PHARMACOGNOSTIC STUDIES AND EVALUATION OF ANTI-INFLAMMATORY, ANALGESIC ANDANTIOXIDANT POTENTIAL OF 'MANJAKANTHA' (*Dracaena terniflora* Roxb.)

By

MEERA MOHAN

(2011-09-121)

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 COURSE

DEPARTMENT OF PLANT BIOTECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2016

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DECLARATION

I, hereby declare that the thesis entitled **"PHARMACOGNOSTIC STUDIES AND EVALUATION OF ANTI-INFLAMMATORY, ANALGESIC AND ANTIOXIDANT POTENTIAL OF 'MANJAKANTHA' (*Dracaena terniflora* Roxb.)"**is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellayani
Date:

Meera Mohan

(2011-09-121)

CERTIFICATE

Certified that this thesis, entitled **"Pharmacognostic studies and evaluation of anti-inflammatory, analgesic and antioxidant potential of 'Manjakantha' (*Dracaena terniflora* Roxb.)"**is a record of research work done by Mrs. Meera Mohan, (2011-09-121) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Thiruvananthapuram
Date:

Dr. P. G. Latha
Former Director, JNTBGRI
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CERTIFICATE

Certified that this thesis entitled "**Pharmacognostic studies and evaluation of anti-inflammatory, analgesic and antioxidant potential of 'manjakantha' (*Dracaena terniflora* Roxb.)**" is a record of research work done by Ms. Meera Mohan (2011-09-121) under my guidance and supervision and this is not previously formed the basis for the award of any diploma, degree, fellowship or associateship to her.

Palode
19 -12-2016

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We, the undersigned members of the advisory committee of Ms. Meera Mohan (2011-09-121), a candidate for thedegree of **B.Sc.-M.Sc.(Integrated) Biotechnology**, agree that the thesis entitled **"Pharmacognostic studies and evaluation of anti-inflammatory, analgesic and antioxidant potential of 'manjakantha' (*Dracaena terniflora* Roxb.)"**may be submitted by Ms. Meera Mohan, in partial fulfillment of the requirement for the degree.

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Date: 19.12.16

MEERA MOHAN

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v. LIST OF ABBREVIATIONS AND SYMBOLS USED

DT	*Dracaena terniflora* Roxb.
SSR	Simple sequence repeats
NO	Nitric Oxide
EDT	Ethanolic Extract of DT
Cm	Centi Metre
Hrs	Hours
µM	Micro Molar
M	Molar
Nm	Nanometer
ROS	Reactive Oxygen Species
GI	Gastro-Intestinal
p.o	Per orally
NSAID	Non-Steroidal Antiinflammatory Drug
%	Per cent
mM	millimolar
µl	Micro litre
PL	Phospholipids

ºC	Degree Celsius
Etc	Etcetera
et al.	And other co workers
Fig.	Figure
G	Gram
g-1	Per gram
mg	Milli gram

INTRODUCTION

INTRODUCTION

Medicinal plants produce a wide variety of chemical compounds that perform many important biological function, and to protect against attack of predators for a long time. Thousands of such compounds have been isolated so far but that is less than 10% of the total medicinal plants available (Biljana, 2012). Chemical compounds in plants work in human body through processes identical to those of chemical compounds used in conventional drugs. This enables herbal medicines to have importance in pharmacology. Prehistoric evidences claims the use of plants as food source, in burial sites, fossils, therapeutic agents etc. Many of those plants used for treating many disease ailments at that time were later discovered as powerful drugs. Medicinal plants are considered as a rich resources for drug development. Certain plants were considered as important source of nutrition and have important therapeutic values which include ginger, green tea, walnuts, lime, turmeric and many others plants.

Ethnomedicine is the traditional health care practice of indigenous people to human health. The knowledge of certain herbs, shrubs, trees, animals and minerals that have curative and palliative effects were transferred from one generation to another and it is the outcome of experimentation through trial and error method over many years. Many of the well-established drugs available in market have been developed by the scientists after analysing the chemical constituents of plants used traditionally. Tribal medicine is a part of Traditional medicine and is mainly practiced by the tribes in the rural and forest area.

Pharmacognosyis the study of medicines derived from natural sources. It is important to classify drugs becauseclassification is a prerequisite to study the various aspects of the plant diversity(Gokhele *et al.,* 2008). The main aim is to study the origin of plants and their evolution from a simpler to advanced form or in other words to investigate the phylogenetic relationships of plants (Cardellina, 2002).

Phytochemicals are plant derived chemicals, beneficial to human health and disease prevention. (Chung *et al.,* 1998).Now most of drugs used are derived from these secondary metabolites (Wang *et al.,* 2008).Evaluation of the plants for secondary metabolites includes qualitative, quantitative and biochemical tests (Rangari, 2002) for the detection of inferior or exhausted materials (Jarald & Jarald, 2007). Therefore pharmacognostic and preliminary phytochemical screening of plants is to be carried out in order to discover and develop novel and effective therapeutic agents with improved efficacy.

An antioxidant is a molecule that inhibits the oxidation of the natural products in the body. Antioxidants are nutrients as well as enzymes that play a role in preventing the development of chronic diseases such as cancer and heart diseases.

Inflammation is part of a complex biological response of body tissues against certain stimuli. It is a protective response that involves many cells like the immune cells, blood vessels, and molecular mediators to eliminate the harmful stimulus and to initiate tissue repair.

Analgesics, or painkillers, are a class of drugs that are generally used to reduce or relieve pain, a sensory experience in a human body with tissue damage or unpleasant sensation of tissue damage. The experience of pain has an unpleasant character, that is, it can be distinguished from its sensory aspects and from the long-term experience of 'suffering'.

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the management of oedema, for the treatment of minor pain and tissue damage. Most of the NSAID work by inhibition of prostaglandin biosynthesis (*Buer et al.,2014).*

D. terniflora is a subshrub which have tribal claim for many medicinal purposes including anti-inflammatory and analgesics. Hence *D. terniflora* need to be subjected for further experiments to prove its efficacy.

REVIEW OF LITERATURE

1. REVIEW OF LITERATURE

2.1 MEDICINAL PLANTS

2.1.1 Importance of medicinal plants

Medicinal plants are important in health care needs and requirements of the more than half of the population for these many years. Out of the 2000 drug items recorded in the Indian Materia Medica, 1800 are of plant origin - about 80% of the raw materials are required in the manufacture of drugs (Lambert *et al.,* 1997). According to the FRLHT report, the Indian system of medicine uses around 8,000 species of plants. The maximum numbers of medicinal plants are utilized by the folk traditions, followed by Ayurveda, Siddha, Unani, Homeopathy, Tibetan and Modern medicine respectively (Shankar *et al.,* 2000)

2.1.2 **Characteristics of medicinal plants**

• Synergic medicine- The components of plants all interact simultaneously, so their uses can complement or neutralize their possible negative effects.

• Support of official medicine- In the treatment of cancer, the components of the plants proved to be very effective.

• Preventive medicine- It has been proven that, plants also characterize by their ability to prevent the appearance of some diseases.This will help to reduce the use of the chemical remedies and in turn reduce the side effect of synthetic treatment.

Traditional medicine has been prevalent in almost every country from the beginning of the civilization. According to Siegerist (1951), a medical historian "every culture had developed a system of medicine and medical history is but one aspect of the history of culture".Traditional medical systems vary from one culture to another, there are common elements connecting implications in them (Joshi, 1990). According to Dubos (1969), "ancient medicine was the mother of sciences and played a large role in the integration of early cultures". The term "traditional

medicine" refers to ways of restoring and protectinghealth, before the arrival of modern medicine. Furthermore, they form the unwritten repertory of health practices that have been passed down many generations through word of mouth for at least one century and continue to be passed down even today (Shankar *et al.,* 2001).

2.1.3 Indian context

There existed a system of medicine that professional healers deal in India for several centuries before and after 2000 BC. Harappan culture contained the seeds of later Indian medicine. That apart, nature of medicine are to be found in the earliest literature of India, the Rig Veda. During this period, diseases were believed to be mostly due to wrath of gods and effect of evil spirits. The healing process was by prayers, several hymns and often aided by the herbal remedies, other treatments etc. and they cured some diseases like blindness, lameness and even leprosy. In the late 5^{th} or 6^{th} century before Christ, the traditional Indian medical system formed and references can be found in the Hindu texts. Then the science of medicine, which came to be known as Ayurveda was formed and a line of sages were believed to have carried the original lore of the Ayurveda in various eras, down to historical times. In the later stage, Charaka and Susruta contributed to popularise the Ayurveda in India by bringing out Sanskrit medical manuals (Reddy, 1986). Surgery was also practiced, like the removal of calculi from the bladder, the replacement of bowels exposed as a result of wound, stitching the stomach wall etc. and plant products and herbal medicine were given for fast recovery. The achievements in plastic surgery were unrivalled anywhere in the world until the 18^{th} century (Kutumbiah, 1962). Soon other systems of medicines like Siddha, Unani etc. also emerged and developed along with Ayurveda.

2.1.3.1 ***Folk stream/little traditional medicine***

- Folk medicine

  The concepts and practice in folk medicines are based upon the humoral theories, cosmological speculations, learned/oral medicine and religion. The

practice of this medicine lies in midwifery, bone setting, supernatural cures of diseases with main emphasis is on utilizing natural herbs, roots, plants, mud, insects and other natural things. The knowledge of such medicinal plants and preparation of medicine are still handed down mostly in oral form to the next generation (Chaudhuri, 1986; Reddy, 1986).

- Tribal Medicine/Ethno Medicine

  The tribes or ethnic group who lived in isolation practiced their own system of medicine, this is particularly known as tribal medicine or ethno medicine.

2.1.4 Transfer of knowledge

Tribal medicinal knowledge is passed from generation to generation by means of words like culture and customs according to Mashelkar (2002). The folk medicine is based on traditional and cultural beliefs,practices and norms based on century's old experiences of trials and errors, success and failures at the household level. These are passed through oral traditions and may be called "people's health cultures, home remedies or folk remedies" Johari and Karki (1999).This knowledge which is often regarded as a family treasure, is not accessible even to the rest of the community to which the practitioner belongs and is therefore vulnerable to destruction and loss". According to Saraswati (1987),"the tribal knowledge of different herbal preparation is based on observations and experiment".

2.2 *Dracaena terniflora (*Roxb).

General description:

*Dracaena terniflora (*Roxb). is a stout perrinial subshrub, locally called as "Manjakkanda, Manjakantha, and Elathaani" of family Dracaenaceae [Asparagaceae (formerly in Agavacaea)]. It is less than 1m tall. Stem is covered with annual leaf scar, Stem is sprawling, simple or few branched, internodes are longer. Leaves are spaced along distal part of stem. Stalks are 3-6 cm, widened at base to forming persistent sheath normally concealing internode. Leaves are elliptic, 6-8 cm, acuminate, petiole 5cm long, canaliculated, base expanded. Inflorescence are racemes arrangement, simple, about 15 cm. Flowers are solitary or in clusters. Flowers are white, closely packed, perianth lobes 6, biseriate, similar, unites into a tube below, 2 cm long, stamens 6, free epipetalous, anthers oblong, ovary 3-celled, ovule solitary in each cell, style1, slender stigma terminal . Berry is globose, 1-3-seeded and 1-1.3 cm in diameter. The plant prefers moist marshy land for its growth. It does not usually grow in dry area. This is found in evergreen and semi evergreen forests in India and South- East Asia. In Kerala, the localities are Peechi, Vellanimala, Chooda, Rosemala, Sabarimala, Arippa, Ranni, Thenmalai, Agasthyamala, etc. and districts like Palakkad, Kasargode, Kanoor, Malappuram, Trivandrum, Thrissur, Wayanad.

The root extract is used by the cholanaikan tribes for treating Jaundice. Traditionally, the root extract is used for piles, jaundice (Satya and Usha, 2007). Root is also used for inflammation and fruit used for headache. (Udayan *et al.,* 2000). And also the whole plant has been traditionally used to treat diabetes by Kurunarippullu tribes of wayanad district *(*Raghavendra *et al.,* 2015). It is also reported to have property to cure bed sores (Bhat *et al.,* 2014). The kurichya tribes use it for the treatment of snakebite, spider bite etc.

Root extract in tender coconut water is given for skin rashes, erysipelas and urticaria. Root and sandalwood ground in rice washed water is used for blood discharge through urine, urine block and genito urinary diseases. Root and leaf

Plate 1: *Dracaena terniflora* Roxb.

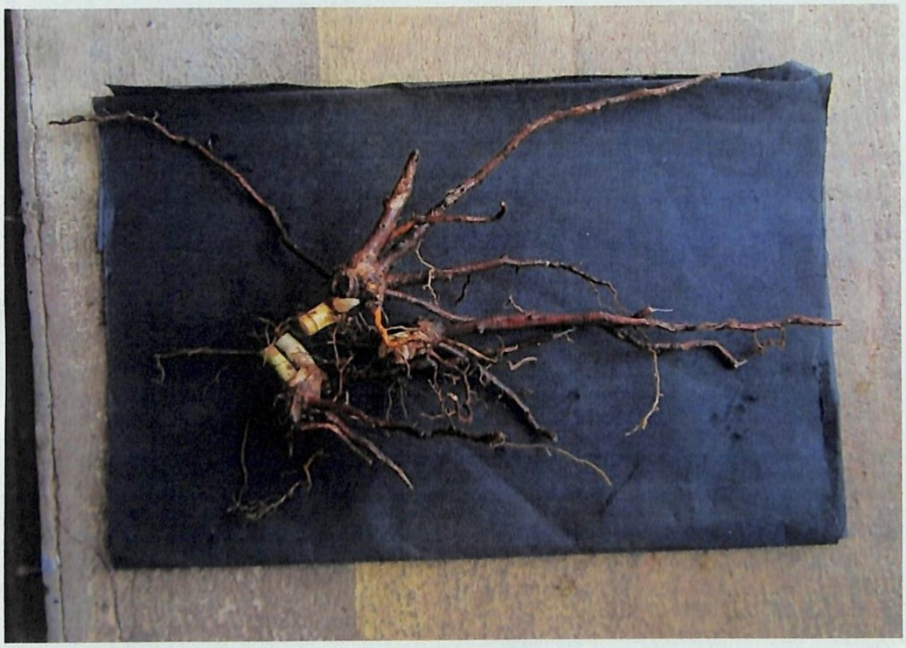


Plate 2: Fibrous root system of *Dracaena terniflora* Roxb.

Plate 3: Herbarium specimen of *Dracaena terniflora* (TBGT/86802(23/10/2015))

paste is applied for Herpes. Root paste with tender coconut water is applied for herpes. Root decoction is recommended for jaundice, leucoderma, urticaria, urine block, bleeding, diabetes, fever and skin diseases.Root extract with lime juice is consumed for pit viper bite. Root decoction is given by adding sugar and milk for protein discharge through urine. The same is used twice a day for a week for biliousness and related itches in the body. However*D. terniflora* need to be subjected for further experiments to prove its efficacy.

A study was conducted to explore the ethno medicinal information of the plants and their method of use adopted to cure diabetes by the tribes of Mananthavady, Bathery and Vythiri taluks of Wayanad district, Kerala. Preparation of medicine using various parts of plants were collected, plant habitat, habit and status of medicinal plants was recorded. The review of literature revealed antidiabetic property of*Dracaena terniflora* Roxb. (Raghavendra *et al.,* 2015)

A study was conducted in 50 selected home gardens of Karrwa, Karnataka to document their floristic diversity and composition with regard to life forms used.The predominance of ornamental species, which include *Dracaena terniflora Roxb.* and few other species makes the home gardens of Karrwa.(Shivanand *et al*., 2005)

(Utsarga *et al.,* 2012) examined mesoscale distributions of endemic, locally significant or rare plant species in plateau or on forest habitats and its escarpments, and assessed the edaphic and hydrological parameters of seasonal plateau micro habitats. In Dracaenaceae family, *Dracaena terniflora Roxb.* is found in protected forests.

2.3 PHARMACOGNOSY

The knowledge of understanding the plant, its features, usage, taxonomic identification of the species, fresh matter, dried or powdered state (Springfield *et al.,* 2005), extraction, purification, plant breeding (Jarald &Jarald, 2007) accurate and standardised identification and quality assurance of sample plant materials is a necessary prerequisite step for reproducible quality of herbal medicine.

Experimetns like Powder microscopic studies, sectioning, maceration and fluorescence studies, were carried out to identify authenticity of the plant sample.In a study the methanolic extract of *Mangifera indica* were examined to investigate the physiochemical, pharmacognostic and phytochemical characters. This investigation was carried out by several methods such as powder analysis, total ash value, acid insoluble ash value, water soluble ash value, loss on drying, and preliminary phytochemical analysis.(Kalpna Rakholiya *et al.,*2012).Similarly in a detailed Macro and Microscopical character study powdered leaves and roots of *Cissus vitiginea* L. have been investigated adopting pharmacognostical studies (Penchala Pratap *et al.,* 2014).

The study on pharmacognostic standardization of Hilleria latifolia, was done with different methods like microscopic and histological analysis, powder microscopy, quantitative microscopy, physiochemical parameters, fluorescence analysis, phytochemical screening, TLC profile, organoleptic characters, and macroscopic evaluation. (Isaac *et al.,* 2014)

2.4 PHYTOCHEMICALS

Phytochemicals appear to neutralize free radicals. It improves the health of an individual by inhibiting enzymes that activate carcinogens (Mamta *et al.,* 2013). Study findings suggest that phytochemicals reduces the risk of coronary heart disease by preventing the oxidation of low-density lipoprotein (LDL) cholesterol(Havsteen, 1983), reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity (Mathai, 2000).

Phytochemical screening of the ethanolic root extract of *Leea indca* (Burm. f.) Merr. was carried out by different methods. Ethanolic root extracts showed the presence of alkaloids, carbohydrates, steroids, triterpenoids, flavonoids, glycosides, anthraquinone glycosides, tannins, resins and saponins. *(*Jose *et al., 2013)*

2.5 ANTIOXIDANT PROTECTION

Medicinal plants are rich sources of secondary metabolites like flavonoids, flavones, isoflavones, flavanones, anthocyanins, catechins, polyphenols and tannins etc. Many of these chemical compounds possess a major biological activity called antioxidation.

Human body produces reactive oxygen species (ROS) as a by-product of many physiological and biochemical processes. Oxygen meditates chemical reactions that metabolize different organic molecules like fats, proteins, and carbohydrates to produce energy. Oxygen is a highly reactive atom that is capable of becoming potentially damaging molecules commonly called “free radicals.” These free radicals are capable of attacking the healthy cells in the body. Antioxidants are the chemicals which scavenge these free radicals and help in preventing occurrenceof several diseases by inhibiting oxidation or reactions promoted by oxygen, peroxidase or free radicals. Cell damage caused by free radicals appears to be a major contributor of many chronic diseases such as atherosclerosis, cancer, diabetics, rheumatoid arthritis, cardiovascular diseases, chronic inflammation, stroke and septic shock, aging and other degenerative diseases in humans. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells.Types of ROS are hydroxyl radical, hydrogen peroxide, the superoxide anion radical, nitric oxide radical, singlet oxygen, hypochlorite radical, various lipid peroxides etc. (Duthie, 1994).

To protect the cells and organ systems of the body from reactive oxygen species, humans have evolved a highly complex and sophisticated antioxidant protection system. It involves a variety of components (endogenous and exogenous in origin)that interact synergistically to neutralize free radicals (Mark, 1996). The most commonly used synthetic antioxidants are Butylated Hydroxy Anisole (BHA) and Butylated Hydroxy Toluene (BHT), but side effects and health risks such as carcinogenic effects has restricted their use therefore the dependence on plantshave increased in the present scenario (Ahmed *et al.,* 2015).

2.5.1 Dietary Antioxidants

Vitamin C, vitamin E, and beta carotene are the most widely studied dietary antioxidants. Vitamin C is considered to be one of the most important water-soluble antioxidant in extracellular fluids. It is able to neutralize ROS in the aqueous phase before lipid peroxidation starts. Vitamin E is a major lipid-soluble antioxidant and is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Vitamin C is capable of regenerating vitamin E. Beta carotene and other carotenoids provide antioxidant protection to lipid-rich tissues. Research suggests beta carotene can work synergistically with vitamin E. Diet which is excessively low in fat can negatively affect beta carotene and vitamin E absorption, and also other fat-soluble nutrients. Fruits and vegetables are major sources of vitamin C and carotenoids, while grains and vegetable oils are major sources of vitamin E (Jacob, 1995). Antioxidants are very important in the treatment of a rare progressive condition that causes damage to the nervous system,fried Reich ataxia (Sies, 1995).

The antioxidant activity of *Pinups dens flora* was determined by measuring the radical scavenging effect on 1, 1,-diphenyl-2-picryl-hydrazyl (DPPH) radicals by (Choi,*et al.,* 2003). The methanol extract of P. densiflora showed strong antioxidant activity when compare to ethyl acetate soluble fraction exhibiting strong antioxidant activity.

In a study, Pourmrad *et al.,*(2006) carried out a record of the relative antioxidant activity in selected Iranian medicinal plant species extracts. The total phenol and flavonoid varied from among those extracts. 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging effect of the extracts was determined using spectrophotometer. The greater amount of phenolic compounds leads to more potent radical scavenging effect as shown by *M. officinalis* extract.

The essential oil extracted from the buds of Eugenia caryophyllata was examined for its free radical scavenging activity, cytotoxicity and in vitro toxicity

the potent free radical assays like DPPH and hydroxyl radical scavenging assays have been carried out with increased efficiency in the results obtained. (Park, 2006)

Antioxidant activity of methanolic and aqueous root extracts of *Heliotropium eichwaldi* was studied by DPPH free radical scavenging assay and hydrogen peroxide scavenging activity. The results concluded that the extracts are a potential source of antioxidants of natural origin (Surendra and Naveen, 2012).

Mohammad, (2013) worked on *Withania somnifera* used in the prevention of different central nervous system (CNS) disorders, especially in neurodegenerative diseases and in conditions of stress. Five different extracts of *Withania somnifera* root is experimented for its antioxidant and free radical scavenging activity and methanol extract and showed significant activity.

In vitro free radical scavenging activity of *Lea philippinensis,* was investigated. Soxhlet extraction using different solvents like hexane, chloroform, and ethyl acetate, acetone and methanol in which acetone and methanol extracts yielded the highest Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). Fractions with the most TFC and TPC exhibited concentration-dependent. (Bartolome *et al.,* 2015)

Antioxidant and anti-inflammatory phenolic glycosides from *Clematis tashiroi* is used in the study various phytochemicals present like alkaloids, glycosides, terpenoids, steroids, polyphenolic compounds and also the compounds isolated from plants of marine origin , algae and fungi (Li –jie Zhang *et al.,* 2015).

2.6 ACUTE TOXICITY TESTING

Afzal *et al.* in 2005 conducted acute toxicity study experiment with Petroleum ether, Chloroform, Methanolic and Aqueous methanolic extracts of *Alpinia galanga* Willd. adn LD50 value of more than 5000 $mgKg^{-1}$with no behavioural changes.

The acute oral toxicity of C. fistula seeds extract was studied in mice. Highest dose administered was 5000 mg/kg and shows mortality or evidence of adverse effects. Throughout 14 days of the treatment shows no behavioural patternchanges, clinical sign and body weight of mice in both control and treatment groups (Subramanion *et al*., 2011).

2.7 INFLAMMATION

Inflammation is part of the complex biological response of body tissues to harmful stimuli(Slauson and Cooper, 2002).

2.7.1Etiology of Inflammation (Winter, 2005)

- Physical agents which cause inflammations areextreme temperatures, electric shock, radiation, mechanical injures, etc.
- Chemical agents are products or by-products of metabolism, acids, alkalis, drugs, tissue necrosis
- Biological agents include microorganisms (bacteria, viruses, fungi), parasites (helminths, insects), immune cells and complexes.

Celsus's*cardinal signs* of inflammation :*Rubor* (redness), *Tumor* (swelling), *Calor* (heat), *Dolor* (pain), *Functio laesa,* or loss of function.

2.7.2 Acute, Subacute and Chronic Inflammation

Acute inflammation lasts from several days up to several months. The major events taking place is the neutrophil attack and intravascular platelet activation. Subacute inflammation lasts from several weeks up to several months. The major cells involved are neutrophils, lymphocytes, plasmocytes and macrophages (approximately in equal proportions).Chronic inflammation lasts from a few months up to tens of years. The cells involved are mononuclear cells (lymphocytes, plasmocytes, macrophages) in case of exacerbations neutrophils are added.

2.7.3 Vascular changes

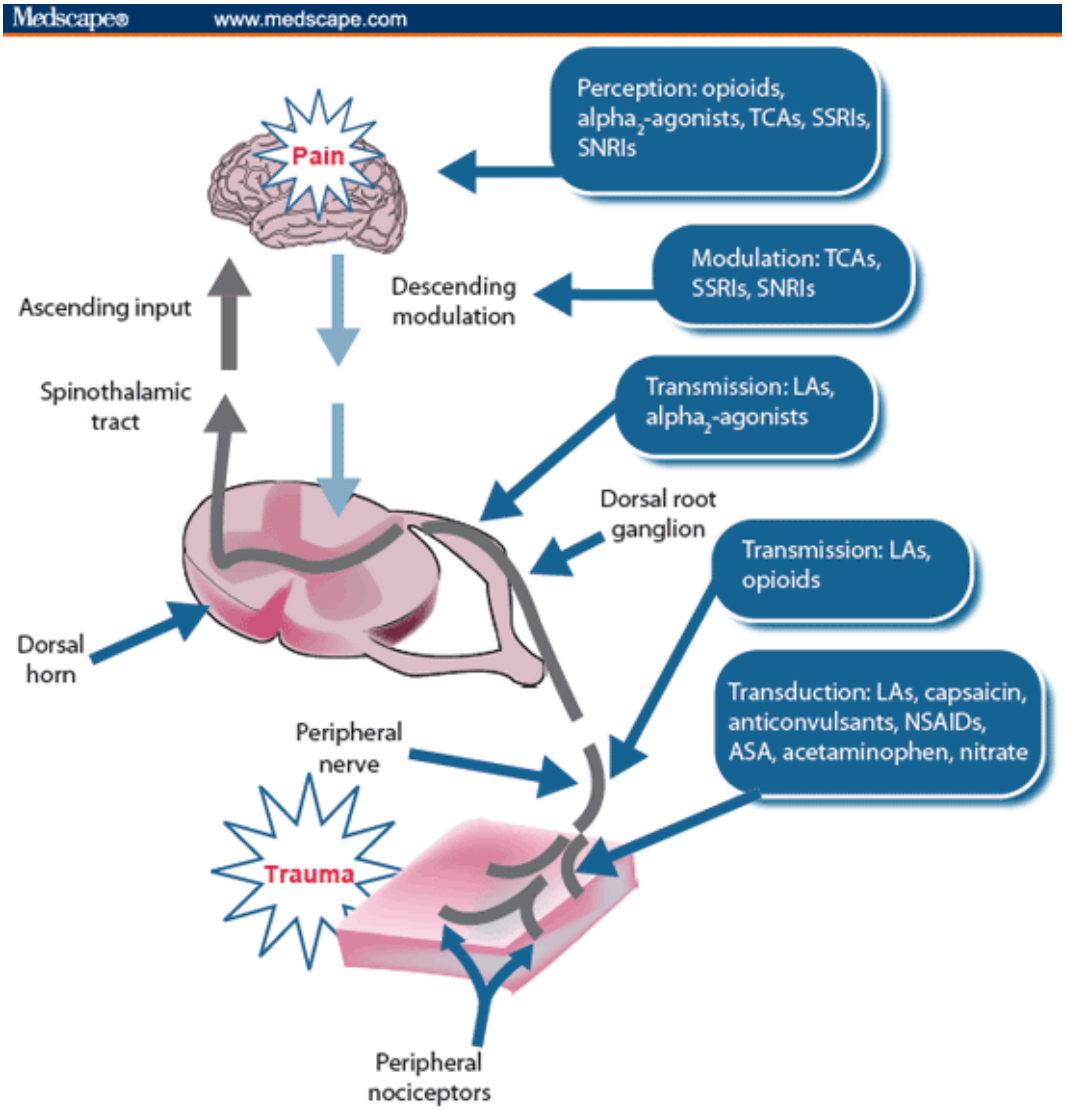
Early phase of vasodilation, then secondly permeability. A transient vasoconstriction that precedes these changes. The small arteries and the capillary bed that control blood flow into the capillary bed .There are sphincters controlling the flow.

2.7.4 Cellular changes

The major events in cellular changes are extravasation, followed by margination,
Accumulation of leukocytes to the epithelial cells of blood vessel walls at the site of injury in the early stages of inflammation. Then rolling, the process of transient adhesion of leukocytes with endothelial cells (selectins are the most important molecule responsible). They interact with the complimentary molecule resulting in transient adhesion. Selectin can be either-E Selectin (CD62E), L Selectin (CD62L), P Selectin (CD62P).Adhesion refers to the firm attachment of leukocytes to the endothelial cells. Integrins are most important molecules. Then this is followed by transmigration, chemotaxis , opsonisation, Phagocytosis

2.8 ANALGESICS

Analgesics include paracetamol, (NSAIDs) such as the salicylates, and opioid drugs such as morphine and oxycodone. They interact with the neurotransmitters and modulators of the pain system and alleviate and control of pain. NSAIDs exert their analgesic effect not only through peripheral inhibition of prostaglandin synthesis but also through a variety of other peripheral and central mechanisms.



ANTIINFLAMMATORY AND ANALGESIC STUDIES

An anti-inflammatory and analgesic experiments are conducted in the hydro -alcoholic extract (HAE) of the bark of few plants by (Alexandre *et al.,* 2000).The HAE inhibited mouse ear oedema induced by o-tetrade-canoyl phorbol acetate (TPA) and by capsaicin. HAE showed both anti-inflammatory and peripheral analgesic activities when administered in the mouse by the intraperitoneal route in doses which were at least 12 times lower than its LD50 doses.The HAE also significantly inhibited acetic acid induced writhing and the formalin induced late phase paw licking response in the mice

Liriodendrin from the ethyl acetate (EtOAC) extracts of stem bark of *Acanthopanax senticosus* significantly reduced an acute paw oedema induced by carrageenan in rats at doses 5&10 $mgKg^{-1}$. The acetic acid induced writhing test and hot plate test, Liriodendrin showed a dose dependent inhibition in animal models. (Jung *et al.,* 2002).

In studies for anti-inflammatory activities in rats, oral administration of the acetonitrile (MeCN) and hexane extracts of the leaves of *Teclea nobilis* (TN) at different doses showed significant anti-inflammatory activity inwrithing induced by acetic acid and heat induced pain in mice.(Al- Rehaily *et al.,* 2003)

Anbalagan *et al.,* 2003 experimented in Vacuum dried chloroform and methanol leaf extracts of *Pisonia grandis* to evaluate the anti-inflammatory and analgesic (acute & chronic) in wistar albino mice and wistar albino rats. Significant analgesic and anti-inflammatory activities were at dose level 500 $mgKg^{-1}$dose level.

Choi *et al.,* 2003 studied on MeOH extract of heartwood of *Rhus verniflua*, shows significant antinociceptive activity in writhing and hot plate test assays and effective anti-inflammatory effects in carrageenan induced hind paw oedema in rats. In addition, Sulfureting suppressed the cyclooxygenase-2 (COX-2) activity in lipopolysaccharide activated macrophage cells, this maybe the reason for analgesic / anti-inflammatory mechanism.

The anti-inflammatory and analgesic activities of methanolic leaf extract and various solvent fractions prepared by soxhlet extraction of *Clematis simensis* using carrageenan-induced guinea pig paw oedema, and tail-flick and hot-plate models in mice. The anti-inflammatory and the analgesic activities of HE at a dose of 800 $mgKg^{-1}$were comparable to the activities of the standards indomethacin (10 $mgKg^{-1}$) employed in the study shows effective anti-inflammatory activity of the sample extract (Ashenif Tadele *et al.,* 2004).

Afzal *et al.,* 2005 conducted an experiment in Alpinia galanga Willd.With different extracts like Petroleum ether, Chloroform, Methanolic and Aqueous methanolic (1:1) investigated for anti-inflammatory activity in carrageenan induced paw edema in Wistar rats, and compared to a positive control Ibuprofen. These extracts were given orally at a concentration of 500 $mgKg^{-1}$ 1 hr before carrageenan injection. Methanolic extract showed maximum inhibition of 79.51 % on carrageenan induced rat paw oedema.

The ethanollic extract of the leaves of *Bougainvillea Spectabilis* was screened for both ethanol and aqueous extracts for anti-inflammatory activity. Both extracts showed significant anti-inflammatory activity. (Senapati *et al.,* 2006)

Anti-inflammatory properties of *Leea guineensis* was evaluated using the carrageenan-induced paw oedema, there was a significant reduction in oedema. The phytochemical studies revealed the presence of saponins and glycosides as the secondary metabolites. (Falodun *et al.,* 2007)

A study investigates the inhibitory effects on NO and proinflammatory cytokines by methanol extract of *Polygonimultiflori ramulus*. The results indicate that the 85% methanol extract has inhibitory effect on the production of NO therefore may be beneficial in diseases which related to macrophage- mediated inflammatory disorder (Cha D.S., and Jeon, H., 2009).

A study was designed to evaluate the anti-inflammatory and analgesic activities of ethanolic root extract of *Adhatoda vasica* Linn. (Acanthaceae). The anti-inflammatory potential has been determined byformalin-induced paw oedema assay in albino rats and carrageenan-induced paw oedema assay. The analgesic activity was tested by hot plate method, acetic acid-induced writhing response and tail flick method in albino rats. The experimental data shows that ethanolic root extract of *Adhatoda vasica* Linn. possess remarkable analgesic and anti-inflammatory activities (Wahid, 2010).

The study conducted by Anuja *et al.* evaluated the anti-inflammatory and analgesic properties and phytochemical profile of the ethanolic extract of rhizome of *Drynaria quercifolia* (DQ). The extract produced with significant inhibition of carrageenan-induced paw edema. DQ significantly attenuated acute and delayed phases of formalin-induced pain and acetic acid-induced writhing in mice. Phytochemical analysis gave positive tests for catechin, coumarins, flavonoids, phenolics, saponin, steroids, tannins, and triterpenes. (Anuja *et al.,* 2010).

Sua *et al.,* (2011) conducted the anti-inflammatory activities by formalin induced hind paw oedema method. While the analgesic activity was examined by the acetic acid induced and hot plate method. The petroleum ether factor significant analgesic activity by significantly reducing acetic acid induced writhing response in mice.

Mequanint *et al.,* (2011) conducted the experiment on Aqueous and ethanolic extract for their anti-inflammatory activities in mice using carrageenan induced paw oedema, histamine and serotonin induced mice paw oedema. The extracts showed no toxic effects.

Citral (CIT) which contains the chiral enantiomers, neral is the majority monoterpene from *Lippia alba* and *cymbopogon citratus*. Analgesic and anti-inflammatory effects were studied using acetic acid test and formalin test and carrageenan induced paw oedema studies. All doses had significantly reduced the paw oedema. CIT possess significant central and peripheral analgesic effects. (Quintans *et al.,* 2011)

Crude saponin extracts of five medicinal plants, the whole plant of *Schwenkia americana* Linn, the rhizomes of *Asparagus africanus* Lam, the leaves of *Dichrostachys cinerea* Linn, the stem bark of *Ficus iteophylla* Miq and the leaves of *Indigofera pulchra* Willd used in the treatment of inflammatory diseases were screened for acute toxicity study and anti-inflammatory activity using carrageenan-induced rat paw oedema test (Hassan, H.S., 2011).

The ethanolic extract of L.indica were investigated for its analgesic activity. The extract at a concentration of 200 $mgKg^{-1}$dose exhibited a significant reduction of writhing response in a dose dependent manner. This method was employed by formalin-induced licking response model & acetic acid induce writhing test. (Emran *et al.,* 2012).

Quercetin was studied on different inflammation model like acute, sub-acute and chronic models. Studies were performed using different agent induced-paw oedema viz., Dextran-induced paw oedema, Formalin-induced paw oedema and Cotton Wool Granuloma model. The test dose was 20 $mgKg^{-1}$of Quercetin which was tested orally and Indomethacin was used as standard (Sapna *et al.,* 2013).

In vivo study was conducted in four preparative extracts of clematis terniflora for their antinociceptive activity and anti-inflammatory activity in rat model by carrageenan induced chronic non-bacterial prostatitis. The study result in the high anti-inflammatory and analgesic activity of clematis terniflora. (Qiuhong Wang *et al.,* 2013).

2.8 NON STEROIDAL ANTI-INFLAMMATORY DRUG

Nonsteroidal anti-inflammatory drugs are used to relieve some symptoms caused by inflammation, swelling, stiffness, arthritis and joint pain. However, they do not cure arthritis and will help relieve pain only as long as you continue to take them. NSAIDs may possibly interfere with healing of joints after an injury. Drugs that are included in analgesics work in diverse ways to reduce or relieve pain. They act mainly by two ways one on the central and then on peripheral nervous system. Narcotic drugs such as pethidine, synthetic drugs such as ketorolac, the non-steroidal anti-inflammatory drugs (NSAIDs) such as the salicylates (aspirin), and a variety of drugs are included in analgesics.Peripherally acting agents are Salicylates, Aspirin. Andcentrally acting agents like propoxyphene, Codeine, hydrocodone.

2.7.1 NSAIDs side effects

Many side effects are caused by NSAIDs including toxicity to vital organs like the kidneys, ears, and stomach. They can also interfere and makeimproper healing. They reduce the flow of blood to the kidneys and impair their function. The impairment is most likely to occur in patients with pre-existing impairment of congestive heart failure, or damage in kidney or liver. Healthy people and even children are also susceptible to such harm. People with asthma are more likely to experience allergic reactions to many NSAIDs. Common anti-inflammatory drugs such as ibuprofen and naproxen may increase the chance of miscarriage if they are taken early in pregnancy (Sahelian, 2016).

The major mechanism by which the NSAIDs elicit their therapeutic effects (antipyretic, analgesic, and anti-inflammatory activities) is inhibition of prostaglandin (PG) synthesis. Specifically NSAIDs competitively inhibit cyclooxygenases (COXs), the enzymes that catalyse the synthesis of cyclic endoperoxides from arachidonic acid to form prostaglandins. Two COX isoenzymes have been identified: COX-1 and COX-2. COX-1, expressed constitutively, is synthesized continuously and is present in all tissues and cell types, mainly in platelets, endothelial cells, the GI tract, renal microvasculature, glomerulus, and 2 collecting ducts. Thus COX-1 is important for the production of prostaglandins of homeostatic maintenance, such as platelet aggregation, the regulation of blood flow in the kidney and stomach, and the regulation of gastric acid secretion. Inhibition of COX-1 activity is considered a major contributor to NSAID GI toxicity. COX-2 is considered an inducible isoenzyme, although there is some constitutive expression in the kidney, brain, bone, female reproductive system, neoplasia, and GI tract. The COX-2 isoenzyme plays an important role in pain and inflammatory processes. (Jack, 2002)

MATERIALS AND METHODS

3.MATERIALS AND METHODS

3.1PLANT MATERIAL

The roots of *Dracaena terniflora* Roxb. were collected from the premises of JNTBGRI Palode. A voucher specimen TBGT/86802(23/10/2015) was certified and deposited at institutional herbarium.

3.2 EXPERIMENTAL ANIMALS

Wistar rats (175 to 250g) and Swiss Albino Mice (30-35 g), of either sex, were obtained from animal house of Jawaharlal Nehru Tropical Botanical Garden Research Institute, Palode. They were grouped and housed in Poly-acrylic cages (three animals per cage) and maintained under standard laboratory conditions (temperature 24^0C, relative humidity of 60-70% and 12-hour dark- light cycles). They were fed commercial rat feed (Lipton India Ltd, Mumbai, India) and boiled water (ad libitum). All animal experiments were carried out per **Organisation for Economic Co-operation and Development (OECD, 2001**) guidelines with slight modification, after getting the approval of Institutes Animal Ethics Committee (B1/03/2015/EM &EP/ 27-30).

3.3 CHEMICALS

Magnesium turnings, Sodium hydroxide, Sulphuric acid, Chloroform, Olive oil, Ferric chloride, Lead acetate, HCl, Acetic acid, Ammonia, Copper sulphate, Sodium Nitro Prusside, Acetone, Ethyl acetate, n Hexane, Fehling's solution A and B, Ammonium sulphate, toluene, Gallic acid, Ethanol, Sodium carbonate, Folins ciocalteus reagent, Quercetin, Methanol, Aluminium chloride, Potassium acetate, Carrageenan, Indomethacin, Saline, Formaline, Indomethacin1, DPPH (1-diphenil-2- picrylhydrazyl), Methanol, Ascorbic acid, Acetic Acid, Acetyl salicylic acid, Sodium Nitroprusside, Griess reagent.

PHARMACOGNOSY

3.4.1 Extract preparation

Collected roots were shade dried for 3days, then again kept in oven for 24 hrs at 60ºC for drying. This is then powdered using a mechanical grinder. 40g of powdered plant material was extracted by Cold extraction method using solvent ethanol. Obtained crude extracts were evaporated and removed excess ethanol by using a rotary evaporator.

3.4.2 Macroscopic characterization of plants

The following macroscopic characters for the fresh root were noted:

- Size And Shape
- Colour
- Surfaces
- Tip
- Arrangement
- Odour And Taste

3.4.3 Microscopical Characters

A thin cross-section of root was cut and put in watch glass containing distilled water so as to prevent drying of the section. The sections were stained by using safranin (0.5%V/V) and sections were placed on the slide and few drops of glycerine poured above the section finally place a coverslip above it without allowing any air bubbles to be formed in between. Examine the slide under the microscope in different magnificationBinocular Microscope, (Zeiss).

3.4.4 Physio-chemical evaluation

Physio-chemical parameters are determined for the powdered roots of DT such as total moisture content, total ash, acid insoluble ash, water soluble ash, Hydro

Ethanol soluble extractive, alcohol soluble extractive, and chloroform soluble extractive, hexane soluble extractive and water-soluble extractive values.

3.4.4.1 Moisture content

Approximately, 10g of fresh root was taken and kept in oven to remove moisture from the leaves and set temperature at 60^{o}c. And simultaneously take weight until the weight become constant. Compare the dry weight with fresh to get the total moisture content.

3.4.4.2 Total ash content

3g of root powder was taken in a tared silica crucible and incinerated by Bunsen burner for 2-4 hours and kept the temperature not exceed at 450^{o}c, to obtain carbon free ash. Record the total weight of the ash obtained from it.

3.4.4.3 Acid insoluble ash

Prepare 100ml of 2% HCl. Wash the silica crucible containing the ash with 2% HCl, washout |is collected is again pour into beaker containing acid and boiled for 10 minutes. Filter it by using gooch crucible and kept in oven to dry. Record the weight of acid insoluble ash.

1.4.4.4 Water soluble ash

Wash the silica crucible containing the ash with water, washout is collected is again pour into beaker containing acid and boiled for 10 minutes. Filter it by using Gooch crucible and kept in oven to dry. Record the weight of acid insoluble ash.

1.4.4.5 Extractive values

5 g of the shade dried powder of root of DT was macerated with 500 ml of the solvents (ethanol, chloroform, petroleum ether, hexane and water) and was evaporated to dryness and the percentage of extractives was calculated with reference to the weight of dried leaf powder.

1.5 STANDARD PHYTOCHEMICAL TESTS:

3.5.1 Preliminary phytochemical screening

3.5.2 Test for alkaloids

3.5.2.1 Wagner's Test

1ml of extract was taken and a few drops of Wagner's reagent was added. Observe the formation of a reddish brown precipitate indicating the presence of alkaloids.

3.5.2.2 Hager's Test

1 ml of extract was taken and a few drops of Hager's reagent was added. Yellow colour appears gives the presence of alkaloids.

3.5.2.3 Meyer's Test

1ml extract was taken in a test tube and add a few drops of Meyer's reagent. Observe the formation of yellow or cream precipitate indicating the presence of alkaloids.

3.5.3 Test for flavonoids

3.5.3.1 Shinoda Test

1ml of extract was taken in a test tube. Add a pinch of Magnesium turnings and add 1-2 drops pf conc. HCl. The formation of pink colour indicate the presence of flavonoids.

3.5.3.2 Lead Acetate Test

1ml extract was taken and add a few drops of 10 % lead acetate solution. Observe the formation of yellow precipitate indicating thing presence of flavonoids.

3.5.4 Alkaline Test

1ml extract was taken and add a few drops HCl. Observe the yellow colour which turns colourless in adding few drops of diluted HCl.

3.5.5 Test Of Phenols And Tannin

1ml extract was taken, add 0.5ml of 1 % lead acetate solution and observe the formation of precipitate indicating the presence of tannins and phenolic compounds.

3.5.6 Test for phenols: Lead Acetate Test

1ml extract was taken and then add 3 ml of distilled water. To the mixture, add few drops of 1 %lead acetate. Positive result is confirmed by the appearance of yellowish cream precipitate.

3.5.7 Test for tannins: Braymer's Test

1ml extract was taken, add 1ml water and 2-3 drop ferric chloride. The formation of green precipitate shows the presence of tannins

3.5.8 Test for Saponins

1ml sample was taken in a test tube. To it, 5 ml water was added and heated. Froth appearance indicate the positive result for saponins.

3.5.9 Test for steroids: Salkowski Test

1ml extract was taken and add 1ml chloroform and 1ml sulphuric acid. Observe for the formation of reddish brown ring at the junction indicate the presence of steroids.

3.5.10 Test for coumarins

1ml extract is added to 2ml 10%sodium hydroxide and observe the formation of yellow colour indicating the presence of coumarins.

3.5.11 Test for protein :

3.5.11.1 Xanthoproteic Test

1ml extract was added to 1ml sulphuric acid. Observe the formation of white precipitate showing the presence of protein.

3.5.11.2 Biuret Test

1ml extract was added with 10% sodium hydroxide and is heated. Then add 0.7% copper sulphate. Observe the formation of purplish violet colour indicating the presence of protein.

3.5.12 Test for anthocyanin

1ml extract was taken, add 1 ml 2N HCl and few drops of Ammonia. Observe the colour change from pinkish red to bluish violet, indicating the presence of anthocyanin.

3.5.13 Test for carbohydrates:

3.5.14 Fehling's Test

1ml of extract is added with equal parts of Fehling's solution A and B, heat the mixture and then wait for few minutes. The formation of brick red colour indicate the presence of reducing sugars.

3.5.15 Test for glycosides

1ml extract was taken, then add 1ml chloroform and acetic acid. The formation of violet colour which changes to blue colour which indicate the presence of glycosides.

3.5.14 Killer Killiani Test

1ml extract was taken. Add 1ml of distilled water and sodium hydroxide solution. Observe yellow coloration indicating the presence of Carbohydrates.

3.5.15 Test for phlobatanins

1ml of extract was taken and then add 1ml HCl and heat. Observe the formation of red precipitate indicating the presence of Phlobatanins.

3.6 TOTAL PHENOL CONTENT ASSAY

The total phenol content assay was carried in the ethanolic root extract of DT (Singleton *et al.*, 1965).0.2 ml of Plant extracts of 5mg/ml concentration is transferred to tubes containing 2.5ml 10% Folins ciocalteus reagent. After 10 minutes incubation, 2ml of sodium carbonate (7.5 W/V) was added to the sample. Then the tubes were allowed to stand at room temperature for 30 minutes and read absorbance at 743nm. The concentration of polyphenols in the samples derived from a standard curve of Gallic acid ranging from 5 to 50µg/ml. The total phenolic content was expressed in Gallic acid equivalent in mg of drug extract.

3.7TOTAL FLAVONOID CONTENT

The total phenol content assay was carried in the ethanolic root extract of DT (Zhishen *et al.*, 1991).0.5 ml of Plant extracts of 5mg/ml concentration is transferred to tubes containing 1.5ml Ethanol, 0.1 ml of 10% aluminium chloride, 0.1ml 1M potassium acetate and 2.8ml distilled water. Incubate for 30 minutes. Read absorbance at 415nm. Quercetin is used as standard.

3.8 ANTIOXIDANT ASSAYS

3.8.1 DPPH free radical scavenging assay

The ability of the extracts to annihilate the DPPH radical (1, 1-diphenil-2-picrylhydrazyl) was investigated by the method described by (Blois, 1958). Stock solution of the whole plant extracts was prepared to the concentration of 10 mg/ml. Varying concentration of plant root extract was taken and transferred to tubes. Add 200 µl by methanol and 2ml DPPH (0.02mM) and incubated for 20minutes at dark

at room temperature and read absorbance at 517 nm. The annihilation activity of free radicals was calculated in % inhibition according to the following formula % of Inhibition = (A of control A of Test)/A of control X 100.Ascorbic acid was used as standard controls.

3.8.2 Nitric Oxide Scavenging Activity:

The compound sodium Nitroprusside (SNP) is known to decompose in aqueous solution at physiological pH 7.4 producing nitric oxide radical (NO). Under aerobic condition, NO reacts with Oxygen to produce stable products (Nitrate and Nitrite). The quantities of which can be determined using Griess reagent. The scavenging effect of plant root extract on nitric oxide was measured according to the modified method of (Marconii *et al.,* 1994).

Firstly, 1ml extract solution at different concentration was added in the test tubes to 1 ml of SNP solution (10mM). Then the tubes were incubated at 29ºC for 2.5 hrs. Finally, an aliquot of 1ml of the incubated solution was removed and diluted with 1ml Griess reagent [1% sulphanilamide in 2% H_3PO_4 and 0.1% N-N-Naphthyl Ethylene diamine hydrochloride].

3.8.3 Hydroxyl radical scavenging activity

The assay is based on quantification of the degradation product of 2-deoxyribose by condensation with TBA (Elizabeth *et al.,* 1990). Hydroxyl radical was generated by the Fe3+- Ascorbate- EDTA-H_2O_2 system (the phenton reaction). The reaction mixture contained in a final volume of 1ml, 2 deoxy 2 ribose (2.8mM); KH_2PO_4.KOH buffer (20mM pH 7.4); $FeCl_3$ (100µM); EDTA (100µM); H2O2 (1.0mM); Ascorbic acid (100µM); and concentrations (500µg/ml) of the root extract. After incubation for one hour at 37^0C, 0.5ml of the reaction mixture was added to 1ml 2.8%TCA, then 1ml 1% aqueous TBA was added and the mixture was incubated at 90^0C for 15 minutes to develop the colour. After cooling, the absorbance was measured at 532 nm. Quercetin was used as a positive control. Percentage inhibition was evaluated by comparing the test and the blank solution.

3.9 ACUTE TOXICITY STUDIES

Acute toxicity effect of Rhizome ethanolic extract of *D. terniflora* (EDT) will be evaluated in Swiss albino mice as per OECD guidelines (OECD, 2001) with slight modification of (Suja *et al.,* 2004). Before starting the study all groups (five groups, consisting of two animals each) is fasted for 12 h and weighed. The crude extracts is suspended in a vehicle (0.5% Tween 80) with distilled water (Group 1) before administration. Each animal in Group 2, 3,4 and 5 were administrated p.o. (0.5 ml DT) with the help of gavage No. 16 with 25, 100, 400, 1600 $mgKg^{-1}$ DT respectively. First groupreceived the vehicle only, served as normal control. Food was provided to the mice approximately an hour after treatment. The animals were observed 30min after dosing, followed by hourly observation for 8h and once a day for the next 14 days. Animals were weighed andmortality, behavioural pattern, and changes in physical appearance, injury, pain and signs of illness will be monitored.

3.10 *ANTI-INFLAMMATORY STUDIES:*

3.10.1 Carrageenan induces paw oedema

The anti-inflammatory activity of the *Dracaena terniflora* Roxb. (DT)extract is studied by the method Carrageenan - induced rat paw oedema .The animals is divided into five groups of three animals each and fasted overnight (Amdekar*et al.,* 2011).Group 1, the control group will be administered 1 ml distilled water, Group 2 will receive 1 ml indomethacin (10 $mgKg^{-1}$, standard in saline, p.o) , while Groups 3, 4 and 5 will receive various concentrations of the DT extract (125, 250 and 500 $mgKg^{-1}$, p.o). 30 min after extract administration, 0.1ml, 1% carrageenan in saline (Sigma Chemicals Company, USA) is injected into the right hind paw, under the plantar aponeurosis. The hind paw volume will be measured Plethysmographically before and 3h after carrageenan injection. The difference in the paw volumes will be recorded, which indicates the degree of inflammation. Percentage of inhibition = ((Control – Test) / Control) * 100

3.10.2 Formalin induced paw oedema

The anti-inflammatory activity of the ethanolic root extract of *D. terniflora* (EDT) is studied by the modified method of, Formalin - induced rat paw oedema (Chau, 1989).The animals will be divided into five groups of three animals each and fasted overnight. Group 1, the control group will be administered 1 ml 0.5% Tween 80 (in Dis. H_2O) p.o., Group 2 will receive 1 ml indomethacin(10 $mgKg^{-1}$, standard) (0.5% Tween 80) p.o., while Groups 3, 4 and 5 will receive plant extract (EDT) 50 $mgKg^{-1}$, 150 $mgKg^{-1}$, 450 $mgKg^{-1}$ (in 0.5% Tween-80) p.o. Drug was administered by oral route and after 30 min. of drug administration, 1st and 3rd day Formalin was injected (0.1ml of 2%) in to right hind paw of rats by sub plantar route. Drug was administered once a day for 7 days. And on 7th day after 1hr. of experimental period paw thickness was measured plethysmographically. The difference in paw thickness after and before induction of inflammation was calculated and presented as mean increase in paw thickness (mm).The difference in the paw volumes will be recorded, which indicates the degree of inflammation. Percentage Inhibition: = ((Control-Test)/ Control)* 100

3.11 ANALGESIC STUDIES

3.11.1 Acetic Acid-Induced Writhing in Mice

In this model, the animals were pre-treated with drugs 30 min before induction of writhing. The Group 1 animals received vehicle (0.5%Tween-80, normal control) and Group 2 animals received the reference standard drug Acetyl salicylic acid (10 $mgKg^{-1}$ p.o.). Analgesic activity of root of ethanolic extract of *D. terniflora* at the doses 50,150 and 450 $mgKg^{-1}$ p.o. (Group 3, 4 and 5) was assessed by counting the number of writhes induced by injecting 0.1 ml 0.7% acetic acid (10$mlKg^{-1}$) i.p. The number of writhes (constrictions in the abdomen of the mice) per animal were counted for 20 min. Percent reduction in writhing syndrome was calculated and compared with the standard drug. Percent reduction indicates the percentage protection against abdominal constriction which was taken as an index of analgesic. Percent inhibition of the number of writhing movements was

calculated using the following formula and compared with positive control. Percentage inhibition of writhing = $[1 - (W_T/W_C)] \times 100$ Where W_C and W_T represent the mean number of writhing of control group and test group, respectively (Koster *et al.,* 1959).

3.11.2 Hot plate test

Analgesia is assessed by the modified method of (Eddy and Leimbach, 1953) using Eddy's hot plate (thermal stimulus), at temperature 55 ± 1ºC. Mice will be divided into 5 groups of 3 animals each. Group A, the control group will receive 0.5 ml 0.5% Tween-80 in Dist. H_2O, p.o., Group B, the standard group will receive Acetyl salicylic acid (100 $mgKg^{-1}$) in 0.5% Tween-80. Groups C, D, and E will be administered root ethanolic extract of *D. terniflora (*EDT)(50, 150, 450 $mgKg^{-1,}$ 0.5 ml p.o.) dissolved in 0.5% Tween-80. At 0, 15, 30, 60, 90, 120 min after extract administration mice will be placed on hot plate maintained at 55 ± 1ºC and the time taken by animals to lick the fore or hind paw or jump off the plate will be taken as the reaction time in (sec); 15 sec cut off will be used. The mean reaction time for each treated group was determined and compared with that obtained for each group before treatment. Percentage increase in reaction time

I%, was derived, using the formula I%= {(It – Io)/Io} x 100 Where It = reaction time at time, t, and Io = reaction time at time zero (0).

RESULTS

4. RESULTS

4.1 PHARMACOGNOSTIC STUDIES

4.1.1 Macroscopic characters:

Table 1.Morphological characters of roots of *Dracaena terniflora* Roxb. (Plate 2)

Sl. No	Morphological characters	Observation
1	Shape	Fibrous root
2	Color	Matured roots are dark orangish and others are pale yellowish
3	Tip	Hairy root

4.1.2 Microscopic characters:

Single layered epidermis with unicellular hairs present. Multilayered cortex and parenchymatous cells have leucoplasts and the outer layer gets cutinized to form exodermis on the distinction of epiblema. The parenchymatous cells are oval with inter cellular spaces as in dicots. Endodermis is also the circular inner layer of cortex made of barrel shaped thick walled cells. Endodermis demarcates the cortex from stele. Numerous vascular bundles present and are closed, collateral and scattered in the parenchymatous tissue. Single layer of thin walled cells inner to endodermis only lateral roots arise from pericycle. Pith not well developed.

Abnormal condition in the cambium growth. Secondary vascular bundles are amphivasal and are produced on its inner side and parenchymatous cells on the outer side. The vascular bundles changes the position as they are arranged as concentric circles. (Plate: 4&5)

ANATOMY

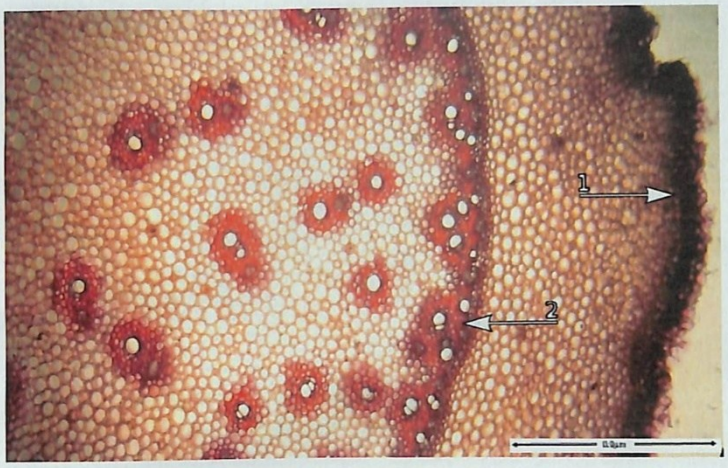


Plate 4: Root cross-section of *Dracaena terniflora* Roxb. Scrattered vascular bundles seen 1: root hairs 2. Sec thickening

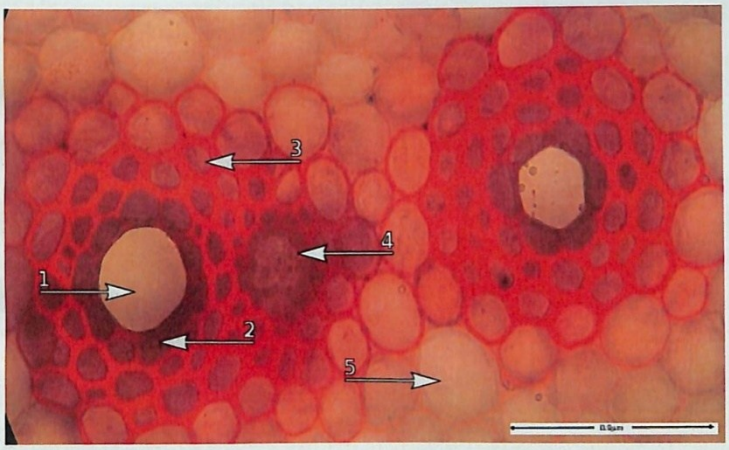


Plate 5: 1.xylem, 2.immature sclerenchyma cells, 3.Mature schlerenchyma cells, 4.phloem, 5. Parenchymatous cells

Powder Analysis:

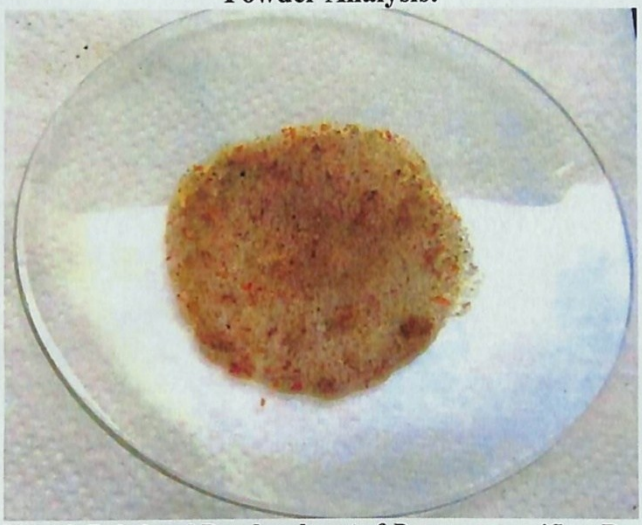


Plate 6: Dried and Powdered root of *Draceana terniflora* Roxb.

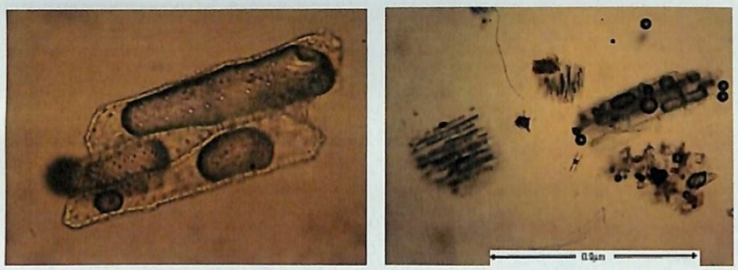


Plate 7: Microscopic powder analysis of DT roots. Pieces of vessels and tracheid are seen.

4.1.3Powder Microscopy

Fragments of root with slightly brownish colour. Pieces of vessels and tracheid with scattered paranchymatous cells present. Fragments of tracheary tissue with helical and scalariform thickenings. Pieces of ground tissue with dense contents and sphaeraphides. (plate 6 & 7)

4.1.4 Physio-chemical parameters (Table 2)

4.1.5 Etractive values of *Dracaena terniflora* in different extracts (Table 3) (Plate 8)

Table 2:Physico chemical parameters of powdered roots of *Dracaena terniflora*

Sl. No	Physio chemical parameters	Values (% w/w)
1	Foreign matters	Nil
2	Total Moisture Content	62.22 ± 0.20
3	Total Ash	12.84 ± 0.32
4	Acid insoluble ash	8.4 ± 0.07
5	Water insoluble ash	41.07 ± 0.02

Values are the mean ± SD, n = 3.

Table 3: Individual Extractive values of powdered roots of *Dracaena terniflora*

Sl. No.	Solvent	Yield (%w/w)	Colour	Consistency
1.	n Hexane	0. 18	Yellow	Sticky
2.	Ethyl acetate	3.5	Red	Sticky
3.	Acetone	7.5	Red	Sticky
4.	Chloroform	0.53 ± 0.057	Orange	Sticky
5.	Ethanol	2.43 ± 0.09	Red	Sticky
6.	Water	1.18 ±0.07	Brown	Sticky

Values are the mean ± SD, n = 3.

Extractive yield:

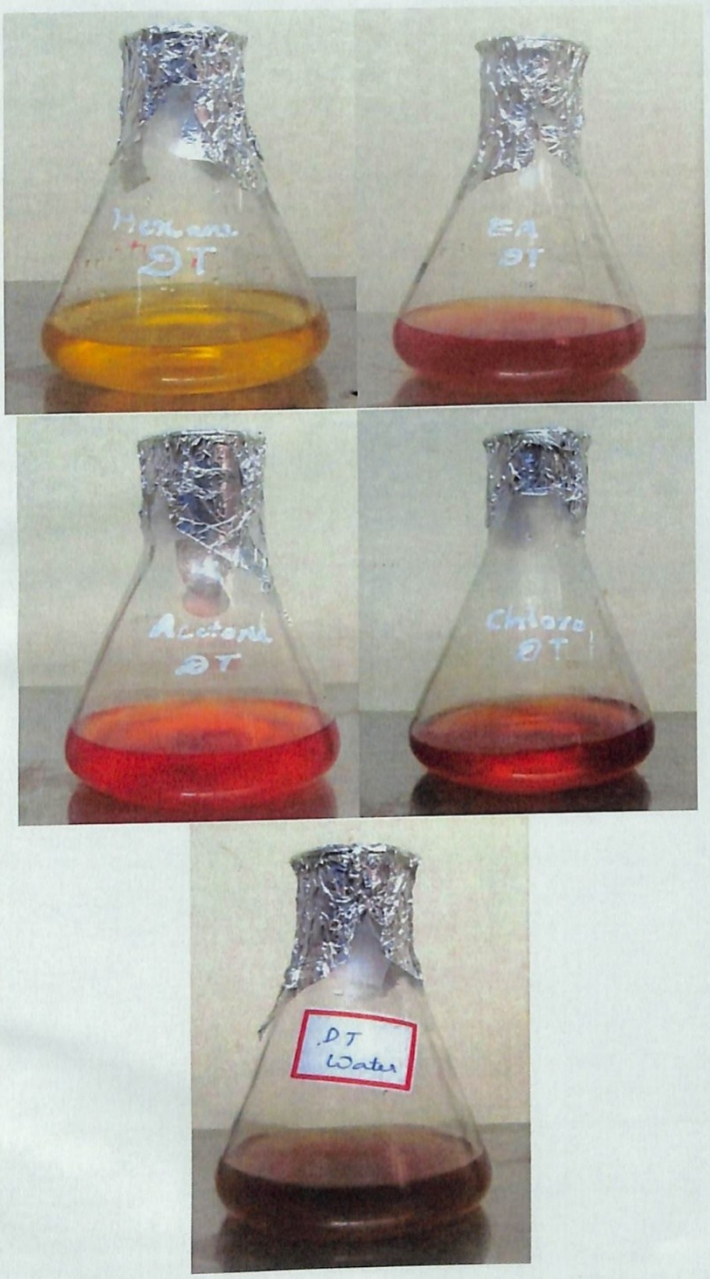


Plate 8: Root extracts of *Dracaena terniflora* in different extracts like 1.Hexane extract , 2.ethylacetate, 3. Acetone extract, 4.chloroform extract and 5.Water extract respectively

4.2 STANDARD PHYTOCHEMICAL TESTS:

4.2.1 Preliminary phytochemical screening

Table 4: Preliminary phytochemical screening of root extract of *Dracaena terniflora*.

Si. No	Reagents	Acetone	Choloform	Ethanol	Ethyl Acetate	Hexane
1	Phenols	+	+	+	+	+
2	Flavonoids	+	+	+	-	-
3	Steroids	+	+	+	+	+
4	Alkaloids	+	+	+	+	+
5	Coumarins	+	+	+	+	+
6	Carbohydrates	+	+	+	-	-
7	Tannins	+	+	-	+	-
8	Saponins	+	+	+	+	+
9	Glycosides	-	-	-	-	-
10	Proteins	-	-	-	-	-
11	Anthocyanin	-	-	-	-	-
12	Triterpenoids	+	+	+	+	+
13.	Anthraquinone	-	+	+	+	+

+ present, – absent

Fig 1: Determination of total phenolic content

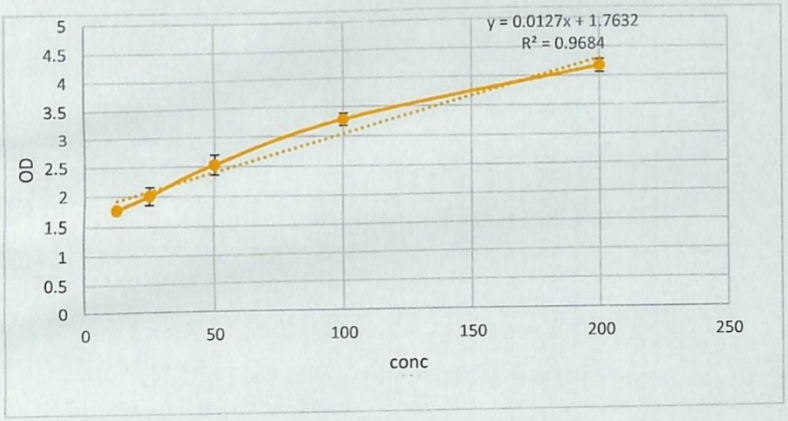
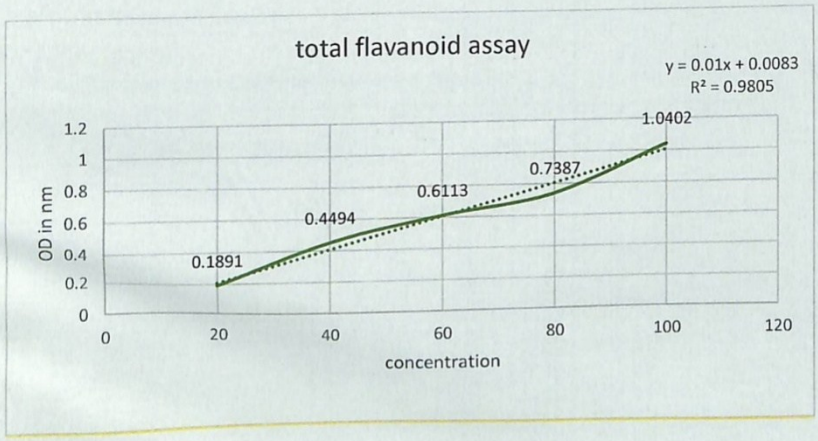


Fig2: Determination of total flavonoid content



4.2.2 Total phenolic content

Table 5: Determination of total phenolic content

Conc. of Gallic acid (µg/ml)	Mean OD (745 nm)
12.5	0.77 ± 0.01
25	2.07 ± 0.15
50	2.57 ± 0.17
100	3.25 ± 0.10
200	4.14 ± 0.011
Sample:	
Ethanol extract (5 µg/ µl)	**0.2823** ± 0.013

The TPC of ethanol extract = 46.64mg GAE / g of extract

The results are expressed as Gallic acid equivalents (QE). From the standard calibration, the total phenol content of the ethanolic root of *Dracaena terniflora* was found that in 500µl sample contains **1.4115**µg/ml (fig 1).

4.2.3 Total flavonoid content

Table 6: Determination of total flavonoid content

Conc. of Quercetin (µg/ml)	Mean OD (745 nm)
20	0.1891 ± 0.004
40	0.4494 ± 0.008
60	0.6113 ± 0.004
80	0.7387 ± 0.006
100	1.0402 ± 0.003
Sample:	
Ethanol extract (5 µg/ µl)	**0.1026** ± 0.013

The TFC of ethanol extract = 3.8mg QE /g of extract

The results are expressed as Quercetin equivalents (QE). From the standard calibration, the total Flavonoid content of the plant ethanolic root of *Dracaena terniflora* was found that in 500µl sample contains **0.1026** µg/ml flavonoid content.

4.3 ANTIOXIDANT ASSAYS:

4.3.1 Free radical scavenging assay

Table 7: Free radical scavenging assay

Conc. (µg/ml)	DPPH radical scavenging activity		NO radical scavenging activity		OH radical scavenging activity	
	% inhibition	EC_{50} (µg/ml)	% inhibition	EC_{50} (µg/ml)	% inhibition	EC_{50} (µg/ml)
100	20.59±0.08	304.98	21.6±0.07	484.68	16.17 ± 0.5	528
300	48.28±0.25		31.5±0.06		21.8± 1.1	
500	71.29±0.10		51±0.05		44.15 ± 0.57	
700	83.05±0.04		33.3±0.01		60.7 ± 0.9	

Values are expressed as mean ± SD (n = 3)

4.3.1.1 DPPH Radical Scavenging Assay

The DPPH antioxidant activity of the ethanolic roots extracts of *Dracaena* ***ternifora*** was determined. The percentage inhibition of scavenging activity was shown in table. And the IC_{50} value of the Standard is 10µg/mland IC_{50} value of the sample is 304.98µg/ml.

Fig3: Determination of DPPH radical scavenging assay

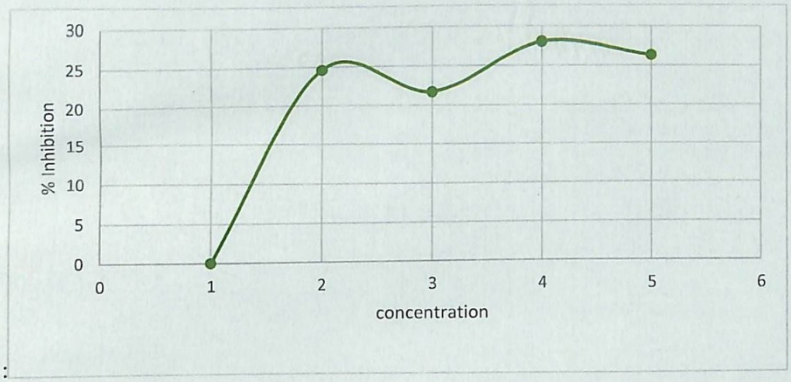


Fig 4: Determination of nitric oxide radical scavenging assay

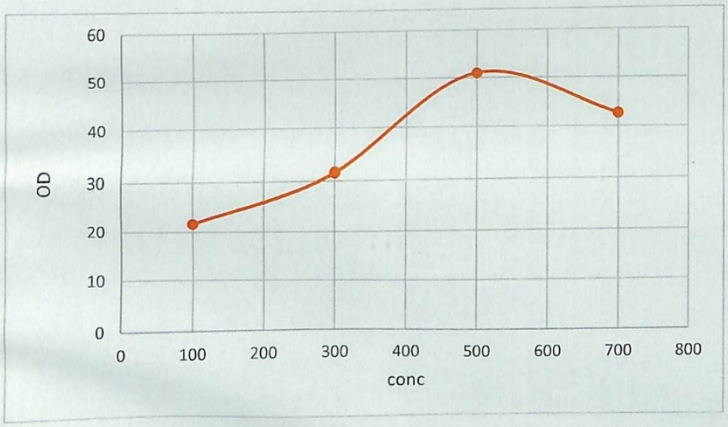
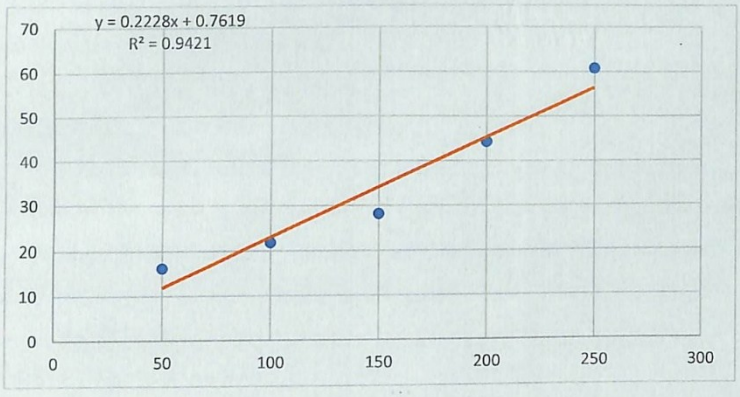


Fig5: Determination of hydroxyl radical scavenging assay



4.3.1.2 Nitric oxide scavenging assay

The Nitric Oxide antioxidant scavenging activity of the ethanolic rootsextracts of *Dracaena ternifora* was determined. The percentage inhibition of scavenging activity was shown in table. And the IC_{50} value of the Standard is 10µg/ml and IC_{50} value of the sample is 484.68µg/ml.

4.3.1.3 Hydroxyl radical scavenging assay

The Hydroxyl radical scavenging antioxidant activity of the ethanolic root extracts of *Dracaena terniflora* was determined. The percentage inhibition of scavenging activity was shown in table and the IC_{50} value of the Standard is 50 µg/mland IC_{50} value of the sample is 528 µg/ml.

4.4 ACUTE TOXICITY STUDIES

Acute toxicity study of the ethanolic root extract *D. terniflora* was administered in mice orally. It doesn't show any mortality at the highest dose of 1600 $mgKg^{-1}$body weight of the animal. And other parameters indicating toxicity of the drug such as Digestion, food intake, fatigue, body weight, behavioural pattern etc. of test animals observed and the result tabulated on the (table 9 & 10).

Table 8: Observation of acute toxicity studies of ethanolic root extract of *Dracaena terniflora*.

Sl. No.	**Parameters**	**EDT 100**mgKg^{-1}	**EDT 400**mgKg^{-1}	**EDT 800**mgKg^{-1}	**EDT 1600**mgKg^{-1}
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 & 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

*Two animals per group

Table 9: Weight of animals (g) during acute toxicity studies:

Days	GRP-1 Control (tween)	GRP-2 100 $mgKg^{-1}$ DT	GRP-3 400 $mgKg^{-1}$ DT	GRP-4 800 $mgKg^{-1}$ DT	GRP-5 1600 $mgKg^{-1}$
DAY 1	24.4	24.8	24.8	27.3	28.5
DAY 7	24.91	24.01	25.41	28.5	27.91
DAY 14	26.25	25.25	26.92	29.02	28.7

4.4 ANTIINFLAMMATORY STUDIES(Plate 9 & 10)

4.4.1 Carrageenan Induced Paw Oedema

At a dose of 450 $mgKg^{-1}$, given the maximum percentage of inhibition in rat's paw oedema obtained was 63%. At this dose the mean number of paw licking was significantly lower than that of control group. Indomethacin exerted a significant protective effect inducing a protection of 70.71% at a dose of 10 $mgKg^{-1}$body weight (fig 6)(table 8).

4.4.2 Formalin Induced Hind Paw Oedema

At a dose of 450 $mgKg^{-1}$of the ethanolic leaves extract of DT reduced the inflammation in rat compared to other doses and the percentage of inhibition obtained was 96.727%. At this dose the mean number of paw licking was significantly lower than that of control group. Indomethacin exerted a significant protective effect inducing a protection of 88% at a dose of 10 $mgKg^{-1}$body weight (Fig 7)(table 9).

ANTI-INFLAMMATORY STUDIES

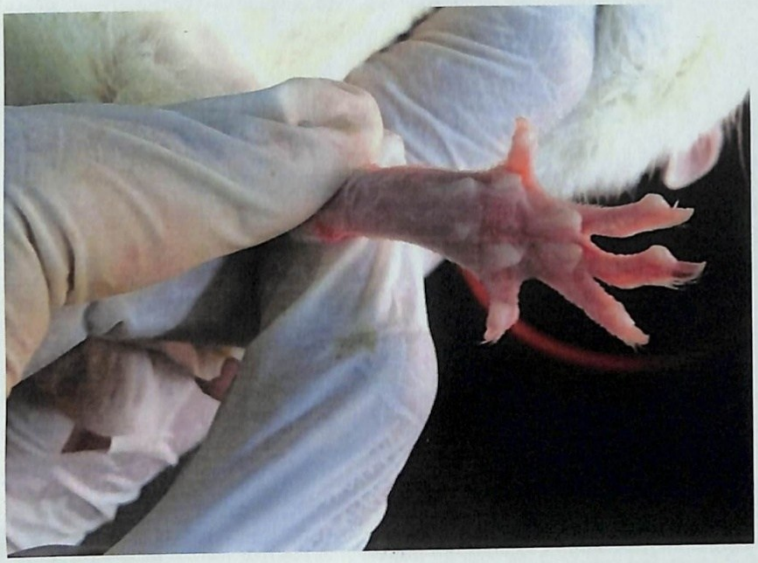


Plate 8: Hind paw of wistar rats before inducing inflammation

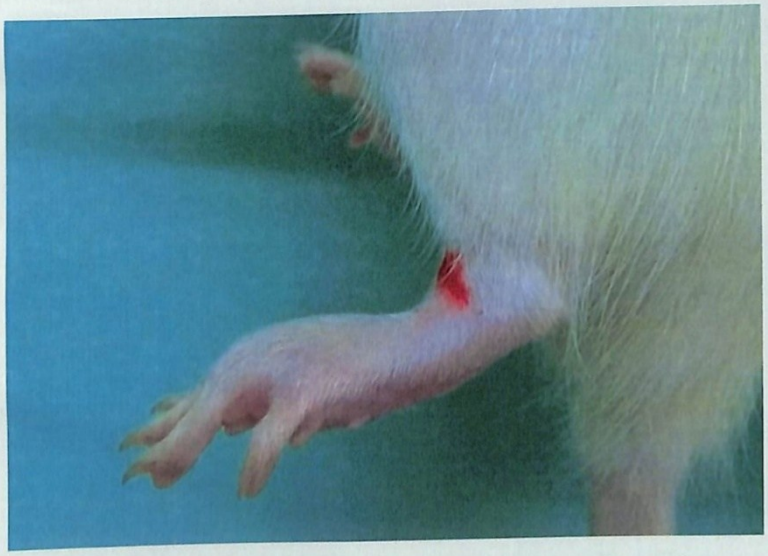
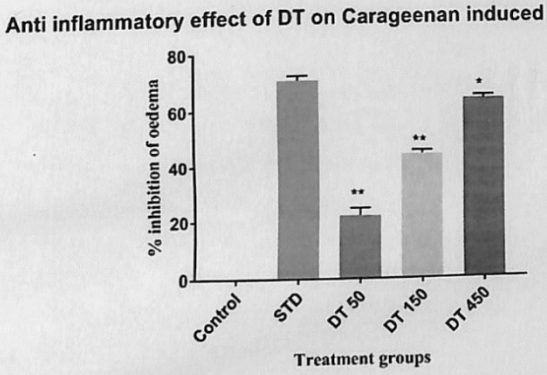


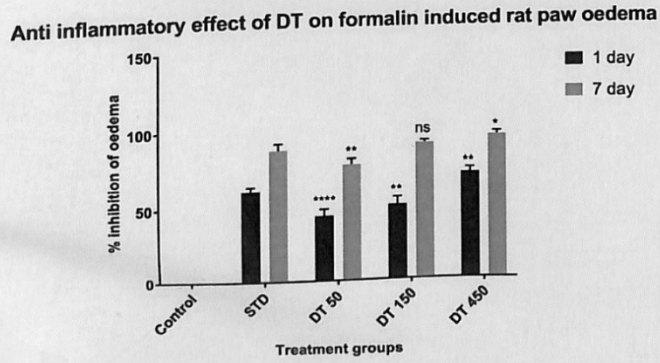
Plate 10: Hind paw of Wistar rats after inducing inflammation

Fig 6: Percentage inhibition in carrageenan induced paw oedema



The *vertical bars* indicate the mean ±SD (n=3).*p< 0.05, **p0.01 compared with standard indomethacin treated group (One way ANOVA, Dunnett's multiple comparison test)

Fig 7: Percentage inhibition in formalin induced paw oedema



The *vertical bars* indicate the mean ±SD (n=3).*p< 0.05, **p0.01, ****p<0.0001 compared with standard indomethacin treated group (Two- way ANOVA, Dunnett's multiple comparison test)

4.5 ANALGESIC STUDIES

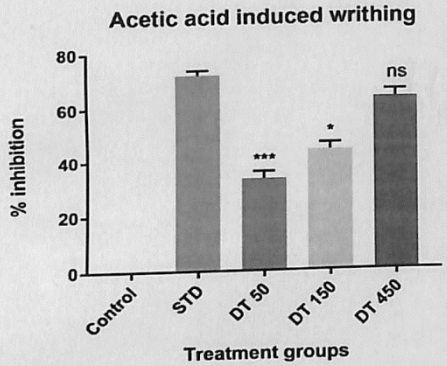
4.5.1 Acetic acid induced writhing in mice

At a dose of 450 $mgKg^{-1}$given the maximum percentage of inhibition in mice around obtained was 62.31%. At this dose the mean number of the writhes was significantly lower than that of control group. Acetyl salicylic acid standard drug which also shows a significant protective effect inducing a protection of 73.76 % at a dose of 10 $mgKg^{-1}$body weight (fig8)(table 10).

4.5.2 Eddy's Hot plate method:

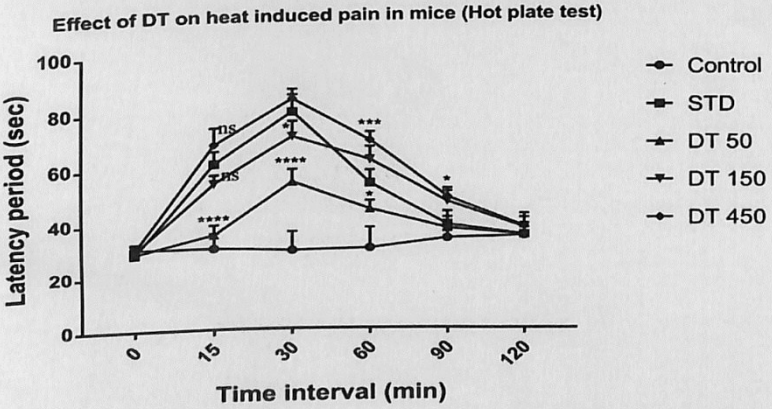
At a dose of 450 mgKg1 given the maximum the percentage of inhibition obtained was 67.4%. At this dose the mean number of paw licking was significantly lower than that of control group. Acetyl salicylic acid exerted a significant protective effect by inducing a protection of 56.66% at a dose of 10 $mgKg^{-1}$body weight (fig 9).

Fig 8: Percentage inhibition in acetic acid writhing test



The *vertical bars* indicate the mean ±SD (n=3).*p< 0.05, **p0.01 compared with standard Acetyl salicylic acid treated group (One way ANOVA, Dunnett's multiple comparison test)

Fig 9: Percentage of inhibition in Eddy's Hot plate method in mice.



The *vertical bars* indicate the mean ±SD (n=3).ns (non significant),*p< 0.05, **p0.01 compared with standard Acetyl salicylic acid treated group (One way ANOVA, Dunnett's multiple comparison test)

DISCUSSION

5. DISCUSSION

5.1 PHARMACOGNOSTIC STUDIES

Macroscopic and Microscopic evaluation is crucial step in the preliminary identification of plant. Miscroscopic studies leads to the proper understanding of the anatomy, or internal structure of plants like spacial arrangement of cells (Jarald and Jarald, 2007).The microscopical examination of root of *D. terniflora*revealed the anatomical features of DT roots including the anamolous secondary thickening exhibited in a monocot plant(Nancy & Dengler, 2000).

In pharmacognpstic studies, moisture content analysis is a critical component of a function of quality control in most products and essentially material quality.This study revealed that the Moisture content of DT root is 62.22%.Ashing is an important tool for analysing the adulteration in crude drugs12.84 ± 0.32 in case of DT. Different methods of ash values are used for detection of crude drugs like total ash, acid insoluble ash and water soluble ash. Extraction with different solvents values are indicative of the presence of the adulterants, defective processing and poor quality of the drug.

5.2 PHYTOCHEMICAL SCREENING

The preliminary phytochemical screening tests is useful for the detection of the bioactive compounds which may lead to the drug discovery and development. Screening tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

Phytochemical screening in this present study, has revealed the presence of alkaloids, anthocyanins, caumarins, glycosides, saponins, phenols, phlobatanins alkaloids, flavonoids, tannins and carbohydrate in the different extracts of roots of DT and so it reveals the Biological and pharmacological activities of DT, based on the phytoconstituents present in it(Varadarajan *et al.,* 2008). These are contributed by the secondary metabolites in the plant body

(Satheesh Kumar *et al.,* 2012). All the six extracts of DT screened were found to possess tannins. Tannins have amazing properties of wound healing Flavonoids arefree radical scavenger, which prevent oxidative cell damage activity(Gibson, 1998). Terpenoids have been found to be useful in anti-cancer agent. Terpenoids possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties Rabi and bishayi. 2003, Wagnor and Elmadfa, 2003). Steroids have immunomodulatory activity (Shah *et.al*., 2009). Coumarins can be suggested to be beneficial for hyperproliferative skin diseases.Natural pigments that are derivatives of anthraquinone are found. Alkaloids represent are involved in the treatment of central nervous system disorders.

Flavonoids have anti-allergic, anti-inflammatory, antioxidant, anti-microbial, anti-cancer, and anti-diarrhoeal activities.*In vitro* studies and *in vivo*studies. From the experiment, it is obtained as 500µl sample contains 0.1026µg/ml flavonoids(Bhajesti *et al.,* 2007).

The major uses ofphenol, involve its conversion to precursors for plastics. Non-ionic detergents are produced by alkylation of phenol to give the alkyl phenols(*Weber*, 2005). Phenol is also a versatile precursor to a large collection of drugs. It is also used in many herbicides and pharmaceutical drugs.Concentrated phenol liquids are commonly used for permanent treatment of ingrown toe and finger nails, a procedure known as a chemical matrixectomy(Svobodova, *et al.,* 2003). Phenol spray is used medically to help sore throat. From the experimental studies it is analysed that 100µl sample contain 0.2823µg/ml Phenolic content.

5.3 ANTI-OXIDANT ASSAYS:

The DPPH free radical scavenging assay is one of the widely used techniques for screening the antioxidant potential of plant extracts. A freshly prepared DPPH solution exhibits a purple colour with absorption maximum at 517 nm . The DPPH radical reacts with suitable reducing agents, the electrons become paired off and solution loses colour depending upon the number of electrons taken

up. The root extract exhibited antioxidant activity at all the concentrations of test solutions. With the increase in concentration of the plant root extract the percentage of antioxidant activity also increased. On evaluating antioxidant activity in *Dracaena terniflora showed* that the DPPH radical scavenging activity increased with the increase in concentration of the extract. DT exhibited the significant free radical scavenging activity of DPPH at 700µg/ml concentration (83.99 %) at700 of DPPH and the IC50 value exhibited at 278.7µg/ml (Soler Rivas *et al.,* 2000)..

Nitric oxide is a potent pleotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signalling, and inhibition of the platelet aggregation and regulation of cell-mediated toxicity (Hagerman, 1998). NO can react with superoxide to form the peroxynitrite anion that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to production of nitrite ions. Large amount of NO will lead to diseased condition by tissue damage. Ethanoic extract of DT roots showed significant NO inhibition activity of 51% at 500µg/ml.

Hydroxyl radical can be formed by the Fenton reaction in the presence of reduced transition metals (such as Fe2+) and H2O2. Scavenging of hydroxyl radical is an important antioxidant activity because of very high reactivity of the OH radical(Wang *et al.,* 2008).The hydroxyl radical scavenging potential of ethanolic root extracts of DT is show increasing hydroxyl radical scavenging activity was with 60.7% inhibition at concentration of 700µg/ml.

5.4 ACUTE TOXICITY STUDY

Acute toxicity study is a technique for toxicity detection in animal body in increasing concentration of the sample drug till symptoms of toxicity appears (Combe *et al.,* 2004). In acute toxicity study, the ethanolic root extract of DT exhibited no toxic effect or lethality in mice. The animals remained healthy even at dose of 1600 $mgKg^{-1}$

5.5 ANTI-INFLAMMATORY STUDIES

Carrageenan model is well suited for comparative bioassay of anti-inflammatory agents in *in vivo* studies. It is generally represented in a biphasic curve (Winter *et al.,* 1962). The first curve happens due to the trauma of injection and also due to the serotonin component and takes place within an hour of injection. Second phase is contributed by the role of prostaglandin which is measured around 3 hrs. The carrageenan induced rat paw oedema models is a sensitive inhibiter of cyclooxygenase and 5-0lipo oxygenase pathway. This is the reason of using it to evaluate the effect of NSAIDs which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis (Di Rosa, 1972). The effect of DT root ethanolic extract and indomethacin on carrageenan induced paw oedema are given in table. In control animals, the injection of carrageenan in the sub plantar region produced a local oedema that increased progressively to reach a maximum intensity at 3 hr after injection. For 10 $mgKg^{-1}$of the indomethacin the percentage inhibition obtained was 95.45%. Ethanolic root extract of DT showed significant dose dependent inhibition of 63% at concentration 450 $mgKg^{-1}$DT.

Formalin induced paw oedema which is a sub-acute inflammation occurs due to the reason of cell damage provoking the production of endogenous mediators. The inflammation produces by the injection of formalin is similar to human arthritis inflammation (Mishra *et al.,* 2010).

Indomethacin is used as the control as it shows uniform inhibition of oedema in all three phases of inflammation namely- early, intermediate and later phases. Arthritis induced by formalin is a model used for the evaluation of an agent with probable antiproliferative activity. From the results the DT fractions significantly inhibited this model of inflammation they can be thought to possess antiarthritic activity similar to indomethacin, a well-known cyclooxygenase inhibitor. The effect of DT root ethanolic extract and indomethacin on formalin induced paw oedema are given in table. In control animals,injection of formalin produced a local oedema in the sub plantar region that increased progressively to

reach a maximum intensity at 3 hr after injection of the phlogistic agent.Ethanolic extract of DT showed a more or less significant inhibition of formaldehyde induced oedema in early phases while significant inhibition at later phases. For 10 $mgKg^{-1}$of the indomethacin the percentage inhibition obtained was 88%. Ethanolic root extract of DT showed significant dose dependent inhibition of 96.72% at concentration 450 $mgKg^{-1}$ DT.

4.6 ANALGESIC STUDIES

The hot plate test has been used widely as a suitable tool for the evaluation of centrally acting analgesics. Swiss albino mice was in this study. The method has the drawback that sedatives and muscle relaxantsmay cause false positives. Latency times comparing the values before and after administration of the DT to the experimental animals compared to the control groups can be used for statistical comparison.

In acetic acid induced writhing test, at dose of 450 $mgKg^{-1}$ the DT root ethanol extract inhibited writhing in response in mice caused by intraperitoneal administration of acetic acid and the percentage inhibition obtained was 62.31%. At this dose the number of the writhes were significantly lower than that compared to the control group. The standard taken was acetyl salicylic acid which excreted a significant protective effect inducing a protection of 73.76 at dose of 10 $mgKg^{-1}$ body weight.

SUMMARY

6. SUMMARY

The study entitled Pharmacognostic studies and evaluation of anti-inflammatory, analgesic and antioxidant potential of 'manjakantha'(*Dracaenaterniflora*Roxb.) was conducted at the Ethnomedicine and Ethnnopharmacolgy 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, analgesic, antioxidant potential of roots of an ethnomedicinal plant *Dracaenaterniflora*Roxb. and to carry out its pharmacognostic studies.

The roots of *Dracaena terniflora* Roxb. were collected from the premises of JNTBGRI Palode Thiruvananthapuram and was used for the studies. Herbarium specimen of *Dracaena terniflora* (TBGT/86802(23/10/2015) has been deposited in JNTBGRI herbarium (Plate 3).

In the pharmacognostical investigation,*Dracaenaterniflora*Roxb. was subjected to microscopic and macroscopic analysis and also physico-chemical tests. The results of this study can be served as a reference material to develop a monograph for further studies and to verify the drug based on this purity. In the preliminary phytochemical screening of roots of *Dracaenaterniflora*Roxb. show the presence of secondary metabolites like phenols, flavonoids, saponins etc. were found and these phytochemicals, can be used for its therapeutic efficiency.

The antioxidant effect of the ethanolic extract of roots of*Dracaenaterniflora*Roxb. (EDT) by DPPH, Hydroxyl and Nitric Oxide free radical scavenging methods in different concentration of the extract. The antioxidant activity 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 roots of *Dracaenaterniflora*Roxb. was tested by acute toxicity study in mice with four

doses 25, 100, 400, 1600 $mgKg^{-1}$ body weight. And the mice were cage side observed for fourteen days and no toxic effect were seen in the tested animals.

The anti-inflammatory effect of the EDT on Carrageenan (Acute inflammation) and formalin (sub chronic inflammation) induced paw oedema on hind limb in rats were tested with three different doses i.e. 50, 150, 450$mgKg^{-1}$of body weight. Treatment with ethanolic extract of roots of DT at above doses which significantly inhibit the inflammation.

The analgesic property of the ethanolic extract of roots of *D. terniflora* on Eddy's Hot Plate method and Acetic acid writhing method in mice were tested with three different doses i.e. 50, 150, 450$mgKg^{-1}$of body weight. Treatment with ethanolic root extracts of *Dracaena terniflora*at above doses which significantly reduced the pain in test animals.As the experiments were carried out accordingly from the microscopic analysis to the in vivo tests. The plant root anatomy, morphology and phamacognosy was analysed, the phytochemicals were screened, the phenol and flavonoid contents were tested, the antioxidants effects were proved and from the in vivo studies the toxicity was also evaluated. Now I conclude that *Dracaena terniflora* Roxb. is having anti-inflammatory and analgesic property and it was be used for commercially for drug preparation. Further studies are needed to explore the exact mechanism of action of *Draceanaterniflora*Roxb.in learning their bioactivities. Some of the phytochemical constituents have been identified, its pharmacognostic and anti-oxidant potential have been studies and also the present study helps to substantiate the tribal claim of using this plant as an anti-inflammatory and analgesic agent.

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7. REFERENCES

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APPENDICES

8. 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

9. ABSTRACT

The study entitled "Pharmacognostic studies and evaluation of antiinflammatory, analgesic and antioxidant potential of 'manjakantha'(*Dracaena terniflora* Roxb.)" was conducted at the Ethnomedicine and Ethnopharmacology Division of Jawaharlal Nehru Tropical Botanical Garden (JNTBGRI), Palode, Thiruvananthapuram, during the year 2015 - 2016. The main objective of the study was to evaluate the anti-inflammatory, analgesic and antioxidant potential of the ethanolic extract of the root of the ethnomedicinal plant *Dracaena terniflora* Roxb. and to carry out its pharmacognostic studies

In the detailed study of pharmacognostical parameters, the microscopic evaluation showed the presence of anomalous secondary thickening in *Dracaena terniflora* roots (Monocot), in powder analysis microscopically scattered tracheids and vessels are seen ash value was low as 12.84%, moisture is obtained as 62.03%. Differential extractive value of *Dracaena terniflora* roots were carried out in which the highest extractive yield was obtained in acetone, which was 7.5% and then Preliminary phytochemical screening revealed the presence of various phytoconstituents like flavonoids, steroids, saponins, alkaloids, carbohydrates and phenols with different extracts. Total flavonoid of 500µl sample is 0.1026µg/ml and phenolic content in 500µl sample is 1.4115µg/ml.

In *in vitro* antioxidant method the methanolic extracts of root showed higher free radical scavenging activity compared to standards with EC50 of 304.98µgml^{-1} (DPPH), 484.68 µgml^{-1} (NO Scavenging Activity) and 528.43 µgml^{-1} (Hydroxyl Free Radical Scavenging Activity).

Toxicity of the ethanolic root extract of *Dracaena terniflora* were tested by acute toxicity study in mice with four doses 25, 100, 400,1600mgKg^{-1} body weight. And the mice were caged and observed for fourteen days and no toxic effect were seen in the tested animals.

Detailed *in vivo*pharmacological studies for anti-inflammatory and analgesics were conducted. Anti-inflammatory activity was determined by Carrageenan (Acute inflammation) and formalin (sub chronic inflammation) induced paw oedema on hind limb in rats with three different doses of ethanolic

root extract of *D.terniflora* with 50, 150 and 450 $mgKg^{-1}$. At the dose of DT 450 mgKg-1 give maximum inhibition of 63% in carrageenan induced paw oedema and 84% and 96%inhibition in first and seventh day of formalin induced paw oedema study respectively.

Analgesic activity was conferred in Eddy's Hot Plate method and Acetic acid induced writhing method in mice with three different doses of 50, 150 and 450 $mgKg^{-1}$. At the dose of DT450$mgKg^{-1}$ gives maximum inhibition 67.4% of inhibition in Eddy's hot plate method and 62.31% in the acetic acid induce writhing study. The anti-inflammatory and analgesic property of *D. terniflora* may be due to the presence of the secondary metabolites present in it, such as flavonoid & phenolic compounds, polysaccharides, steroids, triterpenoids, alkaloids, tannins etc.

The results of current study will help to develop a monograph of the drug by keeping it as a reference. These results substantiate the traditional claim of the plant for its anti-inflammatory action.