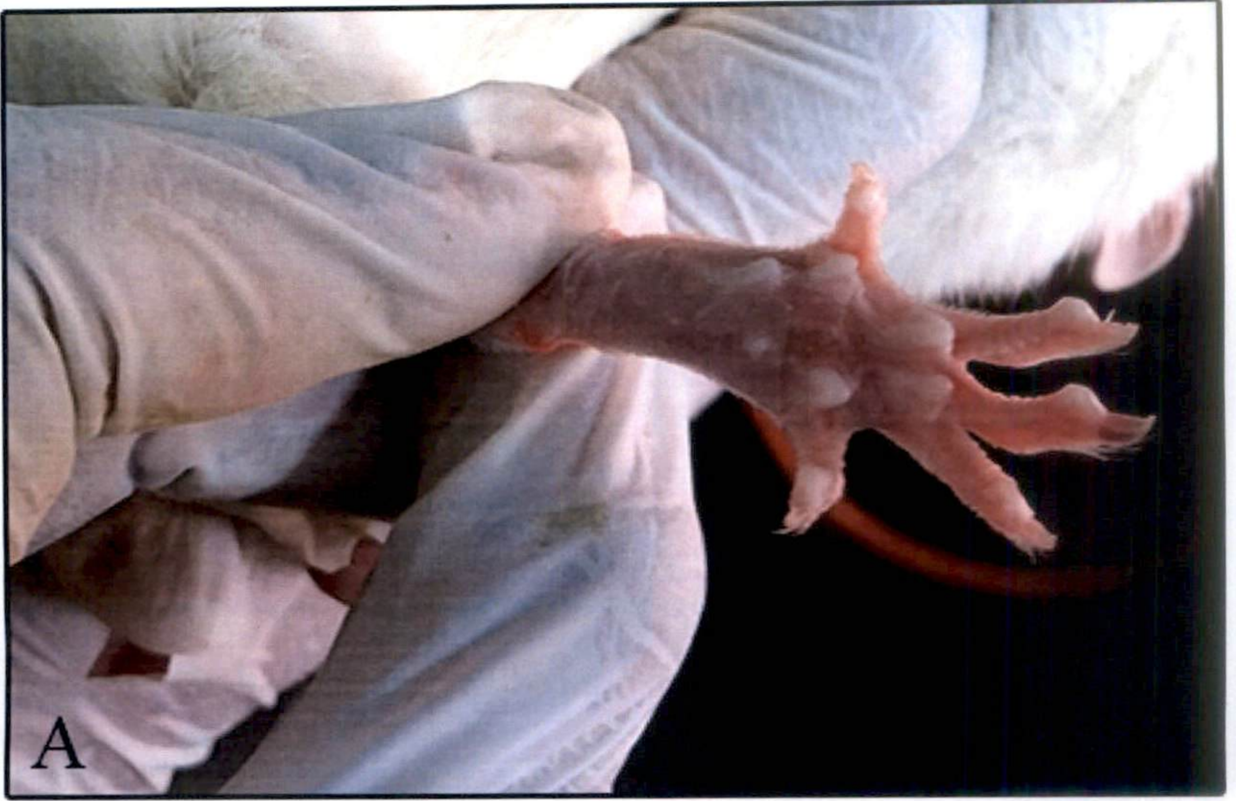
4.6.2 Eddy's Hot plate method

Effect of ethanolic leaf extract of *P. rubiginosum* on Eddy's hot plate method are presented in the Table 13 and graphically represented in figure 7. At a dose of EPR 450 mg/kg was showed the maximum protective effect of 62.02% inhibition was obtained and the mean number of paw licking was significantly showed lesser protective effect than the that of other treatment groups including control and standard group. Acetyl salicylic acid showed a major protective role of 56.66% of inhibition at a dose of 10mg/kg body weight.

Table 13: Effect of aspirin and ethanolic extract of leaves *of P. rubiginosum* on Eddy's Hot plate method in mice.

Sl. No.	Concentration	0th min	15th min	30th min	60th min	90th min	120th min
1	Control	3.3883	3.99	4.7383	5.885	6.5217	5.6533
2	Standard (10mg/kg)	3.37	5.33	7.6017	9.075	11.6433	13.6967
3	EPR 50mg/kg	3.4917	4.6	7.745	8.6017	10.535	11.1883
4	EPR 150mg/kg	3.4317	5.6433	8.045	9.71	11.2	12.5133
5	EPR 450mg/kg	3.6	7.3067	9.7667	10.99	12.1733	14.7367

Values are the mean ± SD, n = 6.



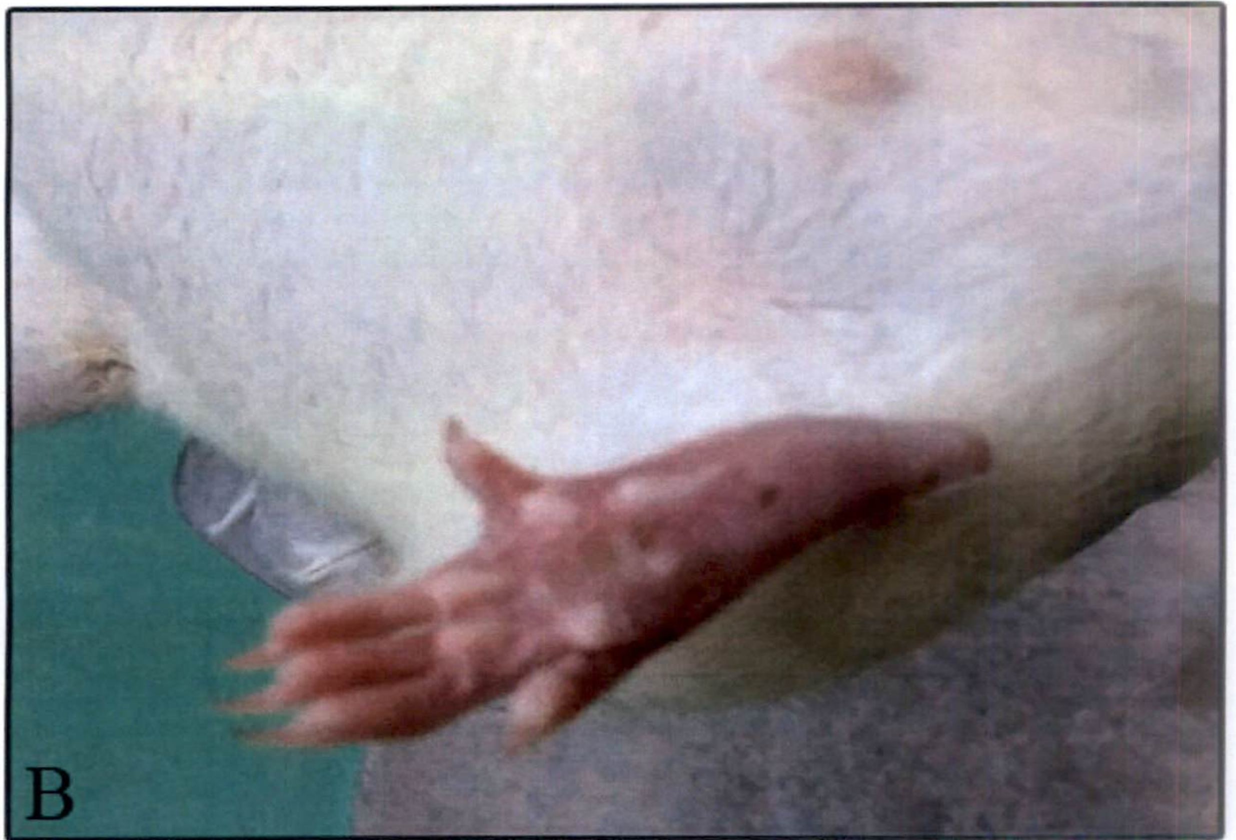


Plate 11: A. Normal paw and B. Inflammation induced paw.

4.7 ANTIINFLAMMATORY STUDIES

Difference in the paw before and after the induction of inflammation are presented in the plate 11.

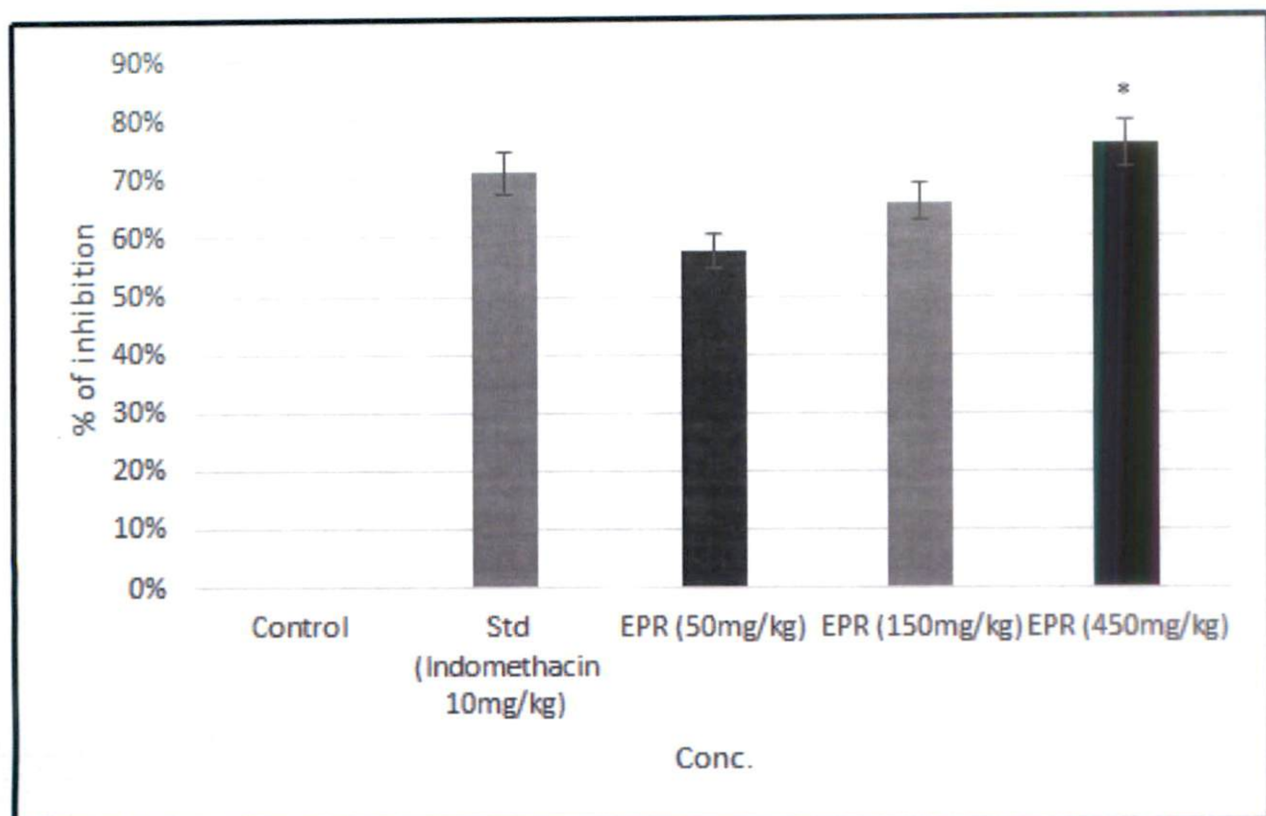
4.7.1 Carrageenan Induced Paw Oedema

Effect of ethanolic extract of leaves *of P. rubiginosum* on carrageenan induced paw oedema in rats are presented in the table 14 and graphically represented in figure 8. At a dose of 450 mg/kg, given the maximum percentage of inhibition in rat's paw oedema obtained was 75.80%. Indomethacin exerted a major protective role by inducing a protection of 71.27% at a dose of 10mg/kg body weight.

Table 14: Effect of Indomethacin and ethanolic leaf extract of *P. rubiginosum* on carrageenan induced paw oedema in rats.

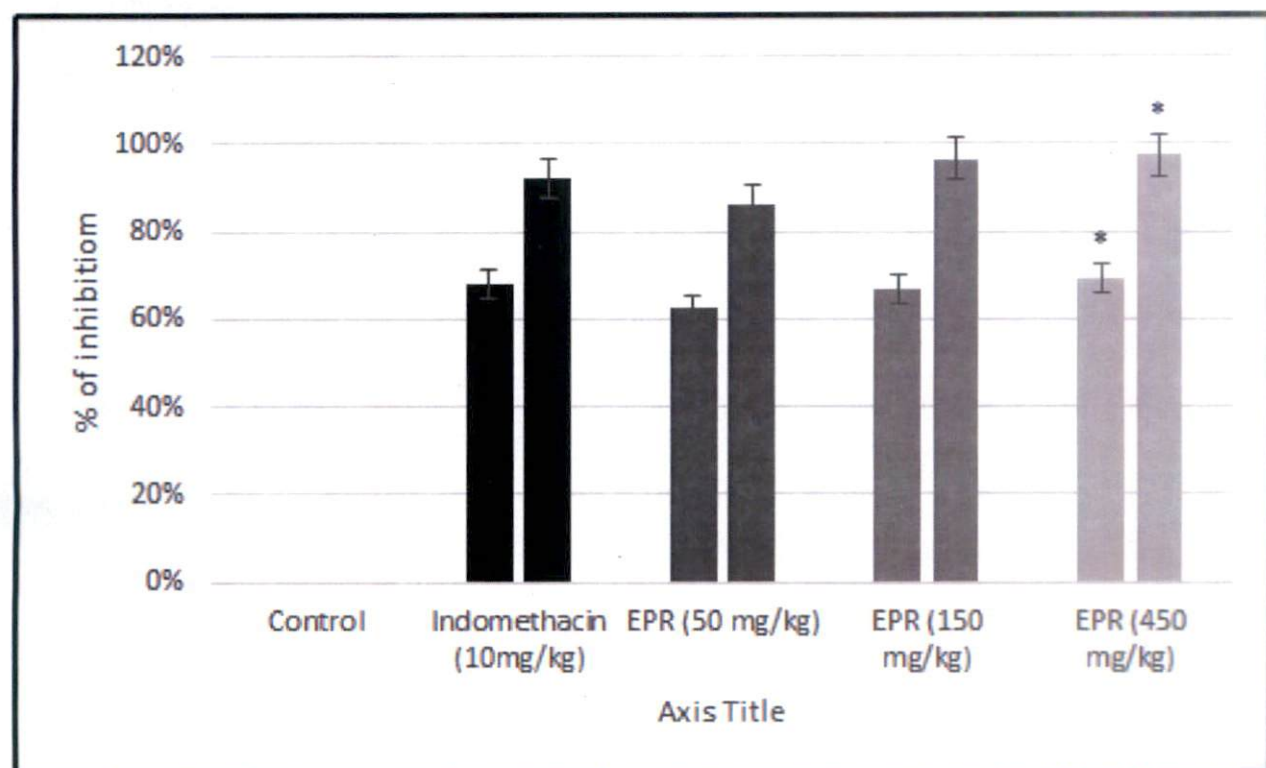
Si. No	Concentration	Difference in paw volume after 3 hours (mL)	Percentage of inhibition
1	Control	1.1 ± 0.0632	----
2	Std (Indomethacin 10mg/kg)	0.32 ± 0.0136	71.20%
3	EPR 50mg/kg	0.46 ± 0.0314	57.63%
4	EPR 150mg/kg	0.37 ± 0.0216	66.06%
5	EPR 450mg/kg	0.27 ± 0.0135	75.80%

Values are the mean ± SD, n = 6.



Each Values is the mean ± SD, for n = 6 mice.
*P<0.05 compared with standard indomethacin treated group .
Data were analysed by using one way ANOVA followed by Duncan's Test.

Figure 8: Effect of ethanolic extract of leaves of *P. rubiginosum* on Carrageenan Induced Paw Oedema in Rats



Each Values is the mean ± SD, for n = 6 mice.
*P<0.05 compared with standard indomethacin treated group .
Data were analysed by using one way ANOVA followed by Duncan's Test.

Figure 9: Effect of ethanolic extract of leaves of *P. rubiginosum* on of Formalin Induced Paw Oedema in Rats

4.7.2 Formalin Induced Hind Paw Oedema

Effect of ethanolic leaf extract of *P. rubiginosum* on of formalin induced paw oedema in rats are presented in the table 15 and graphically in the figure 9. At a dose of 450 mg/kg of the ethanolic leaves extract of PR reduced the inflammation in rat compared to other doses and the percentage of inhibition obtained was 97.27%. Indomethacin exerted a major protective effect by inducing a protection of 88% at a dose of 10mg/kg body weight.

Table 15: Effect of Indomethacin and ethanolic leaf extract of *P. rubiginosum* on formalin induced paw oedema in rats.

Sl. No	Concentration	Difference in paw volume after 1ST Day 3rd hours (mL)	% of Inhibition 1ST Day	Difference in paw volume after 7 Days (mL)	% of Inhibition 7TH Day
1	Control	1.1 ± 0.10	----	1.1 ± 0.10	----
2	Std 10mg/kg	0.35 ± 0.01	72.72%	0.08 ± 0.0081	88%
3	EPR 50mg/kg	0.41 ± 0.09	56.81%	0.15 ± 0.0248	87.27%
4	EPR 150mg/kg	0.36 ± 0.01	67.27%	0.04 ± 0.0043	96.72%
5	EPR 450mg/kg	0.26 ± 0.02	80.01%	0.03 ± 0.0044	97.27%

Values are the mean ± SD, n = 6.

DISCUSSION

5. DISCUSSION

5.1 PHARMACOGNOSY

Ethnobotanically, *Pterospermum rubiginosum* B. Heyne ex G. Don was mostly used by the tribal peoples of Kerala for bone fractures and inflammations. They have used plant parts like bark and leaves for the treatment of inflammation of both exterior and interior part of the body. And also it has a hepatoprotective role in the polyherbal formulation. However, there is no standardization work has done so far in this plant. The methods used to establish pharmacognostical standards are the microscopy and macroscopy, extractive, ash value, fibre content and fluorescence analysis. These parameters help to ensure the quality of the drug.

This plant belongs to family Sterculiaceae and genus *Pterospermum*, the plant in this genus contains some phytochemical which was named as pterospermin and now, there are three types pterospermin isolated and named as Pterospermin a, b and c. Pterospermin has got pharmacological potential i.e. the pterospermin is used to treat bone-related diseases (Preety *et al.*, 2011).

5.1.1 Microscopic Characters

Microscopic evaluation is a crucial step in pharmacognosy for the documentation of plants as well as for the detection of small fragments of powdered drugs or crude and detection of adulterants. So it widely employed in the microscopic evaluation of crude leaf drugs (Jarald and Jarald, 2007).

The microscopic examination of leaf of *P. rubiginosum* revealed the anatomical characteristics of the leaf such as lamina is generally dorsiventral, Single layered epidermis with lysogenous mucilage cavities, abaxial epidermis contains more stomata than adaxial epidermis, stomata's were anomocytic, numerous hairs are present, lamina contains secretory cavities, adaxial hypodermis present, Hypodermis is chlorenchymatous. Mesophyll contains both palisade and

spongy tissues. Vascular conducting tissue xylem and phloem is organized into discrete strands or vascular bundles and it is surrounded by bundle sheath. Bundle sheath which is made up of parenchyma and just above and below the vascular bundles contains parenchymatous and collenchymatous cells up to epidermis.

5.1.2 Physico-chemical parameters

Evaluation of the physical constants of the drug is an essential parameter for detecting adulteration of the drugs.

i. Moisture Content

Moisture content analysis is a critical component of material quality and essentially a function of quality control on most products. In the current study has shown that the moisture content of *P. rubiginosum* leaves is 52.2% is greater than that of some common leafy vegetables such as *Xanthosem sagittifolum* (14.7%), *Vernonia amygdaline* (27.4%) and *Gnetum buchholsianium* (33.8%) (Ladan *et al.*, 1996), but moisture content of *P. rubiginosum* is lower when its compared to *Moringa oleifera* leaves (76.53%) (Oduro *et al.*, 2008). The Moisture content in the leaves indicates the presence of water soluble vitamins.

ii. Ash values

Determining the ash value of the plant powder is a significant tool for identifying the adulterants in crude drugs. There are various types of ash values are taken for the detection of crude drugs such as total ash, acid insoluble ash and water soluble ash. The total ash value is useful for detection of the adulterants like lime chalk powder, siliceous contamination or other earthy matter. Total ash value of *P. rubiginosum* is 12.84 %; it is because of the presence of calcium crystals in the plant leaves which was as already confirmed in the microscopical studies. Acid soluble ash value of *P. rubiginosum* is 3.65% was the minimum and its value nearly four times less than total ash. In acid insoluble ash, the presence of earthy materials

detected and also varying the amount of calcium oxalate crystals in the cells. In water soluble ash, the value of *P. rubiginosum* is 9.12% and this value has given there is an existence of water exhausted materials in the leaves (Jarald and Jarald, 2007).

iii. Crude Fibre Content

The crude fibre content *P. rubiginosum* was 7.86% in the leaves. The crude fibre content of *P. rubiginosum* leaves are high when the value is compared to the fibre content in the leaves of sweet potatoes, which is 7.20%, but the fibre content is low compared to *Moringa oleifera* leaves 19.25% and *Tribubus terrestris* leaves 13% (Oduro *et al.*, 2008).

iv. Swelling index

In many of the herbal materials of specific pharmaceutical or therapeutic utility because of their swelling properties. Swelling of the herbal materials is because of the presence of gums and those having a significant amount of mucilage, hemicellulose or pectin. In this investigation swelling index of leaves of *P. rubiginosum* was found to be 9.8% and this is because of the presence of mucilage is the cause of swelling. And the swelling index is more when compared to *Pyrus pashia* (Vikrant *et al.*, 2011) and other plant materials which lack in mucilage, pectin or hemicellulose etc.

5.1.3. Extractive values

Extractive values are one of the important tools for determining the physio chemical parameters and plays an imperative part in the assessment of the crude drugs. Extraction of the drug from plant powder with different solvents gives a particular amount yield of crude drugs. This helped to identify the presence several types of adulteration and exhausted materials. For example, the yield of ethanol and water soluble extractive values are indicative, the changes in the values of the extracts are because of the defective processing and poor quality of the drug.

Petroleum ether soluble extractive value shows the presence of lipid contents in the crude drug (Madhavan *et al.*, 2009). Hydro ethanol gives more yield than other solvents. This gives 8.5% of yield from the plant powder and the least yield was obtained from Acetone 1.69%.

5.1.3 Fluorescence study

The plant powder has exhibited fluorescence phenomenon primarily due to its chemical constituents. In different wavelength of light, the same material in different reagents may appear similar and dissimilar. Some of the constituents show fluorescence in the visible range in daylight while some other shows fluorescence only in ultraviolet light. If the plant material does not show any fluorescence phenomena, then they may be made fluorescent by applying various reagents into it. Through this analytical technique quality of the crude drug can be assessed which ultimately help the people to identify the adulterants in the drug. And this parameter can be used for standardizing the quality of the Drug. Therefore, this fluorescence property can be used as a fingerprint for identification of plant material (Reddy and Chaturvedi, 2010).

Fluorescence properties of leaf powder of *P. rubiginosum* established using different chemical reagents and it has shown a diverse colouration in visible light and short and long UV light. One of the significant features of fluorescence is that UV light induces a fluorescent nature in many natural products (e.g. Alkaloids like berberine) where fluorescence is not seen in natural daylight. Several solvents and reagents tested, HCl, H_2SO_4, HNO_3, 5% $FeCl_3$, 5% iodine, 1M NaOH, 1M NaOH+ D.H_2O, dil. Ammonia, 1M HCl 1M H_2SO_4 and KCr_2O_7 did not how any fluorescence in leaf powder. Whereas, in acetone, methanol, toluene chloroform, ethanol and 1M NaOH + methanol showed characteristic colouration in the leaf powder (Table 3). Some of the substances may be often converted into fluorescent derivatives by using different chemical reagents, though they are not fluorescent, hence we can often assess qualitatively some crude drugs using

fluorescence as it is the most important parameter of pharmacognostical evaluation (Ansari, 2006).

5.2 Preliminary Phytochemical Screening

Plants having medicinal properties are playing an essential role in the drug discovery all over the world. Preliminary phytochemical screening helps in the finding of the bioactive compound, which might lead to the discovery and development of new drugs. Screening tests facilitate qualitative separation and quantitative estimation of pharmacologically active phytochemical from extracts (Varadarajan *et al.*, 2008). Most of the developing countries are reliant on plants for their primary health care and significant progress in the field of synthetic organic chemistry. In developed countries, more than 25% of prescribed drugs are derived indirectly or directly from plant sources (Newman *et al.*, 2000).

During phytochemical screening investigation, has revealed the occurrence of alkaloids, anthocyanins, coumarins, saponins, phenols, phlobatannins, glycosides, alkaloids, flavonoids, tannins and carbohydrate in the different extracts of leaves of *P. rubiginosum*. Phytochemicals present in this plant may be the responsible for its pharmacological potential.

5.3 ANTI-OXIDANT STUDIES

The observed anti-oxidative activities can be recognized to either the different mechanisms which exhibited by different polyphenolic compounds obtained, especially from plants having tocopherols, flavonoids and other organic acids alone or synergistic effects of these different compounds (Demla and Verma, 2012). These compounds can act as highly effective free radical scavengers due to their redox potential, which can play an important role in adsorbing and neutralizing free radicals produced in our body by quenching singlet and triplet oxygen or decomposing peroxides (Hasan *et al.*, 2008).

Total flavonoids and phenolics retain a broad spectrum of chemical and biological activities including radical scavenging activities. The medicinal potential of plants is often attributed to the antioxidant property of phytochemicals mainly flavonoids, phenolics and flavonols. Phenolic compounds are one of the powerful chain-breaking antioxidants (Adithya, 2012).

Flavonoids are also effective free radical scavengers for most of the oxidizing molecules such as singlet oxygen and several other free radicals produced during various diseases (Bravo, 1998). Flavonoids inhibit scavenging reactive species, chelated trace elements and formation of reactive oxygen that is involved in the production of free-radical and up-regulate antioxidant defense in the body (Agati *et al.*, 2012). Crude extracts from herbs, fruits, vegetables, cereals and other plant materials rich in flavonoids are progressively being used in the food industry because of their antioxidant potential and health benefits. The concentrations of flavonoids in ethanolic leaf extracts of *Pterospermum rubiginosum* is 11.341 µg QE / 0.5 mg mass of extract. The highest flavonoid content was identified in methanol extracts (containing 5 mg/mL of extract).

One of the major groups of compounds performing as main antioxidants or free radical terminators is phenolics (Padmanabhan and Jangle, 2012). A reasonable amount of phenolics was detected in the crude extracts. The content of total phenols in ethanolic extract of *Pterospermum rubiginosum* expressed as gallic acid equivalents (GA) per / 0.5 gram of dry extract is 22.68 µg GAE extract. The highest phenolic content was found in methanol extracts (with 5 mg/mL extract concentration).

5.3.1 DPPH Radical Scavenging Activity

DPPH radical scavenging activity is one of the most extensively used technique for screening the antioxidant potential of plant extract. In this study, Antioxidant activity was determined in the ethanolic extract of leaves of *Pterospermum rubiginosum* (EPR) showed a better antioxidant potential in a higher concentration of 250 µg/mL by DPPH radical scavenging method and in some of

the concentrations of EPR showed a significant percentage of scavenging when compared to standard ascorbic acid. IC 50 value found to be 22.50 μg/mL and 128.42 μg/mL for ascorbic acid and ethanolic extract of *Pterospermum rubiginosum* respectively.

5.3.2 Nitric oxide scavenging activity

Nitric oxide (NO) is a significant chemical intermediary which is produced by macrophages, neurons, endothelial cells, etc. and is involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases (Lalitha *et al.*, 2013). The antioxidant activity of ethanolic extract of leaves of *Pterospermum rubiginosum* (EPR) showed a better result and it is due to the scavenging of nitric oxide. At EPR 250 μg/mL showed similar antioxidant potential with the standard at a concentration of 100 -150 μg/mL L-Ascorbic acid (AA). IC 50 value found to be 16.56 μg/mL and 146.87 μg/mL for ascorbic acid and ethanolic extract of *Pterospermum rubiginosum* respectively.

5.3.3 Hydroxyl radical (OH) scavenging activity

During Fenton's reaction hydroxyl free radicals are formed in the presence of reduced transition metals such as Fe^{2+} and H_2O_2, those are supreme reactive species of all the reduced forms of dioxygen is thought to initiate cell damage *in vivo* (Duan *et al.*, 2007). The hydroxyl radical scavenging potential of ethanolic extracts of *Pterospermum rubiginosum* leaves was increased with increasing concentration. The hydroxyl radical scavenging was observed in the range of 45.91–55.88% from 50 to 250 μg/mL concentrations. At 250 μg/mL showed the maximum percentage of scavenging for both L-ascorbic acid and ethanolic leaf extracts and IC 50 value was found to be 17.91 μg/mL and 163.23 μg/mL for L-ascorbic acid and ethanolic extract of *Pterospermum rubiginosum* respectively.

5.4 Oral Acute toxicity study

Oral acute toxicity study is an essential method used for revealing of toxicity by rising dose till the appearance of toxicity signs. The use of animal models in toxicity studies is for evaluating the safety and toxicological evaluations in acute systemic toxicity testing. Nowadays this practice is still used in toxicological studies (Combe *et al.*, 2004). This will help to understand the effect of the drug in a biological system. There are no behavioural changes happened during the observations made at different time intervals after the administration of EPR of higher concentrations.

3.5 ANTINOCICEPTION STUDIES

3.5.1 Acetic acid induced writhing

The writhing response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. Local peritoneal receptors were involved during this response. This technique is mainly done to evaluate analgesics, which act peripherally, the maximum analgesic activity of aspirin was observed in this model. Simvastatin also exhibited significant analgesic activity, though less than aspirin (Barsante *et al.*, 2005).

Bradykinin, (BK), tumour necrosis factor-a (TNF- a), interleukin-1b (IL-1b) and the chemokine CXCL induce about the same intensity of nociception and pre-treatment with atorvastatin reduced each of these hyper nociceptive states about the same level (Garzon *et al.*, 2006) and it also reported that it reduces prostaglandin E2 (PGE2) induced hyper nociception (Nakamura and Ferreira, 1989). Ethanolic leaf extracts of *Pterospermum rubiginosum* (EPR) was significantly inhibited writhing response induced by acetic acid has shown maximum inhibition of 85.24% at EPR 450mg/kg. So EPR also has the potential to inhibit these mediators involved during the nociception.

5.5.2 Eddy's Hotplate Test

The hot plate method is employed to study the thermal stimuli, the paw licking in test animal in hot plate response is due to complex supraspinal integrated behaviour (Chapman *et al.*, 1983). The nociceptors are sensitized by sensory nerves. The participation of endogenous substances such as Prostaglandins may be reduced. In centrally acting analgesic techniques, the drug in EPR 50 mg/kg, EPR 150 mg/kg and EPR 450 mg/kg was found to be significantly effective when compared to the standard drug. EPR 450 mg/kg was found to be more effective. In this antinociception study was done in evaluating centrally acting drugs. It also shows more percentage inhibition than the standard drug (10mg/kg of Aspirin). These neuronal fibres are sensitive to both narcotics and non-steroidal anti-inflammatory drugs (Collier *et al.*, 1968).

Ethanolic extract of *Pterospermum rubiginosum* showed antinociceptive activity at the maximum dose (450 mg/kg), it could alleviate pain in all times of paw licking test, whereas the lower doses (50 and 150 mg/kg) reduced only late pain.

5.6. ANTI-INFLAMMATORY STUDIES

The inflammatory process is a complex process. During this process several mediators such as prostaglandins, interleukins, platelet activating factor etc. are involved. The presence or accumulation of these inflammatory mediators in the body are reported, during different types of inflammatory diseases.

For determining the anti-inflammatory diseases, there are various *in vitro* and *in vivo* models in animals. For the present study I used *in vitro* techniques for the confirmation of anti-inflammatory disease, and they are carrageenan and formalin-induced hind paw oedema. Indomethacin is used as the standard for the anti-inflammatory studies.

5.6.1 Carrageenan-Induced hind paw oedema

Carrageenan-induced hind paw oedema is normally used as an experimental animal model for acute inflammation studies and it has two phases (biphasic) such as early phase and late phase. During the initial phase (1-2 h) of the carrageenan model is primarily mediated by serotonin, histamine, and synthesis of prostaglandins in the injured tissue surroundings was increased. And in the late phase is sustained by prostaglandin release and mediated by polymorph nuclear cells, leukotrienes, bradykinin, and prostaglandins produced by tissue macrophages (Gupta *et al.*, 2006).

The ethanolic leaf extracts of *Pterospermum rubiginosum* was significantly inhibited carrageenan-induced paw oedema after 3 hrs. It has shown a maximum inhibition of 75.80% at a dose of EPR 450mg/kg, this finding suggests a possible inhibition of cyclooxygenase synthesis by the ethanolic leaf extracts of EPR and the effect of EPR is similar to that of nonsteroidal anti-inflammatory drugs indomethacin and whose mechanism of action is by inhibiting the production of cyclooxygenase enzyme.

5.6.2 Formalin-induced hind paw oedema

Formalin-induced paw oedema is a sub-acute inflammation result from cell injury, which initiates the production of endogenous mediators such as, prostaglandins, histamine, bradykinin and serotonin are examples of some endogenous mediator's produced during the inhibition of oedema induced by formalin in rats is one of the suitable protocol to screen anti-inflammatory agents (Mishra *et al.*, 2010). Arthritis induced by formalin is a model is also used for the evaluation of an agent with probable anti-proliferative activity.

Indomethacin showed more or less inhibition of oedema in early, intermediate and later phases. Ethanolic extract of *Pterospermum rubiginosum* showed a more or less significant inhibition of 80.01% and 97.27% at EPR

450mg/kg in formalin-induced oedema has shown significant inhibition in the early phases and also in later phases respectively.

SUMMARY

6. SUMMARY

The study entitled "Pharmacognostic studies and assessment of antiinflammatory, antinociceptive and antioxidant potential of 'Ellooti' (*Pterospermum rubiginosum* B. Heyne ex G. Don)" was conducted at the Ethnomedicine and Ethnopharmacology Division of Jawaharlal Nehru Tropical Botanical Garden (JNTBGRI), Palode, Thiruvananthapuram, during the year 2015 to 2016. The objective of the study was to scientifically evaluate the antiinflammatory, antinociceptive, antioxidant potential of leaves of an ethno medicinal plant *Pterospermum rubiginosum* B. Heyne ex G. Don. and to carry out its pharmacognostic studies.

Pterospermum rubiginosum B. Heyne ex G. Don leaves were collected from various localities of Thiruvananthapuram and Wayanad district and it was maintained at JNTBGRI, to carry out pharmacognostic and pharmacological studies.

In the pharmacognostical investigation, physico-chemical parameters, fluorescence analysis and preliminary phytochemical screening of *Pterospermum rubiginosum* were carried out. The results of the current study will help us prepare a reference material and to develop a monograph and to check the purity of the drug. The preliminary phytochemical screening of leaves of *Pterospermum rubiginosum* has shown the occurrence of secondary metabolites like phenols, flavonoids, saponins etc. and these phytochemicals, may play a vital role in medicine for healing different kinds of diseases.

The antioxidant effect of the ethanolic leaf extract of *P. rubiginosum* (EPR) were carried out by DPPH, Hydroxyl and Nitric Oxide free radical scavenging methods in different concentration of the EPR. The antioxidant activity of *P. rubiginosum* was compared with a standard (Ascorbic acid) and the result shown in some of the concentration gives the same effect and relatively equal effect.

Toxicity of the ethanolic extract of leaves of *P. rubiginosum* were tested by an acute toxicity study in mice with four doses 25, 100, 400, 1600 mg/kg body weight, and the mice were cage side observed for 14 days and no toxic effect was seen in the tested animals.

The anti-inflammatory potential of the ethanolic leaf extract of *P. rubiginosum* investigated by carrageenan (acute inflammation) and formalin (sub chronic inflammation) induced paw oedema on hind limb in rats with three different doses i.e. 50, 150, 450mg/kg of body weight. Treatment with different ethanolic extract of leaves of *P. rubiginosum* at the above doses significantly inhibit the inflammation to a greater extend.

The anti-nociception potential of the ethanolic leaf extract of *P. rubiginosum* investigated by Eddy's Hot Plate method and Acetic acid writhing method in mice with three different doses, i.e. 50, 150, 450mg/kg of body weight. Treatment with ethanolic extract of leaves of *P. rubiginosum* at above doses significantly reduced the pain in test animals.

The present study revealed that *P. rubiginosum* exhibited, the satisfactory pharmacological effects such as anti-oxidant antiinflammatory and anti nociceptive studies. From the detailed pharmacology studies, the effective dose of the drug was found to be 450 mg/kg for all experiments because it has shown the maximum percentage of inhibition.

REFERENCES

7. REFERENCES

Adithya, E. S., Sasikumar, J. M., Krishnakumar, K. A., Lakshmi, M. S., and Christabel, H. 2012. *Invitro* antioxidant activity, mineral content and HPLC analysis of *Talinum portulacifolium* (Forssk.) Asch. ex schweinf. leaf and stem. *Intl. J. pharmacy pharma. sci.* 4: 423-429.

Agati, G., Azzarello, E., Pollastri, S., and Tattini. M. 2012. Flavonoids as antioxidants in plants: location and functional significance. *Plant Sci.* 196: 67-76.

Lowe, H., Payne-Jackson, A., Beckstrom-Sternberg, S. M., and Duke, J. A. 2000. Jamaica's Ethnomedicine: Its potential in the healthcare system. University of the West Indies, Kingston, Jamaica.170p.

Agnel, N., Arul, J., and Shobana, G. 2012. Anti-inflammatory activity of *Talinum fruticosum* L. is evaluated by formalin induced paw oedema in albino rats. *J. Appl. Pharma. Sci.* 02 (1): 123-127.

Amal, K. G., Ha, Y. L., Young, H. K., Eun, K. R., and Moon, S. C. 2015. Evaluation of Antioxidant and Anti-Inflammatory Effect of *Rhododendron brachycarpum* Extract Used in Skin Care Product by *In-Vitro* and *in Vivo* Test. *Technol, Investment.* 6: 105-111.

Amir, F., Golbarg, G., Peyman, M. K., Hosein, F., and Amin, N. 2009. Antinociceptive effect of Promethazine in Mice. *Iranian J. Basic Med. Sci.* 12: 140-145.

Ansari, S. H. 2006. Essential of Pharmacognosy (1st Ed.) Birla publications Pvt. Ltd. New Delhi.

Anthony, S. F. 2008. *Harrison's Principles of Internal Medicine* (17th ed.). McGraw-Hill Professional. United States.

Ashutosh, K. 2007. Pharmacognosy and Pharmacobiotechnology. New Age International Publisher. India, New Delhi. 20p.

Axelrod, F. B. and Hilz, M. J. 2003. Inherited Autonomic Neuropathies. *Semin Neurol.* 23(4): 381-390.

Barsante, M. M., Roffe, E., Yokoro, C. M., Tafuri, W. L., Souza, D. G., and Pinho, V. 2005. Anti-inflammatory and analgesic effects of atorvastatin in a rat model of adjuvant-induced arthritis. *Eur J. Pharmacol.* 516: 282-289.

Bhutani, K. K. 2003. Herbal medicines enigma and a challenge for science and guidelines for new initiatives. *J. Nat Product* 19(1): 3-8.

Biljana, B. P. 2012. Historical review of medicinal plants' usage. *Pharmacogn Rev.* 6(11): 1-5.

Bravo, L. 1998. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. *Nutr. Rev.* 56: 317-333.

Calixto, J. B. and Barz, J. 2000. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Med. Biol. Res.* 33: 179-89.

Cena, C., Lolli, M. L., Lazzarato, L., Guaita, E., Morini, G., Coruzzi, G., McElroy, S. P., Megson, I. L., Fruttero, R., and Gasco, A. 2003. Antiinflammatory, gastrosparing, and antiplatelet properties of new NO-donor esters of aspirin. *J. Med. Chem.* 46: 747.

Chakraborty, R., Biplab, D., Devanna, N., and Sen, S. 2012. Antiinflammatory, antinociceptive and antioxidant activities of *Phyllanthus acidus* L. extracts. *Asian Pac. J. Trop. Biomed.* 2: 953-961.

Chakraborty, R., Biplab, D., Devanna, N., and Sen, S. 2012. Antiinflammatory, antinociceptive and antioxidant activities of *Phyllanthus acidus* L. extracts. *Asian Pac. J. Trop. Biomed.* 2: 953-961.

Chapman, C. R., Casey, K. L., Dubner, R., Foley, K. M., Gracely, R. H., and Reading, A. E. 1985. Pain measurement: an overview. *Pain*, 22: 1-31.

Collier, H. O., Dinneen, L. C., Johnson, C. A., and Schneider, C. 1968. The abdominal constriction response and its suppression by analgesic drugs in mouse. *Br Pharmacol.* 32: 295-310.

Costa, M. A., Zia, Z. Q., Davin, L. B., and Lewis, N. G. 1999. Chapter Four: Toward Engineering the Metabolic Pathways of Cancer-Preventing Lignans in Cereal Grains and Other Crops. In: Romeo, J. T. (ed.). In Recent Advances in Phytochemistry, Phytochemicals in Human Health Protection, Nutrition and Plant Defence. New York. 33: pp.67-87.

Cox, P. A. and Balick, M. 1994. The ethnobotanical approach to drug discovery. *Sci. Am.* 270: 82-87.

Duan, X., Wu, G., and Jiang, Y. 2007. Evaluation of the antioxidant properties of litchi fruit phenolics in relation to pericarp browning prevention. *Molecules.* 12: 759-771.

Edriss, A. E., Alabjar, Z. A., and Satti, A. A. 2012. Phytochemical screening of important secondary metabolites in some extracts of two Sudanese plants. *Glo. Adv. Res. J. Environ. Sci. Toxicol.* 1: 199- 202.

Garzon, T. S., Cunha, T. M., Verri, W. A., Jr, R., Valerio, D. A., Parada, C. A., and Poole, S. 2006. Atorvastatin inhibits inflammatory hypernociception. *Br. J. Pharmacol.* 149: 14-22.

Ghani, A. 1998. *Medicinal Plants of Bangladesh: Chemical Constituents and Uses.* Asiatic Society of Bangladesh, Dhaka. 460p.

Goldfarb, A. H. 1993. Antioxidants: role of supplementation to prevent exercise-induced oxidative stress. *Medicine and Sci. in Sports and Exercise.* 25: 232- 236.

Grifo, F. and Rosenthal, J. 1997. *Biodiversity and human health.* Island Press, Washington DC, USA.

Hasler, C. M. and Blumberg, J. B. 1999. Symposium on Phytochemicals: Biochemistry and Physiology. *J. Nutri.* 129: 756S-757S.

Ialenti, S., Moncada, M., and DiRosa. 1993. Modulation of adjuvant arthritis by endogenous nitric oxide. *Br. J. Pharmacol.* 110: 701-705.

IUCN. 2011. Selected medicinal plants of Chittagong hill tracks Dhaka, Bangladesh, pp xii, 33-36,116, 978-984, 50-7.

Jacob, R. A. 1992. The integrated antioxidant system, Nutr. Res. 15: 755-766.

Harris, E. D. 1995, Regulation of antioxidant enzymes, *J. Nutr.*, 122: 625-626.

Jesupillai, M. and Thillai, A. P. 2014. Evaluation of anti-inflammatory activity of *phyllanthus longiflorus* in experimental animal models. *Int. J. pharm. Bio. Sci.* 5(1): 691-695.

Joshi, P., Priya, B. and Gahlot, M. 2014. Anti-inflammatory and analgesic activities of methanol extract of *Vallaris solanacea* leaves. *Int J. Pharm. Sci.* 6: 622-624.

Kamala, P. E., Narendra, K., Suman, J., Swathi, J., Sowjanya K. M., Rathnakarreddi, K. V. N., Emmanuel, S. J., Padmavathi, C., and Krishna, S. A. 2014. Studies on qualitative, quantitative, phytochemical analysis and screening of in vitro biological activities of *Leucas indica* (L) VAR. Nagalapuramiana. *Int. J. Herbal Med.*. 2(3): 30-36.

Karmakar, R., Kumar, S., and Prakash, H. S. 2013. Antioxidant, Anti-Inflammatory, Antimicrob'al and Cytotoxic Properties of Fungal Endophytes from Garcinia Species. *Int. J. Pharm. Sci.* 5(3): 889-897.

Kelly, K. 2009. *History of medicine*. Facts on file, New York. 29-50.

King, A. and Young, G. 1999. Characteristics and Occurrence of Phenolic Phytochemicals. *J. American Dietetic Assoc.* 24: 213-218.

Ladan, M. J., Bilbis, L. S., and Lawal, M. 1996. Nutrient composition of some green leafy vegetables consumed in Sokoto. *Niger. J. Basic Appl. Sci.* 5: 39-44.

Lalitha, E. M., Vijaya, B. R., and Jayshree, N. 2013. Preliminary Phytochemical Screening and Heavy Metal Analysis of Leaf Extracts of *Ziziphus oenoplia* (L) Mill. Gard. *Intl. J. Pharma. Sci. Drug Res.* 5(1): 38-40.

Lata, H. and Ahuja, G. K. 2003. Role of free radicals in health and disease. *Ind. J. Physiol. Allied Sci.* 57: 124-28.

Loeser, J. D., Butler, S. H., Chapman, C. R., and Turk, D. C. eds. *Bonica's Management of Pain*. 3rd ed. Philadelphia, Pennsylvania, USA: Lippincott Williams and Wilkins; 2003:73.

Lowe, H., Payne-Jackson, A., Beckstrom-Sternberg, S. M., and Duke, J. A. 2000. Jamaica's Ethnomedicine: Its potential in the healthcare system. Canoe Press. University of the West Indies, Kingston, Jamaica. 170.

Lucy, H. and Edgar, J. D. 1999. Medicinal Plants: A re-emerging Health aid. Electronic. *J. Biotechnol.* 2(2): 1-15.

Mathai, K. 2000. Nutrition in the Adult Years., (10th ed.). In: Mahan, L. K. and Escott-Stump, S. *In Krause's Food. Nutrition. and Diet Therapy.* 271: 274-275.

Meagher, E. and Thomson, C. 1999. Vitamin and Mineral Therapy. In Medical Nutrition and Disease, (2nd ed.). In: Morrison G and Hark L. Malden, Massachusetts: Blackwell Science Inc, 33- 58.

Modi, D. C., Patel, J. K., and Shah, B. N. 2010. Pharmacognostic Studies of the seed of Syzygium Cumini Linn. *Pharma sci. monitor.* 1(2): 20-26.

Mohideen, S., Sasikala, E., and Aruh, A. J. P. 2005. Pharmacognosy of Cassia Alata Linn-leaves. *Ancient Sci. of Life.* 24 (4): 192-198.

Nakamura, M. and Ferreira, S. H. 1987. A peripheral sympathetic component in inflammatory hyperalgesia. *Eur. J. Pharmacol.* 135: 145-153.

Narasinga, R. 2003. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. *Asia Pacific J. of Clin. Nutr.* 12(1): 9-22.

Newman, D. J., Cragg, G. M., and Snader, K. M. 2000. The influence of natural products upon drug discovery. *Nat. Prod. Rep.* 17: 215-234.

Nidal J., Fatima, H., and Anas, A. *2015.* Preliminary Phytochemical Screening, Quantitative Estimation of Total Flavonoids, Total Phenols and Antioxidant Activity of *Ephedra alata* Decne. *J. Mater. Environ. Sci.* 6(6): 1771-1778.

Nilofer, S., Yogendra, K., Misra, A. K., and Lokho, P. 2013. Phytochemical screening to validate the ethnobotanical importance of root tubers of Dioscorea species of Meghalaya, North East India. *J. Medicinal Plants Studies.* 1(6): 62-69.

Obi, R. K. and Chiegboka, N. 2011. Ethanolic extraction and phytochemicals screening of two Nigerian herbs n pathogens isolated from wound infections. *Int. J. Comp. Pharm.* 2(10): 1-5.

Oduro, I., Ellis, W. O., and Owusu, D. 2008. Nutritional potential of two leafy vegetables: *Moringa oleifera* and *Ipomoea batatas* leaves. *Sci. Res. Essay.* 3: 57-60.

Okigbo, R. N., Eme, U. E., and Ogbogu, S. 2008. Biodiversity and conservation of medicinal and aromatic plants in Africa. *Biotechnol. Mol. Biol. Rev.* 3(6): 127-134.

Padmanabhan, P. and Jangle S. N. 2012. Evaluation of DPPH Radical Scavenging Activity and Reducing Power of Four Selected Medicinal Plants and Their Combinations. *Intl J. of Pharma. Sci. Drug Res.* 4(2): 143-146.

Pandya, D. J., Desai, T. R., Nadpara. N. P., Mehta, H. A., and Modi, A. M. 2010. Pharmacognostic study and establishment of quality parameters of leaves of *Bombax insigne* Linn. *Int. J. Pharmaco. Phytochem. Res.* 2(3): 1-5.

Patel, P. M., Patel, N. M., and Goyal, R. K. 2006. Quality control of herbal products. *Indian Pharmacist.* 5(45): 26-30.

Poongothai, A., Sreena, K. P., Sreejith, K., Uthiralingam, M., and Annapoorani, S. 2011. Preliminary phytochemicals screening of *Ficus racemosa* linn. Bark. *Intl. J. Pharma. Bio Sci.* 2(2): 431- 434.

Prakash, C. G., Nisha, S., and Rao, C. V. 2012. Pharmacognostic studies of the leaves and stem of Careya arborea Roxb. *Asian Pac. J. Trop. Biomed.* 2(5): 404-408.

Priya, A. and Aparna, S. 2012. Chemical profile studies on the secondary metabolites of medicinally important plant *Zanthoxylum rhetsa* (Roxb.) DC using HPTLC. *Asian Pac. J. Trop. Biomed.* 1293-1298.

Radha, C., Kumar, P. R., and Prakash, V. 2008. Enzymatic modification as a tool to injure the functional properties of heat processed soy flour. *J. Sci. Food Agric.* 88(2): 336-43.

Rajalkshmi, G., Ramarendran, R., and Gomathi, N. 2013.Phytochemical Screening and Antifungal Activity of Medicinal Plant. *Global Res. Analysis.* 2(3): 8-9.

Rajnish, K. S., Vaishali, S., Kumar, P., Murthy, P. N., Panigrahi, G., Sharma, P. K., and Gupta, R. K. 2014. Evaluation of anti-inflammatory potential of *Nardostachys jatamansi* rhizome in experimental rodents. *J. Coastal Life Med.* 2(1): 38-43.

Reynolds, J. F., Noakes, T. D., Schwellnus, M. P., Windt, A., and Bowerbank, P. 1995. Non-steroidal anti-inflammatory drugs fail to enhance healing of acute hamstring injuries treated with physiotherapy. *S Afr Med J.* 85:517-522.

Ross, R. 1993. The pathogenesis of atherosclerosis: a perspective for the 1990's. *Nature*, 362: 801.

Sannigrahi, S., Parida, S., Patro, J. V., Mishra, U. S., and Pathak, A. 2010. Antioxidant and anti-inflammatory potential of *Pterospermum acerifolium. Int. J. Pharma. Sci. Rev. Res.* 2: 1-5.

Santos, E. N., Lima, J. C., Noldin, V. F., Cechinel-Filho, V., Rao, V. S., Lima, E. F., and Martins, D. T. 2011. Anti-inflammatory, antinociceptive, and antipyretic effects of methanol extract of *Cariniana rubra* stem bark in animal models. *An. Acad. Bras. Cienc.* 83: 557-566.

Savithramma, N., Linga, R. M., and Suhrulatha, D. 2011. Screening of Medicinal Plants for Secondary Metabolites. *Middle-East J. Sci. Res.* (3): 579-584

Setzer, M. C., Werka, J. S., Irvine, A. K., Jackes, B. R., and Setzer, W. N. 2006. Biological activity of rainforest plant extracts from far north Queensland, Australia. In: Williams, L.A.D. (ed.) *Biologically Active Natural Products for the 21st Century*. Research Signpost, Trivandrum, Kerala, India. 21-46.

Sharma, S. K. and Singh, H. 2013. Pharmacognostical standardization of *Jatropha integerrima* Jacq. (Euphorbiaceae) roots. *Der Pharmacia Lettre*. 5(1): 155-159.

Shinde, V., Dhalwal, K., Paradkar, A. R., Mahadik, K. R., and Kadam, S. S. 2006. Evaluation of *in vitro* antioxidant activity of human placental extract, *Pharm. Online*. 3: 172-179.

Sies, H. 1991. *Oxidative Stress: Oxidants and Antioxidants*. Academic Press, London. 56-59.

Singh, R. P., Sharad, S., and Kapur, S. 2004. Free radicals and oxidative stress in neurodegenerative diseases: Relevance of Dietary Antioxidants. *J. Ind. Academy of Clin. Med.* 5: 218-225.

Stadtman, E. R. 1992. Protein oxidation and aging. *Sci.* 257: 1220-1225.

Syed S. G., Mohib, K., and Rana, T. 2015. Anti-Inflammatory Activity of *Ficus Dalhousiae* Miq Roots Ethanolic Extract in Wistar Albino Rats. *Asian J. Pharm Clin Res.* 8(1): 117-119.

Tamilselvan, N., Thirumalai, T., Elumalai, E. K., Balaji, R., and David, E. 2011. Pharmacognosy of *Coccinia grandis*: a review. *Asian Pac. J. Trop. Biomed.* 1: 299-302.

Terman, G.W. and Bonica, J.J. 2003. Spinal mechanisms and their modulation. In: Loeser, J.D., Butler, S.H., Chapman, C.R., and Turk, D.C., (eds.)

Bonica's Management of Pain. (3rd Ed.). Lippincott Williams and Wilkins, Philadelphia, Pennsylvania, USA. 73p.

Tiwari, P., Bimlesh, K., Mandeep, K., Gurpreet, K., and Harleen, K. 2011. Phytochemical screening and Extraction: A Review. *Int. Pharm. Sci.* 1: 98-106.

UNESCO. 1996. Culture and Health, Orientation texts- World Decade for Cultural Development Documents CLT/DEC. PRO-1996, Paris, France. 29p.

UNESCO. 1998. Terminal Report: Promotion of Ethnobotany and the sustainable use of Plant Resources in Africa. 60p.

Valko, M., Leibfritz, D., Moncola, J., and Cronin, M. D. 2007. Free radicals and antioxidants in normal physiological functions and human disease. Review. *Int. J. Biochem. Cell Biol.* 39: 44-84.

Varadarajan, P., Rathinaswamy, G., and Asirvatahm, D. 2008. Antimicrobial properties and phytochemical constituents of Rheo discolor. Ethnobotanical Leaflet. *J. Pharmacol. Phytochem.* 12: 841-845.

Vikrant, A., Narender, T., and Kashyap, C. P. 2012. Preliminary Phytochemical Analysis of the Extracts of Psidium Leaves. *J. Pharmacol. Phytochem.* 1(1): 1-5.

Wahid, A, M., Suyog, D. M., Suraj, B. J., Ajinkya, M. P., Mukhtar, S. K., and Madhukar, R. V. 2010. Evaluation of antiinflammatory and analgesic activities of ethanolic extract of roots *Adhatoda vasica* Linn. *Intl J. Pharm. Tech Res.* 2(2): 1364-1368.

Waleed, K. G., Al-Hejjaj., Numan, I. T., Raghdan, Z., Al-Saad, and Saad, A. 2011. Anti-inflammatory activity of telmisartan in rat models of experimentally-induced chronic inflammation: Comparative study with dexamethasone. *Saudi Pharm. J.* 19: 29-34.

Wang, H., Gao, X. D., Zhou, G. C., Cai, L., and Yao, W. B., 2008. In vitro and in vivo antioxidant activity of aqueous extract from *Choerospondias axillaris* fruit. *Food Chem.* 106: 888-895.

Wichers, M. C. and Maes, M. 2004. The role of indoleamine 2, 3-dioxygenase (IDO) in the pathophysiology of interferon-alpha-induced depression. *J. Psychiatry Neurosci.* 29(1): 11-17.

Yoshikawa, T., Toyokuni, S., Yamamoto, Y., and Naito, Y. 2000. *Free Radicals in Chem. Bio. Med.* OICA International, London.

Young, I. and Woodside, J. 2001. Antioxidants in health and disease. *J. Clin. Pathol.* 54: 176-186.

Young, I. and Woodside, J. 2001. Antioxidants in health and disease. *J. Clin. Pathol.* 54: 176-186.

APPENDICES

APPENDIX I

Wagner's reagent

Iodine	2.00g
Potassium iodide	6.00g
Water	$100cm^3$

APPENDIX II

Mayer's reagent

Mercuric chloride	1.36g
Potassium iodide	5.00g
Water	100ml

APPENDIX III

Hager's reagent

Picric acid	1.00g
Water	100ml

APPENDIX IV

DPPH free radical scavenging assay

0.1 mM DPPH solution

- DPPH 4mg
- Ethanol 100ml

APPENDIX V

Hydroxyl scavenging activity

Iron-EDTA

- Ferrous ammonium sulphate 0.13%
- EDTA 0.26%

EDTA 0.018%

Ascorbic acid 0.22%

TCA 17.5%

Nash reagent

- Ammonium acetate 7.5g
- Glacial acetic acid 0.5ml
- Acetone 0.2ml
- Distilled water 100ml

APPENDIX VI

Griess reagent

Naphthylethylenediamine dihydrochloride 0.1%

Sulfanilamide 1% (5% concentrated phosphoric acid)

ABSTRACT

PHARMACOGNOSTIC STUDIES AND ASSESSMENT OF ANTIINFLAMMATORY, ANTINOCICEPTIVE AND ANTIOXIDANT POTENTIAL OF 'ELLOOTI' (*Pterospermum rubiginosum* B. Heyne ex G. Don)

Submitted
by
SHAHASAD SALAM

(2011-09-120)

ABSTRACT

Submitted in partial fulfilment of the
requirement for the degree of

MASTER OF SCIENCE (INTEGRATED) IN BIOTECHNOLOGY

Faculty of Agriculture

Kerala Agricultural University, Thrissur

B.Sc. M.Sc. (INTEGRATED) BIOTECHNOLOGY
DEPARTMENT OF PLANT BIOTECHNOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM - 695 522
KERALA, INDIA

2016

ABSTRACT

The study entitled "Pharmacognostic Studies and Assessment of Antiinflammatory, Antinociceptive and Antioxidant Potential of 'Ellooti' (*Pterospermum rubiginosum* B. Heyne ex G. Don)" was conducted at the Ethnomedicine and Ethnopharmacology Division of Jawaharlal Nehru Tropical Botanical Garden (JNTBGRI), Palode, Thiruvananthapuram, during the year 2015 to 2016. Objective of the study was to scientifically evaluate the antiinflammatory, antinociceptive, antioxidant potential of leaves of an ethnomedicinal plant *Pterospermum rubiginosum* B. Heyne ex G. Don. and to carry out its pharmacognostic studies.

The detailed study of pharmacognostical parameters revealed the presence of anomocytic stomata and adaxial hypodermis microscopically, ash value, is less because the presence of the oxalate crystals is low, moisture is 52.03% and fibre content 7.86% revealed by physico chemical parameters and macroscopic characters of the leaves. Fluorescence analysis revealed presence of fluorescence in solvents like chloroform, methanol, toluene and ethanol in the powdered leaves. Preliminary Phytochemical examination in different solvents revealed the presence of various phytoconstituents such as flavonoid, saponins, alkaloids, carbohydrates, saponin, coumarin, glycoside, tannin, phlobatanin, steroid and phenols.

In *in vitro* antioxidant method the methanolic extracts of leaf showed higher free radical scavenging activity compared to standards with IC_{50} of 25μg/mL (DPPH), 150 μg/mL (NO Scavenging Activity) and 100 μg/mL (Hydroxyl Free Radical Scavenging Activity).

Toxicity of the ethanolic extract of leaves of *P. rubiginosum* were tested by acute toxicity study in mice with four doses 25, 100, 400, 1600 mg/kg body weight. And the mice were cage side observed for fourteen days and no toxic effect were seen in the tested animals.

173922

In the detailed *in vivo* pharmacological studies for antiinflammatory and anti nociception were conducted. Antiinflammatory activity was determined by Carrageenan (Acute inflammation) and formalin (sub chronic inflammation) induced paw oedema on hind limb in rats with three different doses 50, 150 and 450 mg/kg. At the dose of EPR 450mg/kg give 76.36% of inhibition in carrageenan induced paw oedema and in the formalin induced paw oedema (sub-acute) study a dose of EPR .150mg/kg gives maximum inhibition of 86.01% and 97.27% of inhibition in the first and seventh day respectively.

Anti nociception activity was determined by Eddy Hot Plate method and Acetic acid writhing method in mice with three different doses 50, 150 and 450 mg/kg. At the dose of EPR 450mg/kg gives maximum inhibition 62.02% of inhibition in Eddy's hot plate method and in the acetic acid induce writhing study a dose of EPR 450mg/kg gives maximum inhibition of 85.24% of inhibition.