ASSESSMENT OF ANTIINFLAMMATORY AND ANTIOXIDANT PROPERTIES OF *Chlorophytum laxum* R. Br.

Submitted by

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(2013-09-115)

THESIS

Submitted in partial fulfilment of the

requirement for the degree of

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KERALA, INDIA

2018

DECLARATION

I hereby declare that this thesis entitled '**Assessment of antiinflammatory and antioxidant properties of *Chlorophytum laxum* R. Br.**' is a bonafide record of the research work carried out by me under the supervision and guidance of Dr. S. R. Suja, Scientist & HOD (i/c), Division of Ethnomedicine and Ethnopharmacology, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, during the course of research. No part of the work embodied in this thesis has been submitted earlier for the award of any other degree, diploma or title.

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16-11-2018

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കെ എസ് സി എസ് ടി ഇ - ജവഹർലാൽ നെഹ്റു ട്രോപ്പിക്കൽ ബൊട്ടാണിക് ഗാർഡൻ ആന്റ് റിസർച്ച് ഇൻസ്റ്റിറ്റ്യൂട്ട്
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National Centre of Excellence, Govt. of India

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CERTIFICATE

This is to certify that this thesis entitled **"Assessment of antiinflammatory and antioxidant properties of *Chlorophytum laxum* R. Br."** is a record of research work done by **Ms. Arundhathy G. B.** (2013-09-115) under my guidance and supervision and that this has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Dedicated to Almighty, my Family, Friends and Teachers

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Percent
°C	Degree Celsius
µg	Microgram
µL	Microliter
µM	Micromolar
AA	Ascorbic acid
AAE	Ascorbic acid equivalents
ANOVA	Analysis of variance
cm	Centimeter
DNA	Deoxyribonucleic acid
DPPH	2, 2-diphenyl 2-picrylhydrazyl
EC_{50}	Effective concentration 50 percent
ECL	Ethanolic extract of *Chlorophytum laxum*
EDTA	Ethylene Diamine Tetra Acetic Acid Disodium salt

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et al.	And other co workers
etc.	Etcetera
$FeCl_2$	Ferric chloride anhydrous
$FeSO_4$	Ferrous sulphate
g	Gram
GAE	Gallic Acid Equivalents
h	Hour
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HRBC	Human Red Blood Cell
IC_{50}	Inhibitory concentration 50 percent
KCL	Potassium Chloride
Kg	Kilogram
L	Litre
M	Molar
MDA	Malondialdehyde
mg	Milligram

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min	Minute
mL	Millilitre
mm	Millimetre
mM	Millimolar
N	Normality
ng	Nanogram
NIH	National Institute of Health
nm	Nanometer
nmol	Nanomole
NO	Nitric Oxide
NSAID	Non-steroid antiinflammatory drug
OD	Optical Density
OECD	Organization of Economic Cooperation and Development
OH	Hydroxyl
pH	Potential of hydrogen
QE	Quercetin Equivalent
rpm	Rotation per minute

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RB	Round Bottom
RBC	Red Blood Cell
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SD	Standard Deviation
SDS	Sodium Dodecyl Sulphate
STD	Standard
TAC	Total Antioxidant Capacity
TBA	Thio Barbituric Acid
TCA	Trichloro Acetic acid
TPC	Total Phenolic Content
UV	Ultraviolet
w/v	Weight/Volume
WHO	World Health Organization

INTRODUCTION

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1. INTRODUCTION

Food and medicines are inseparable combinations not only to human beings but also to all living beings in our planet. Early man explored his surroundings and identified suitable flora and fauna used as food and medicine for sustenance as they knew the need for consuming seasonal food to enhance the functioning of immune system and to prevent diseases. Ethnic food and medicine are mostly location specific and is the best suited for the local climate and environment.

The plants capable of contributing to the health security of humans and their domesticated animals, have always fascinated human beings. They called such herbage as medicinal plants, and made use of its different parts- roots, leaves, bark, stem, flowers, fruits, and seeds for curing many ailments varying from simple discomforts to serious diseases. WHO's finding that about the 80% of people in developing countries rely on plants for their primary health care needs still remains true, particularly in villages of countries in the tropical world.

Plants have been utilized in various traditional medicinal systems throughout the world that provide people with medicines - to prevent disease, maintain health or cure ailments. They are the backbone of traditional medicine which are considered as a rich resources of ingredients that have substances utilized for therapeutic purposes or as antecedents for the preparation of essential drugs. The therapeutic potency of medicinal plants is because of the presence of bioactive compounds, namely tannins, vitamins, flavonoids, glycosides, alkaloids and coumarins compounds. Traditional medicine refers to the health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being (WHO, 2008). The use of traditional medicine

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and medicinal plants in most developing countries, as the basis for maintenance of good health, have been widely observed by UNESCO (1996).

Inflammation is an integral part of the body's immune response. It is a physiological response towards deleterious stimulus, suchlike tissue damage, irradiation or pathogenic organisms, to eliminate harmful stimuli and to initiate the healing cascades. It is featured by redness, heat, pain, swelling and disturbance of function. Inflammation signifies the reaction of body tissues towards physical trauma, leading to the aggregation of fluids and blood cells; and that the entire mechanism, viewed across the wide purview of evolution, is a beneficial one, its prime consideration being (in every prospect) that of a defense against microscopical assailants.

Antioxidants are defense mechanism produced by the body to neutralize the effects of ROS and reduce or diminish the oxidative stress. Antioxidants inhibit the free radical production early in the oxidation process. Absence of antioxidants can satiate the reactive free radicals that aids to the development of degenerative disorders, such as neurodegenerative diseases, cancers (Gerber *et al.*, 2002), cardiovascular diseases, Alzheimer's disease and inflammatory diseases. Plants are known to contain variety of natural antioxidants that protect their physical and metabolic integrity.

Chlorophytum laxum R. Br. (commonly called as "Neeruvatti") belongs to the family of Liliaceae. It is an important medicinal plant used by Kani tribes of Kerala, Karnataka, Maharashtra and Madhya Pradesh for inflammation, insect bites and snake bites. Ethnobotanically the tubers of *Chlorophytum laxum* have been used as a folk medicine for the treatment of traumatic injury, poisonous snake bites, swelling and pain, diarrhoea, dysentery and insect bites.

The present study aims to scientifically evaluate the antiinflammatory and antioxidant potential of *Chlorophytum laxum* R. Br.

REVIEW OF LITERATURE

Plate 1. Habit and tubers of *Chlorophytum laxum* R. Br. (Neeruvatti)

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2. REVIEW OF LITERATURE

2.1 MEDICINAL PLANTS

Medicinal plants are those plants which are used in various traditional systems of medicines throughout the world. These plants virtually benefits humans with medicines to prevent disorders, maintains their health and cure maladies. The medicinal plants consists of various species of plants which is used in herbalism and possess medicinal activities. Medicinal plants are the backbone of traditional medicine. These medicinal plants are considered as a rich resources of ingredients that contain substances which can be used for therapeutic purposes or which are the precursors for the synthesis of useful drugs. The medicinal plants therapeutic properties are adapted by the presence of bioactive constituents, namely coumarins, flavonoids, glycosides, alkaloids, vitamins and tannins compounds, that physiologically influence animals and humans or which are biologically dynamic with respect to the causative agents of various illness. Large proportion of such therapeutic compounds have been discovered with the aid of ethno-botanical knowledge of their traditional uses.

The utilization of medicinal plants and traditional medicine in developing nations, as a footing for the sustainment of good health, have been widely observed by UNESCO (1996). Moreover, an increase in the dependency on the usage of medicinal plants in industrialized societies for the synthesis and development of various chemotherapeutics and drugs from these medicinal plants apart from traditionally used herbal therapies have been traced (Singh, 2015). India is well endowed with the knowledge and practice of herbal medicine and can be considered as one of the largest producers of medicinal herbs.

Herbs are setting a revival and herbal 'renascence' is occurring globally. Today the herbal products exemplify safety in contrary to the synthetic products which is

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regarded as perilous to human and environment. A variety of biochemical products are synthesized and preserved by plants, much of which are extractable and utilized as chemical feed stocks or as basic material for several scientific researches. These secondary metabolites of plants discloses their commercial importance as they find use in several pharmaceutical products. Nonetheless, a constant supply of the raw material habitually becomes strenuous due to the factors such as diversified geographical distribution, changes in environment, over exploitation by pharmaceutical industries, labour cost, cultural practices and superior plant stock selection.

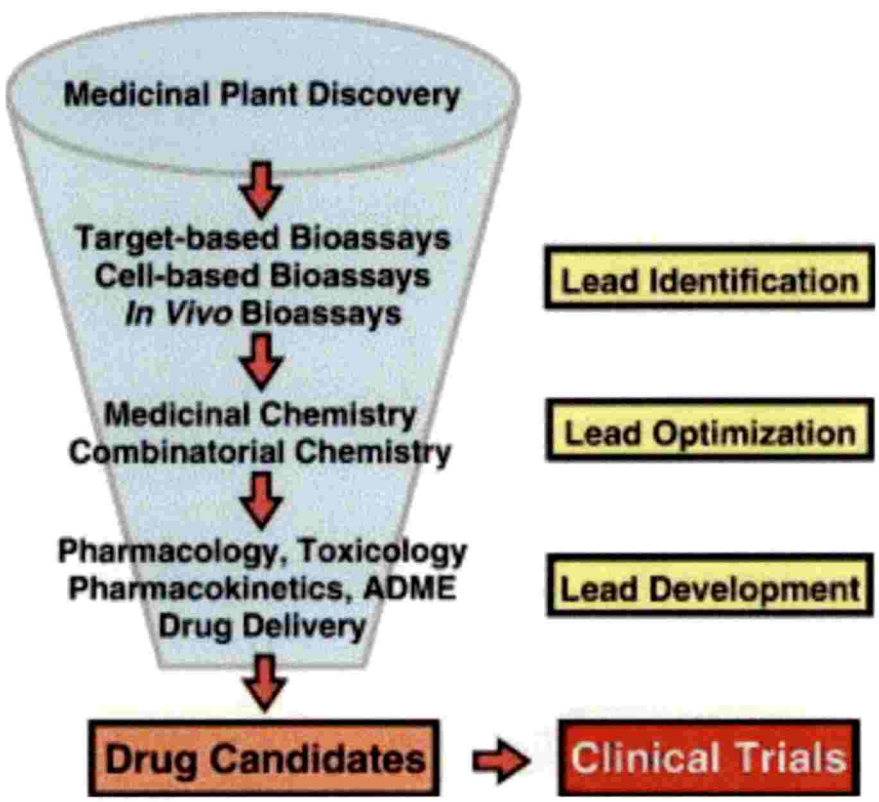

Figure 1. Schematic representation of a typical medicinal plant drug discovery process and development (Balunas and Kinghorn, 2005)

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2.2 ETHNOBOTANY, ETHNOMEDICINE AND ETHNOPHARMACOLOGY

Ethnobotany is the study of the direct relationships between human and plants (Ford, 1978). It as a unit of an ecological study specialization in the interaction of man and the plant world. Ethnobotany can also be defined as an anthropocentric approach towards botany, and is typically concerned with collection of literature on plants and their uses (Rajendran, 1997). The Ethnomedicine concept was evolved from the necessity for studies in the modern sciences on the drugs used in the traditional medicines. Ethnomedicine is described as the study of traditional medical practice that is concerned with the cultural decipherment of health, illness, and diseases which addresses the healthcare pursuing process and healing practices (Pushpangadan, 1995). Many plant-derived drugs used in modern medicine are developed through ethno botanical approach which leads to subsequent ethno pharmacological studies (Phillipson, 2001). Scientific studies accessible on number of medicinal plants reveal that propitious bioactive constituents could be developed to resolve many problems pertaining to health (Gupta, 1994).

The explosion of the ethnomedicinal studies have been stimulated by an increased awareness of the consequences of the forced displacement and/or acculturation of indigenous people, the recognition of indigenous health concepts as a means of maintaining ethnic identities, the search for new medical treatments and technologies (Krippner, 2003). However, it is fascinating to observe that the pharmaceutical industries explore the ethnomedicinal properties of plants as their victorious norm for finding novel therapeutic agents in various domains of biomedicine (Cox *et al.*, 1994).

2.3 PHYTOCHEMICALS

Phytochemicals are biologically active, powerful group of naturally occurring chemicals that are derived from plants, which contribute health benefits for humans.

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They protect the plants from disease and damage caused from environmental hazards such as pollution, stress, UV exposure and pathogenic attack. Phytochemicals are concentrated in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits, tubers or seeds. These are secondary plant metabolites that are produced by plants to protect themselves, and has biological properties like modulation of detoxification enzymes, antioxidant property, antimicrobial effect, immune system stimulation, anticancer property, hormone metabolism modulation and decrease of platelet aggregation (Saxena *et al.*, 2013).

Phenols are the largest group of phytochemicals which is abundantly distributed in the kingdom Plantae (Singh *et al.*, 2007). Phenolic compounds are complex category of phytochemical constituents observed in plants. Phenols evince numerous properties which is advantageous to human beings and its antioxidant characteristics are essential in determining their part as protecting agents against free radical-mediated disease processes (Walton *et al.*, 2003).

Study by Mathai (2000) propose that phytochemicals lessens the threat of coronary heart disease by inhibiting the oxidation of low density lipoprotein (LDL) cholesterol, regulating high blood pressure and clotting, reducing the synthesis or absorption of cholesterol, and enhancing arterial elasticity. Phytochemicals turn up to neutralize free radicals thereby detoxifying the substances that cause cancer, hinder enzymes that stimulate the carcinogens, and switch on enzymes that detoxify the carcinogens.

Phytochemical analysis of leaves and stem of *Chlorophytum borivilianum* carried out by Ahmad *et al.* (2014) showed the presence of alkaloids, glycosides nucleus, saponins and tannins in leaves and stem.

The total flavonoid, phenolic contents and the antioxidant efficacy of methanolic extracts of *Argemone mexicana* was analysed by Sharma *et al.* (2013). The

results revealed the presence of phenols and flavonoids in the extracts. The total phenols and flavonoids was observed to be 23.50 mg GAE/gdw and 34.5 mg QE/gdw respectively, owing the potential of the plant to be used as a medicine against the diseases caused by free radicals.

Phytochemical screening and antioxidant activities in different solvent extracts of *Thymus satureioides* was carried out by Labiad *et al.* (2017). The phytochemical screening of indicated the presence of steroids, flavonoids, alkaloids, saponins, and catechic tannins. The average total phenol content of hydroethanolic extracts were significantly higher when compared with the hexane, ethyl acetate and dichloromethane extracts. Results of the phytochemical screening confirms the potent source of antioxidant activity of the solvent extract.

Screening of phytochemicals present in stems of *Smilax zeylanica* L. were investigated by Uddin (2015). Preliminary phytochemical screening disclosed the existence of tannins, flavonoids and glycosides in methanolic and petroleum ether extracts and presence of polyphenolic constituents, have been reported to have multiple biological effects, including antioxidant activity.

Solvent extracts of leaf and stem of *Gynochthodes ridsdalei* were used for physicochemical, fluorescent analysis and phytochemical screening (Ramachandran, 2016). Analysis of the plant extracts showed the presence of phytochemicals such as alkaloids, carbohydrates, glycosides, phenols, tannins, saponins, steroids, terpenoids and anthraquinones.

2.4. ANTIOXIDANTS

Oxidation is defined as a chemical reaction wherein an atom within a molecular entity transports hydrogen or electrons to an oxidizing agent. Oxidation process give rise to reactive free radicals which sequentially can start chemical reactions that cause damage or mortality to the living cell. Free radicals are molecular species with one or

more unpaired electrons and is therefore unstable and highly reactive. The free radicals particularly, reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are emancipated during metabolic reactions, plays a crucial role at lower concentration against mutagenic activity and response to pathogen attack (Ahmad *et al.,* 2014). However, at higher concentration it gives rise to oxidative stress leading to oxidative degradation of vital biomolecules like lipids, proteins and DNA (Figure 2).

Antioxidants regulates and diminishes the oxidative impairment by inhibiting or hindering oxidation induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS). Antioxidants scavenge the free radicals and enable the cells to rejuvenate or stabilize the process of life. Virtually every organisms are protected against reactive free radicals by certain enzymes like superoxide dismutase and catalase or by dietary antioxidant compounds suchlike *ß-carotene,* tocopherols and ascorbic acid (Sies, 1997). When the antioxidant protection mechanisms become unbalanced, worsening of physiological functions might result in development of degenerative or pathological processes such as cancer, aging, rheumatoid arthritis and coronary heart diseases (Halliwell, 1984).

Antioxidants appear as scavengers of free radicals, reducing agents, singlet molecular oxygen quenchers and antioxidant enzyme activators to suppress the ruin caused by reactive free radicals in the biological system (Sharma *et al.*, 2013). Over the last years, there is an increasing heed in the arena of determination of antioxidant phytochemicals, as they could impede the development of oxidative stress mediated by free radical and protect the body from disorders (Forman *et al.*, 2014). A lot of natural and synthetic reactive oxygen species scavengers and antioxidants has been developed and studied that protects the biomolecules from the ROS attack and/or to suppress the resultant damage.

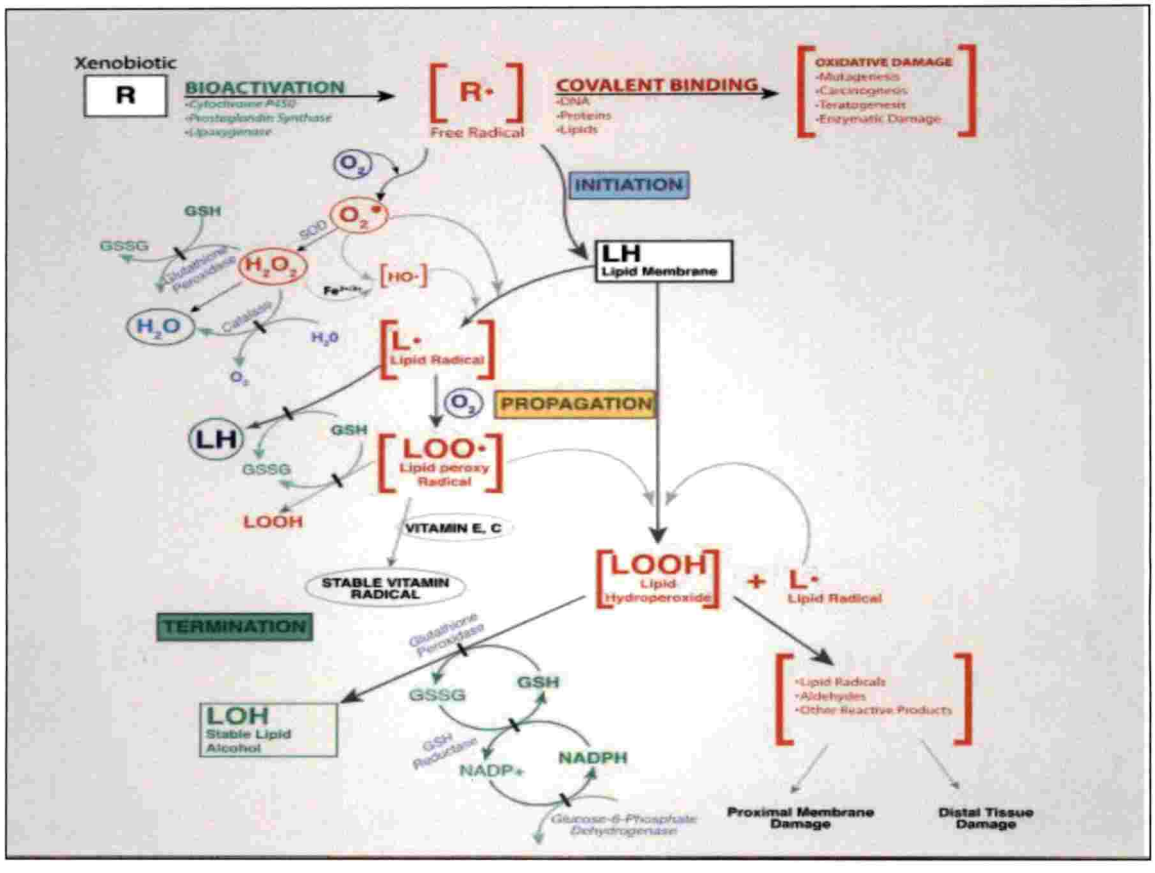

Figure 2. Oxidative stress mechanisms in tissue injury

By using different methods such as superoxide, hydroxyl, and DPPH scavenging activity, evaluation of antioxidant activity of *Vitex negundo* Linn seed has been performed (Singh *et al.,* 2005). It was observed that both raw and dry heated seed extracts exhibits highest antioxidant activity, whereas hydrothermally processed samples showed a lower antioxidant potential.

Visavadiya *et al*. (2009) showed *Carpesium abrotanoides* L. dose dependently suppressed the quantity of *malondialdehyde* spawned and thereby inhibited the peroxidation of lipid in liver microsomes, due to the scavenging of free radical and antiatherogenic potency of *Sesamum indicum* seed extracts in both chemical and biological models.

The ethanol distillate of the *Dalbergia sissoo* bark were evaluated for antioxidant potency (Kumari, 2008). Nitric oxide quenching potential and lipid peroxidation inhibitory (LPO) potential of *Dalbergia sissoo* was also scrutinized. The bark distillate displayed 69.1% LPO inhibitory potential per 10 µg of extract, and trolox was the positive control used. The superoxide dismutase (SOD) mimetic potential was observed to be 116.62 unit/min/mg.

Studies made by Chittam *et al.* (2016) showed the potent antioxidant activity of roots of *Chlorophytum tuberosum*. The aqueous and ethanolic extracts of roots of *Chlorophytum tuberosum* showed potent antioxidant activity in 2, 2-diphenyl-1, 1-picrylhydrazyl (DPPH) radical scavenging, nitric oxide radical scavenging assay and reducing assay methods. This study suggest that the antioxidant activity of the extract might be because of the presence of phytochemical constituents like phenolic compounds, saponins and alkaloids in roots.

Ashraf *et al.* (2013), experimented on cytotoxicity and antioxidant estimation of crude and total saponin extract of *Chlorophytum borivilianum*. It was observed from the three different antioxidant assays namely 2, 2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH), ferrous ion chelating (FIC), and β-carotene bleaching (BCB) activity, crude extract seems possess high free radical scavenging activity as well as bleaching activity in comparison with the total saponin fraction of *Chlorophytum borivilianum* tubers.

Karimi (2015) made studies in *in vitro* antioxidant capability and total phenolic content of unrefined methanol concentrate of *Quercus brantii* L. acorn. Crude methanol extract were taken to gauge the total phenolic, total flavonoids and antioxidant activities also. The study concluded that the methanolic concentrate of *Quercus brantii* acorn could be utilized as genesis of natural antioxidants and constitutes high phytochemical constituents.

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The methanolic and acetone extracts of the *Marrubium vulgare* leaves, were investigated for their phytochemical constituents and antioxidant capacity (Amessis-Ouchemoukh, 2014). The antioxidant potential assessed by DPPH radical scavenging activity, total antioxidant capacity, H_2O_2 scavenging activity and iron-reducing power showed potent antioxidant capacity in methanolic leaf extract than acetone. The UHPLC– MS/MS strategy for the methanolic extricate uncovered the presence of phytochemical constituents. Thus this study indicates the use of *Marrubium vulgare* as a vital source of antioxidants for preservation of food and prevention of oxidative-stress-related sicknesses.

Shah (2015) investigated phytochemicals, antioxidant, total phenolic contents (TPC) and phytotoxic activities of methanolic extract from the *Cornus macrophylla* bark. Phytochemical analysis discovered the existence of glycosides, tannins, reducing sugar, anthraquinones, saponins and flavonoids. The percentage of DPPH radical scavenging potential revealed that the crude methanolic extract of *Cornus macrophylla* is a potential fount of natural antioxidants and herbicidal.

The antioxidant activity of alcoholic extract of *Digitalis purpurea* was calculated using DPPH radical scavenging and total antioxidant capacity with ascorbic acid as the standard (Al-Snafi, 2017). The results signifies the importance of *Digitalis. purpurea* as a promising source of antioxidants.

2.5 ANTIINFLAMMATORY

The word inflammation is sprang from the Latin phrase – *Inflammare*, means burn. Any shape of bruise to the living body can wring a progression of chemical changes within the injured region. The indications of inflammation are heat, swelling, redness, pain and loss of function. Inflammation includes a progression of events which are classified into acute transient phase, delayed sub-acute phase and chronic proliferate phase. In the foremost phase, inflammatory exudates develop because of

improved vascular permeability and prompts to edema. It is accompanied by the relocation of leukocytes and phagocytes from blood to vascular tissues which is the second one phase and in the third phase, tissue degradation is trailed by fibrosis. Inflammation effects in the deliverance of endogenous mediators such as histamine, serotonin, prostaglandins, bradykinin etc. These mediators even in small quantities can elicit pain response. Pain results in dropped muscular activities.

The study done by Muhammad, *et al.* (2012) aimed to provide scientific evidence to the ethnobotanical application of the methanol concentrate of *Viola betonicifolia* (VBME) in the treatment of inflammation in different animal models. Their analysis concluded that the methanolic concentrate of *Viola betonicifolia* was proved as a natural safe antidote for the treatment of inflammation.

Saleem *et al.* (2011) assessed the anti-inflammatory calming attribute of the leaf of *Gendarussa vulgaris* Nees which have been utilized in the remedy of ulcers, inflammation, sores, dyspepsia, healing of wounds, and so on. The *in vitro* (HRBC) and *in vivo* method (by carrageenan induced paw oedema) showed significant anti-inflammatory property. The alcoholic extract showed potent activity that is comparable with the standard drug diclofenac sodium and is considered to be safe since no sign of toxicity was observed. Their outcomes showed the efficiency of *Gendarussa vulgaris* Nees as an effectual therapeutic source for acute inflammation treatments.

Nagaharika *et al.* (2013) evaluated the anti-inflammatory activity of *Jatropha gossypifolia* L. leaves by using *in vitro* HRBC membrane stabilization assay. The aqueous extract indicated significant stabilization activity when contrasted with the standard Diclofenac sodium. The study suggests that the anti-inflammatory activity might be because of alkaloids or steroidal compounds present in *Jatropha gossypifolia.*

Hendra *et al.* (2011) evaluated antioxidant and anti-inflammatory activities of the pericarp and mesocarp of *Phaleria macrocarpa* fruit. The study revealed that the

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activities may be because of the existence of phenolic and flavonoid compounds in substantial quantities. The cytotoxicity activity demonstrated potential anticancerous property of *Phaleria macrocarpa* seed.

Oueslati (2012), investigated the biological properties of the shoot extracts of *Suaeda fruticosa*. The estimation of antioxidant capacities of extracts showed the highest antioxidant activities in shoot methanolic extract. It was also observed that the extract flaunted the uttermost anti-inflammatory activity by inhibiting nitric oxide (NO) release in lipopolysaccharide (LPS)-stimulated macrophages. The extract indicated most astounding anticancer action against human lung carcinoma and colon adenocarcinoma cell lines. Thus it was inferred that this edible halophyte has valuable source of antioxidants which exhibit anti-inflammatory and anticancer capacities.

Research reports suggests that plant extracts of *Ocimum labiatum* and their constituents have possible anti-inflammatory potential. Kapewangolo (2015) made *in vitro* studies of *Ocimum labiatum*'s immune-enhancing and antioxidant properties. *Ocimum labiatum* validated a promising anti-inflammatory and antioxidant properties even as the terpenoid indicated anti-inflammatory activity. They inferred that the mechanism of anti-inflammation of the terpene was due to inhibition of activator protein-1.

Majid *et al.* (2015) assessed the antioxidant and anti-inflammatory activities of *Euphorbia dracunculoides extracts*. Phytochemical components of the plant assured the existence of tannins, polyphenolics, terpenoids, steroids and coumarins. They urge that presence of numerous polyphenolics, terpenoids and steroids offer *Euphorbia dracunculoides* with therapeutic potency for oxidative stress and inflammation associated diseases.

Low *et al.* (2015) made studies on andrographolide main secondary metabolite of *Andrographis paniculata* (Acanthaceae) that contributes the anti-inflammatory activity. The antioxidant potential of the extracts had been carried out using DPPH

free radical scavenging, oxygen radical antioxidant capacity (ORAC) and a Folin-Ciocalteu (FC) antioxidant assays and their anti-inflammatory activity was evaluated by analyzing their inhibitory effect on the discharge of tumor necrosis factor alpha (TNF-α) in the human monocytic cell line THP-1. They inferred that the extracts contains flavonoid, phenlycarboxylic acid compounds that contribute to antioxidant potential. Also they found andrographolide have significant effects on the release of TNF-α. Thus, their outcomes bolster the development of andrographolide or andrographolide-derived compounds as anti-inflammatory capsules.

Nigella sativa a widely used medicinal plant as food flavoring, preservative and an herbal treatment for several ailments from old time. Gholamnezhad (2015) made clinical documentation of *Nigella sativa* and its components on preventive (anti-inflammatory, antioxidant, and immunomodulatory) and relieving (bronchodilatory) effects on obstructive respiratory infections. He analyzed that *Nigella sativa* stifles inflammatory mediators thereby enhancing immunomodulatory activity in respiratory diseases. *Nigella sativa* also showed increases in the activity of antioxidant enzymes.

Chakraborty *et al.* (2004) evaluated the antiinflammatory activity of the aqueous extract of *Spilanthes acmella* (SPA), is an indigenous herb belonging to the family Compositae. They evaluated for antiinflammatory action by carrageenan-induced rat paw edema in albino mice. The results of the study suggest that the presence of flavonoids contributes to the antiinflammatory activities of aqueous SPA. The study signifies that SPA has significant antiinflammatory properties.

Saponins extract of *Sesbania sesban* leaves showed significant antiinflammatory activity by carrageenan induced rat paw edema method (Dande, 2010). Phytochemical studies on leaves of *Sesbania sesban* has shown the presence of triterpenoidal, steroidal saponins, tannins and flavonoids. Thus it was analyzed as a promising anti-inflammatory therapeutic applications.

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The anti-inflammatory effect of *Salacia oblonga* and *Azima tetracantha* was examined in animal models using carrageenan-induced paw oedema (acute inflammation) and cotton pellet granuloma (chronic inflammation) methodology by Ismail (1997). They found that medications could stifle the transudative, exudative and proliferative components of chronic inflammation. And they also inferred that the drugs could bring down the lipid peroxide content of liver, γ-glutamyl transpeptidase activity in the exudate. The study suggest that the drugs may exert their activity by antiproliferative, antioxidative and lysosomal membrane stabilization.

Patgiri (2014) evaluated anti-inflammatory activity of *Guduchi Ghana* which is a part of the distinctive Ayuvedic traditional formulation prepared from *Guduchi* (*Tinospora cordifolia* Miers.) stem extract. It was observed that classically prepared *Guduchi Ghana* produced significant anti-inflammatory activity.

MATERIALS AND METHODS

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3. MATERIALS AND METHODS

The present study entitled "Assessment of antiinflammatory and antioxidant properties of *Chlorophytum laxum* R. Br." was carried out in the Ethnomedicine and Ethnopharmacology division of Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, during 2017-2018 academic year.

3.1 *Chlorophytum laxum* R. Br.

3.1.1 Plant Description

Chlorophytum laxum is one of in excess of 200 species of the genus Chlorophytum, greatly cultivated over India for its consumable leaves and roots that are utilized as traditional medicine. It is an evergreen herbaceous perennial, which is reasonably rapid-developing and diminutive in height, almost 30cm tall with a range of 60 cm or greater on ground yet less whenever contained in pots. Displaying tuffet of charming lime-green grass-like recurving leaves with thin ivory-white. The leaves are willowy and elongated with decreasing tips, paper- skinny, linear-oblong, acute, glabrous, bifarious, sheath open, smooth and shiny, distichous and sessile, ranging 10-25 x 0.8 cm long and up to 1.5 cm wide. Peduncle is abbreviate so the lower flowers/fruits are located within the leaf-rosette. It exhibits minute subtle star-molded 6-petaled flowers in white on a 10-20 cm non-arching scape (flowering stalk) that is relatively erect. Inflorescence is a simple racemose, seldom branched below, 2–5 cm long. Flowers are paired at the nodes, with the pedicel 8 mm long that are jointed at the middle (Plate 2). Stigma is terminal. The tepals ranging 2 x 1 mm are oblong with chartaceous and white. The ovary is 3-lobed and each cell have one ovule. Capsule is profoundly triquetrous and larger as compared to the plant size and is 8–10 mm long and 5–6 mm wide with a shriveled perianth at the pedestal and is 3-lobed. It do not yield 'baby spiders' or plantlets on the wiry scape but set seeds though hardly. Seeds are saucer-shaped and diameter range of 2 mm. It has a minuscule rhizome that

produces fleshy tuberous roots as a bunch of whitish bulblets from which new plantlet arise. The rhizomes are short, and the roots are tinny at the proximal parts which are bloating to elongated tubers peripherally.

3.1.2 Propagation

Chlorophytum laxum is effectively propagated through dividing the plants that consist of the rhizomes and tuberous roots at the crown.

3.1.3 Uses

In India, the roots of *Chlorophytum laxum* is utilized as traditional medicine for treating dysentery and diarrhoea and also consumed as demulcent and galactogogue.

3.1.4 Global distribution

Chlorophytum laxum is indigenous to South Africa but widespread across tropical Africa, Myanmar, Sri Lanka, India, China, Malaysia, Thailand, Indonesia and Northern Australia. It is mainly found in localities such as Kollam, Idukki, Pathanamthitta, Thiruvananthapuram, Malappuram and Kozhikode (Plate 3).

Systemic position of *Chlorophytum laxum* R. Br.

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Liliopsida

Subclass: Liliidae

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Order: Liliales

Family: Liliaceae

Genus: *Chlorophytum* Ker Gawl.

Species: *Chlorophytum laxum*

Basic information

Habit: Herb

Medicinal: Yes

Habitat: Grasslands

Flowering and fruiting: September-December

Distribution: Tropics

Localities: Kollam, Idukki, Pathanamthitta, Thiruvananthapuram, Malappuram and Kozhikode

Morphological information about it features is given in plate 1.

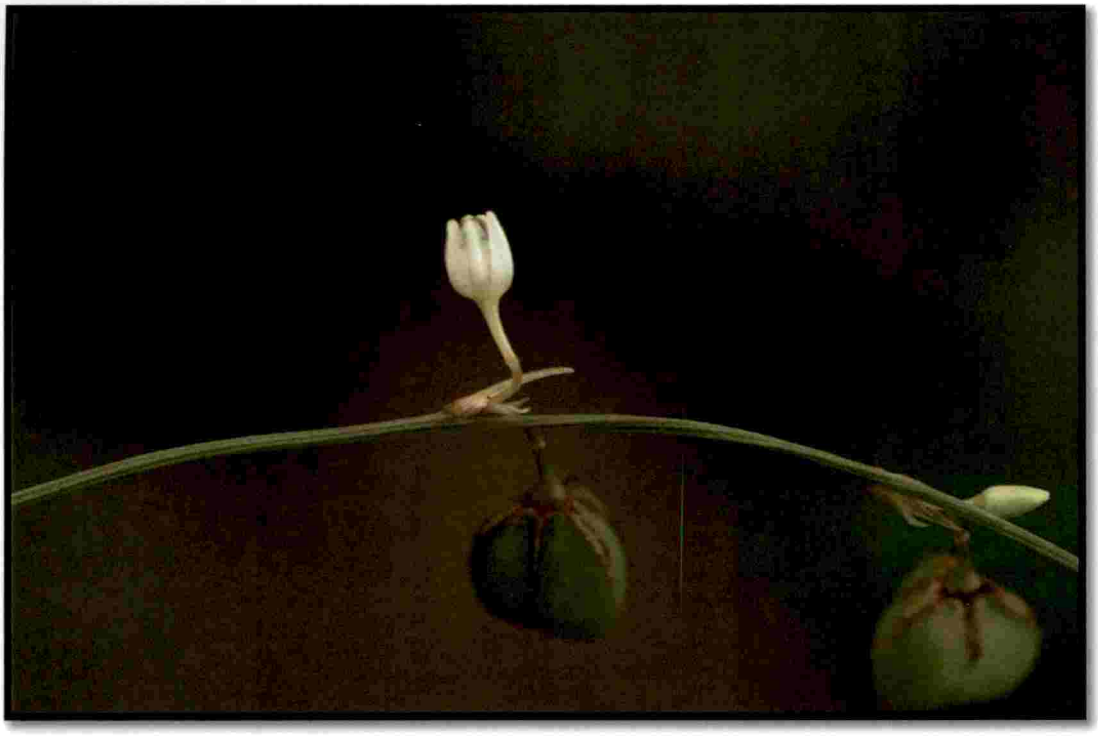

Plate 2. Flower of *Chlorophytum laxum* R. Br. (Neeruvatti)

Plate 3. Distribution of *Chlorophytum laxum* R. Br.

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3.2 MATERIALS

3.2.1 Plant material

In the present study, the tuber of *Chlorophytum laxum* R. Br. was selected based on the ethnobotanical claim of Kani tribe of Thiruvananthapuram district, Kerala. The tubers of *Chlorophytum laxum* R. Br. were collected from the hills of Western Ghats. The plant material was identified and authenticated by Dr. E. S. Santhosh Kumar, Technical Officer, Plant Genetic Resources, JNTBGRI. A Herbarium of the plant specimen were deposited in the Herbarium Collection at JNTBGRI (TBGT 86816/ dated 25/07/2018) (Plate 4).

3.2.2 Experimental animals

Swiss Albino Mice (20-30 g) and Wistar rats (150 to 250 g) (Plate 5) of both sex, were obtained from the Animal House of Jawaharlal Nehru Tropical Botanical Garden Research Institute, Palode. The experimental animals were divided into different groups and caged in polypropylene cages and maintained under standard environment conditions with temperature 25 ± 2 °C, the relative moisture of 60 ± 10 %, room air changes 15 ± 3 times/h and 12-hour dark- light cycles. The animals were supplied with commercial rodent feed (Lipton India Ltd; Mumbai, India) and water. Animals were acclimatized for one week before the initiation of an experiment. All animal experiments were carried out based on the guidelines of the NIH and only after getting approval of the Institute Animal Ethics Committee.

3.2.3 Drugs and chemicals

Carrageenan, (Formaldehyde) Formalin, Ethanol, Indomethacin, Acetyl salicylic acid (Aspirin), Acetic acid, Furosemide, Sodium Chloride, Magnesium turnings, Sodium hydroxide, Sulphuric acid, Chloroform, Ferric chloride, Lead acetate, HCl, Ascorbic acid, Hydrogen peroxide, Fehling's solution A and B, Sodium nitroprusside, Griess reagent, Ammonium sulphate, Folins ciocalteus reagent, Thio

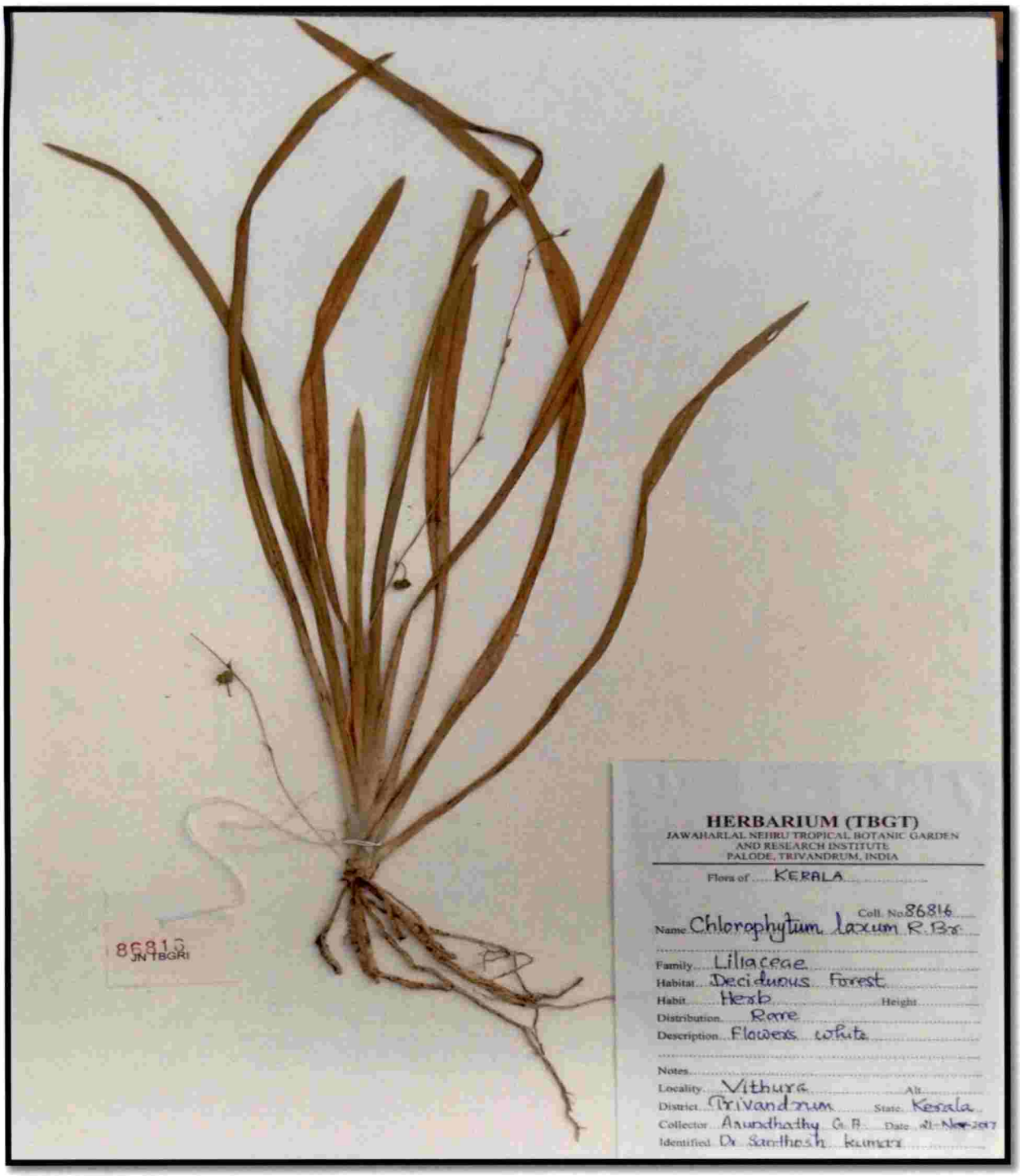

Plate 4. Herbarium specimen of *Chlorophytum laxum* R. Br. (TBGT 86816/ dated 25/07/2018)

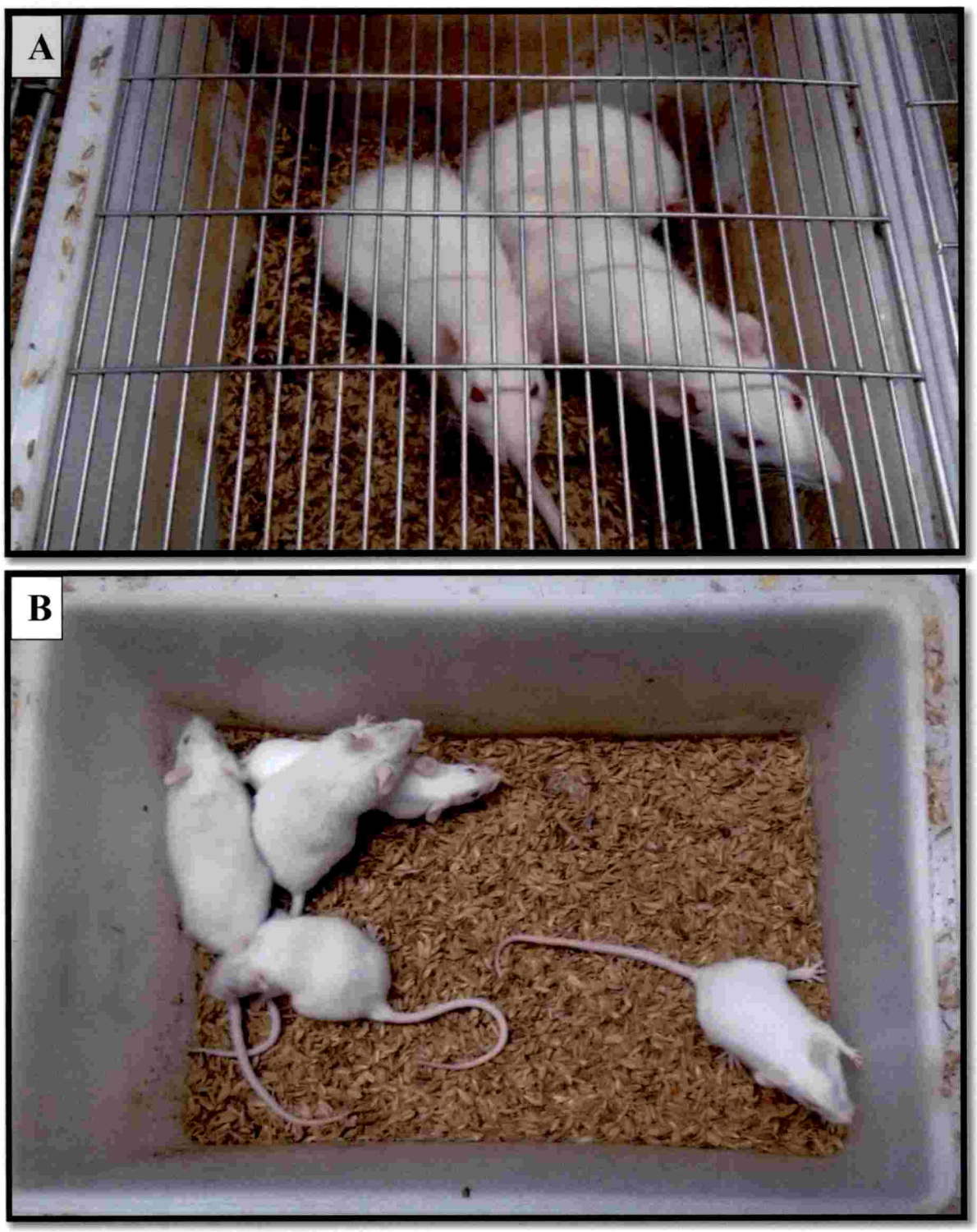

Plate 5. Experimental animals used: (A) Wistar rats (B) Swiss Albino mice

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barbituric acid (TBA), Methanol, Saline, Acetic acid, Sodium carbonate, Tween-80 (All chemicals were of analytical grade).

3.2.4 Instruments

Shaker (Remi. Ltd., India), Rotary vacuum evaporator (Perfit, India), Hot air oven (Beston Industries, Cochin), Electronic balance (Shimadzu, Japan), Plethysmometer, Cooling Centrifuge (Remi Scientifics, Mumbai), Spectrophotometer (Agilent- Cary 100, Hungary), pH Meter (Eutech-ION 2700, Singapore), Centrifuge (Remi Scientifics, Mumbai) and Water Bath (Beston Industries, Cochin) were used.

3.3 METHODS

3.3.1 Preparation of extract

The collected plant material were thoroughly washed under running tap water and distilled water to remove adhering dust. They were shade dried for almost 10-12 days and were made into fine powder. 100g of the plant powder was weighed out and was subjected to cold extraction. For the extraction, the plant powder was mixed with distilled ethanol in the ratio 1:10 and was incubated in a shaker at a uniform speed for 48 hours. It was then filtered through Whatman coarse filter paper and the filtrate was collected. A fresh round bottom (RB) flask was taken and its weight was determined by electronic weighing balance and was recorded as initial weight. The collected filtrate was transferred to the round bottom flask and was concentrated using a rotary evaporator (Buchi, rotavapor R – 215) and was kept in a desiccator for drying at room temperature until use.

3.3.2 Determination of extract yield

Extract yield was calculated by the difference between the initial weight and final weight of the round bottom flask in which extract was concentrated.

3.3.3 Determination of solubility

A pinch of extract was mixed in few drops of distilled water, 0.1 % Tween-80, 0.5 % Tween-80 and 1% Tween-80 in each mortar and pestle respectively. The extent of solubility was observed in each medium.

3.3.4 Acute toxicity study

Acute toxic effect of ethanolic extract of *Chlorophytum laxum* (ECL) was evaluated in Swiss albino mice as per OECD guidelines on acute oral toxicity test (Suja *et al.*, 2004). Before starting the study, all groups were fasted for 12 h and were weighed. The crude extracts were suspended in a vehicle (0.5 % Tween 80) with distilled water before administration. Five groups (A- E), consisting of six animals each were used for the study (Table 1). Oral administration of the drug was carried out in 4 groups with the help of gavage No. 16. One of the group was maintained to serve as control and was devoid of extract. The animals were then observed for 30 min after dosing. This was then followed by hourly observation for 8h and once a day for the next 14 days. Animals were weighed and observations for mortality, behavioral pattern, and changes in physical appearance, injury, pain and signs of illness had been monitored. The observation was scientifically recorded and all records for individual mice were maintained for each test group.

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Table 1. Drug administration pattern for oral acute toxicity study in mice

Groups	Treatment	Volume (per orally) (mL)	No. of animals/ group
1	ECL (5 mg/kg)	0.5	6
2	ECL (50 mg/kg)	0.5	6
3	ECL (300 mg/kg)	0.5	6
4	ECL (2000 mg/kg)	0.5	6
5	0.5% Tween-80	0.5	6

3.4 PRELIMINARY PHYTOCHEMICAL TEST

3.4.1 Standard phytochemical screening tests

The qualitative phytochemical screening was carried out by using standard procedures of Harborne (1998). Phytochemical evaluation of ECL were carried out according to the following procedures.

1. ***Detection of Alkaloids***

   a) **Mayer's test**

   1 mL of filtrate was taken in a test tube and a few drops of Mayer's reagent was added to it. A yellow crystalline precipitate formation stipulates the presence of alkaloids.

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b) Wagner's test

A few drops of Wagner's reagent was added to a few mL of filtrate. The reddish brown precipitate confirms the occurrence of alkaloids.

c) Hager's test

1 mL of filtrate was taken, a few drops of Hager's reagent was added. A prominent yellow precipitate confirms the presence of alkaloids.

d) Dragendorff's test

To the test tube containing a few mL of filtrate, 2mL of Dragendorff's reagent were added. A prominent brownish orange precipitate substantiates the alkaloids presence.

2. Flavonoids

a) Alkaline reagent test

To the test tube containing 1mL of extract, a few drops of diluted HCl was added. Yellow fluorescence which turned colorless when drops of Conc. HCl were added to it showed the presence of flavonoids.

b) Shinoda test

The extract were suspended in 5 mL of alcohol and a pinch of magnesium turnings and a drop of concentrated hydrochloric acid (drop wise) was added. Formation of crimson colour indicates the presence of flavonol glycosides.

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c) Lead Acetate test

A few drops of 10% 1M lead acetate were added to a tube containing 1 mL of extract. Formation of yellow precipitate indicates the occurrence of flavonoids.

3. Phenols

a) Lead acetate test

50 mg of extract was dissolved in distilled water and 3 mL of 1% lead acetate solution were added to it. Occurrence of yellowish precipitate showed the presence of phenolic compounds.

4. Tannins

a) Braymer's Test

To a clean test tube containing 1 mL of plant extract, 1 mL of distilled water and 2-3 drops of ferric chloride was added. The development of a green precipitate shows the presence of tannins.

5. Saponins

a) Foam test

1g sample was added to the test tube containing 5 mL water. The suspension was shaken in a graduated cylinder for 15 min. The formation of foam indicated the presence of saponins.

b) Forth test

1g sample was added to the test tube containing 5 mL water and heated. Forth appearance reveals the presence of saponins.

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6. ***Carbohydrates***

a) Fehling's test

1 mL of the extract were added with equal amount of Fehling's solution A and B and boiled in a water bath. A reddish precipitate formation shows the presence of sugar.

7. ***Proteins***

a) Xanthoproteic test

1 mL of filtrate was added to 1 mL of Sulphuric acid. A white precipitate indicated the presence of proteins.

b) Biuret test

2% copper sulphate solution was added to the test tube containing 2 mL of extract. Then, 1 mL of 95% ethanol and potassium hydroxide pellets was added consecutively. Pink colour formation indicated the presence of proteins.

8. ***Steroids***

a) Salkowski's test

The extract was treated with chloroform and a drop of concentrated sulphuric acid was added. The formation of reddish brown ring in lower layer indicated the presence of steroids.

9. ***Anthocyanin***

To a test tube of 1 mL extract, 1 mL 2N HCl and a few drops of Ammonia was added. Change in pinkish red to bluish violet coloration indicated the presence of anthocyanin.

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10. Glycosides

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

Killer killiani test

To a test tube of 1 mL extract, 1 mL of distilled water and sodium hydroxide solution was added. Yellow coloration indicates the presence of glycosides.

11. Phlobatannins

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

3.4.2 Total phenolic content

The total phenolic content (TPC) was determined by spectrophotometry according to the method of Sakat *et al.* (2009). 0.2 mL of the extract ECL (1mg/mL) was transferred in tubes containing 1.0 mL 10 % Folin-Ciocalteu's reagent. After 10 min, 0.8 mL of sodium carbonate solution (7.5% w/v) were added to the mixture. The tubes were kept at room temperature for 30 min and the absorbance was read at 743 nm. The concentration of polyphenolic compounds in the samples was descended from a standard curve of gallic acid ranging from 25 to 400 µg/mL.

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3.5 ANTIINFLAMMATORY STUDIES

3.5.1 *In vitro* antiinflammatory study

HRBC membrane stabilization have been utilized as a method to evaluate the antiinflammatory activity (Gandidasan, 1991). The blood was collected from healthy individuals and were blended with equivalent amount of sterilized Alseiver solution (0.8% sodium citrate, 0.05% citric acid, 2% dextrose and 0.42 % sodium chloride in water). The blood was centrifuged at 3000 rpm and the packed cells were washed with 0.85 % isosaline (pH 7.4) and a 10% v/v suspension were made with isosaline. Various concentrations of extracts were prepared (50, 100 and 200 µg/mL) using distilled water. The assay mixture comprises the drug, 1 ml of 0.15 M phosphate buffer (pH 7.4) and 2 ml of 0.36% hypo saline and 0.5 ml of the HRBC suspension. Diclofenac sodium was utilized as the reference standard. As an alternative of hypo saline, 2 ml of distilled water was used in the control. All the assay mixes were incubated at 37°C for 30 minutes and were centrifuged. The haemoglobin content in the supernatant solution were evaluated utilizing spectrophotometer at 560 nm. The percentage of hemolysis was computed by assuming the hemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection were calculated by using the following equation,

$$\% \text{ Protection} = \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

3.5.2 *In vivo* antiinflammatory studies

i. Carrageenan induced paw oedema study

The anti-inflammatory activity of the ethanolic extract of *Chlorophytum laxum* (ECL) was studied by the method, Carrageenan - induced rat paw oedema (Amdekar *et al.*, 2011). The animals have been separated into five groups with six animals in each cages and were fasted overnight. The drug administration pattern for each group

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was included in Table 2. 30 min after ECL/STD administration, 0.1 ml 1% carrageenan (in saline) was injected into the right hind paw, under the plantar apo-neurosis sub-cutaneously. The volume of hind paw was measured by using a plethysmometer before and 3h after carrageenan injection. The paw volumes difference were documented, which indicates the degree of inflammation. The percentage inhibition of paw oedema was calculated by the given formula;

Oedema volume = (Oedema volume of control animals – Oedema volume of ECL treated animals)

% inhibition = [(control-test)/ control)] * 100

Table 2. Drug administration pattern for Carrageenan induced paw oedema

Groups	Treatment	Volume (per orally) (mL)	No. of animals/ group
1	0.5% (V/V) Tween-80	1	6
2	10(mg/kg) Indomethacin	1	6
3	50 (mg/kg) ECL	1	6
4	150 (mg/kg) ECL	1	6
5	450 (mg/kg) ECL	1	6

ii. Formalin induced paw oedema study

The anti-inflammatory activity of the ethanolic extract of *Chlorophytum laxum* (ECL) was studied by the method, Formalin induced paw oedema in wistar rats (Chau,

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1989). The animals were made into five groups with six animals each and was fasted overnight and the oral drug administration pattern was as followed in Table 3. 30 min after ECL/STD administration, 0.1 mL formalin (2%) was injected into the right hind paw of the rats by sub plantar route. The drug was dispensed during an interval of 24h for consecutive 7 days. Formalin injection was given on the 3rd day after the 1st day injection. The paw thickness was measured plethysmo graphically on the 7th day after 1h of experimental period. The mean difference in paw thickness (mm) was obtained from the change in paw thickness before and after the induction of inflammation for each groups. The difference in the paw volumes were recorded, which indicates the degree of inflammation.

Oedema volume = (Oedema volume of control animals – Oedema volume of ECL treated animals)

% Inhibition = [(Control-Test)/ Control]* 100

Table 3. Drug administration pattern for Formalin induced paw oedema study

Groups	Treatment	Volume (per orally) (mL)	No. of animals/ group
1	0.5% (V/V) Tween-80	1	6
2	10 (mg/kg) Indomethacin	1	6
3	50 (mg/kg) ECL	1	6
4	150 (mg/kg) ECL	1	6
5	450 (mg/kg) ECL	1	6

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3.6 *IN VITRO* ANTIOXIDANT ACTIVITY

In vitro antioxidant activity of ECL was evaluated using standard procedures as mentioned below.

3.6.1 Estimation of Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity were measured using the standard procedure (Marcocci *et al.*, 1994). 1mL of 10 mM sodium nitroprusside was combined with 1 mL of various concentration (50 to 250 µg/mL) of plant extract (ECL)/serial fractions in the phosphate buffer (pH 7.4). The test tubes were incubated at 25°C for 2.5 hrs. 1 mL of Griess' reagent (1% Sulphanilamide, 2% O-phosphoric acid and 1% Napthylethylenediamine dihydrochloride) were added to I mL of incubated solution. The absorbance was read at 546 nm and percentage of inhibition was estimated using the formula,

$$\text{Percentage of inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}} \times 100$$

Ascorbic acid was used as standard.

3.6.2. Hydroxyl radical scavenging assay

Hydroxyl radical (Elizabeth *et al.*, 1990) assay is based on quantification of degradation of 2-deoxy ribose by condensation with TBA. Various concentrations of extract (ECL) / standard (Ascorbic acid) (50-200 µg/ml) were added to the reaction mixture containing 100 µL of 2-Deoxyribose (2.8 mM), 100 µL of Ferric Chloride (100 µM), 100 µL of EDTA (104 µM) and 100 µL of Hydrogen peroxide (1 mM) in phosphate buffer (20 mM, pH-7.4) to make the final volume of 1.5 mL. The reaction mixtures were incubated at 37 °C for 1 hr. After incubation, the reaction was ceased by adding 1.5 mL of 2.8 % TCA followed by the addition of 1.5 mL of 1 % aq. TBA

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to each mixture. Intensity of the colour formed was measured spectrophotometrically at 530nm. The reaction mixture without sample were utilized as control. Percentage inhibition was calculated as:

$$\% \text{ inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of sample}]}{[\text{Absorbance of control}]} \times 100$$

3.6.3 Total antioxidant activity

0.1 mL of test solution containing a reducing species (in water or ethanol) were mixed in an eppondroff tube with 1 mL of reagent solution (4mM ammonium molybdate, 28 mM sodium phosphate and 0.6M sulphuric acid) (Prieto *et al.,* 1999). The tubes were incubated in a thermal block for 90 min at 95°C. When the samples had cooled to room temperature, the absorbance of the aqueous solution were quantified at 695nm against blank. 1mL of reagent solution and equal volume of same solvent used for the sample was utilized as blank solution. α- tocopherol or ascorbic acid was used as positive control.

3.6.4 Anti-lipid peroxidatant activity

The anti-lipid peroxidation effects of ECL was evaluated *in vitro*, by the method (Suja *et al.*, 2004). 0.5 g of rat liver tissue were sliced and homogenized with 10 mL of 150 mm KCl-Tris-HCl- buffer (pH 7.2). The reaction mixture contained 0.25 mL of liver homogenate, 0.05 mL of 0.1 mM ascorbic acid (AA), 0.1 mL Tris-HCl buffer (pH 7.2), 0.05 mL of 4 mM $FeCl_2$ and 0.05 mL of various concentrations of plant extract/serial fractions. The mixture were kept for incubation at 37° C for 1h. After that 0.2 mL of 9.8% sodium dodecyl sulphate (SDS), 0.5 mL of 0.1 N HCl, 0.9 mL of distilled water and 2 mL of 0.6% thiobarbituric acid (TBA) were added to each of the tube and were shaken vigorously. The tubes were placed in a boiling water bath at 100°C for half an hour. The flocculent precipitate formed after cooling was removed

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by the addition of 5 mL of n- butanol and was centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was measured at 532 nm.

The antioxidant activity is calculated as follows:

$$\% \text{ Inhibition of lipid peroxidation} = \frac{(\text{Control-Test})}{\text{Control}} \times 100$$

3.7 STATISTICAL ANALYSIS

The results obtained were expressed as mean ± standard deviation (SD) and presented as graphs and tables. Data were analyzed by using a statistical software called GraphPad 5.0 (GraphPad Software, Inc., San Diego, CA) with one way analysis of variance (ANOVA) followed by Duncan's test. ANOVA was performed to compare the significant differences between groups and Duncan's test was carried out for pair-fed comparisons between groups. The level of significance was set at $***p<0.05$.

RESULTS

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4. RESULTS

4.1 MORPHOLOGICAL CHARACTERISTICS

Chlorophytum laxum is an evergreen herbaceous perennial, which grows upto 30 cm height. The mode of propagation of *C. laxum* is by the rhizomes and tuberous roots at the crown. The morphological characters of the plant and the medicinally important tubers were studied and observations were tabulated (Table 4).

Table 4. Morphological characters of the plant Neeruvatti (*Chlorophytum laxum*)

Sl. No.	Morphological characters	Observation
1	Habit	Herb
2	Mode of propagation	Rhizomes
3	Leaves	Green grass like 6-12 in number, linear-oblong, 10-25 x 0.8 cm in length and up to 1.5 cm broad.
4	Root	Tuberous root
5	Venation	Parallel
6	Colour	Green on ventral side and light green on dorsal side
7	Leaflet width	Ranges from 0.5-1 cm
8	Leaflet length	Ranges from 15-35 cm
9	Odour	No characteristic odour
10	Taste	Slightly sour

Each values is the mean, for n = 10

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4.1.1 Extract yield

The percentage yield of ethanolic extract of the whole plant was found to be 5.02.

4.1.2 Extract miscibility

The extract showed maximum solubility in distilled water and this solvent was selected as the vehicle for drug preparation.

4.1.3 Oral acute toxicity studies

The ethanolic extract of *C. laxum* (ECL) was administered to mice orally for the acute toxicity study at doses of 5, 50, 300 and 2000 (mg/kg body weight) for each groups. No mortality and behavioural changes were observed even at the highest dose of 2000 mg/kg. The other parameters such as respiration, skin conditions, behavioural pattern etc. of test animals were monitored and the results are tabulated (Table 5).

Table 5. Acute toxicity studies of ethanolic extract of *Chlorophytum laxum*

Sl. No.	Parameters	Normal	ECL 5 (mg/kg)	ECL 50 (mg/kg)	ECL 300 (mg/kg)	ECL 2000 (mg/kg)
a) Difference in body weight						
1	Difference in body weight	Nil	Nil	Nil	Nil	Nil
b) Changes in food and water intake						
2	Food and water intake	Normal	Normal	Normal	Normal	Normal
c) Cage side observations						

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3	Respiration	Normal	Normal	Normal	Normal	Normal
4	Breathing abnormalities	Nil	Nil	Nil	Nil	Nil
5	Condition of the fur	Normal	Normal	Normal	Normal	Normal
6	Skin	Normal	Normal	Normal	Normal	Normal
7	Subcutaneous swelling	Nil	Nil	Nil	Nil	Nil
8	Abdominal distension	Nil	Nil	Nil	Nil	Nil
9	Pupil diameter	Normal	Normal	Normal	Normal	Normal
10	Wetness or soiling of the perineum	Nil	Nil	Nil	Nil	Nil
11	Condition of teeth	Normal	Normal	Normal	Normal	Normal
12	Gait	Normal	Normal	Normal	Normal	Normal
13	Color and consistency of the feaces	Normal	Normal	Normal	Normal	Normal

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4.2 PRELIMINARY PHYTOCHEMICAL TEST

4.2.1 Standard phytochemical screening tests

Qualitative analysis of the roots indicated the presence of alkaloids, phenols, saponins, tannins, proteins, sugars, glycoside and steroids (Table 6).

Table 6. Phytochemical study of *Chlorophytum laxum* R. Br.

Sl. No.	Test	Results
1	**Alkaloids**	
	Mayer's test	+
	Wagner's test	+
	Hager's test	+
	Dragendorff's test	+
2	**Flavonoids**	
	Alkaline reagent test	–
	Shinoda test	
	Lead Acetate test	
3	**Phenols**	
	Lead acetate test	+
4	**Tannins**	

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	Braymer's Test	+
5	**Saponins**	
	Foam test	+
	Forth test	+
6	**Carbohydrates**	
	Fehling's test	+
7	**Proteins**	
	Xanthoproteic test	+
	Biuret test	+
8	**Steroids**	
	Salkowski's test	+
9	**Anthocyanins**	–
10	**Glycosides**	
	Killer killiani test	+
11	**Phlobatanins**	–

+ present, - absent

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4.2.2 Total phenolic content

The values are expressed as Gallic acid equivalents (GAE). The total phenolic content of ethanolic extract of tubers of *C. laxum* was found to be 5.12 mg GAE/g of extract.

Table 7. The total phenolic content of ethanolic extract of *C. laxum* R. Br.

Conc. of Gallic acid (µg/mL)	Mean Absorbance (743 nm)
25	0.1019 ± 0.003
50	0.5675 ± 0.005
100	0.9939 ± 0.002
200	1.4142 ± 0.006
400	1.8814 ± 0.004
Ethanolic extract (0.5 mg/mL)	0.2185 ± 0.011

Values are expressed as mean ± SD, for n=6

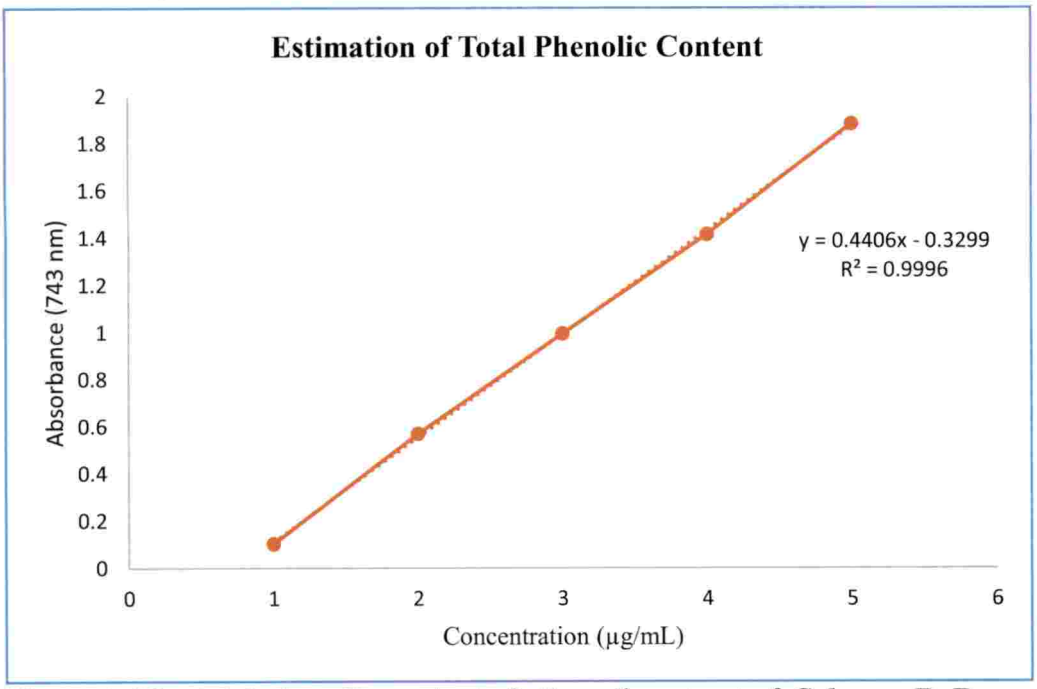

Figure 3. The total phenolic content of ethanolic extract of *C. laxum* R. Br.
Values are expressed as mean ± SD, for n=6

The TPC of extract = 5.12 mg GAE/g of extract

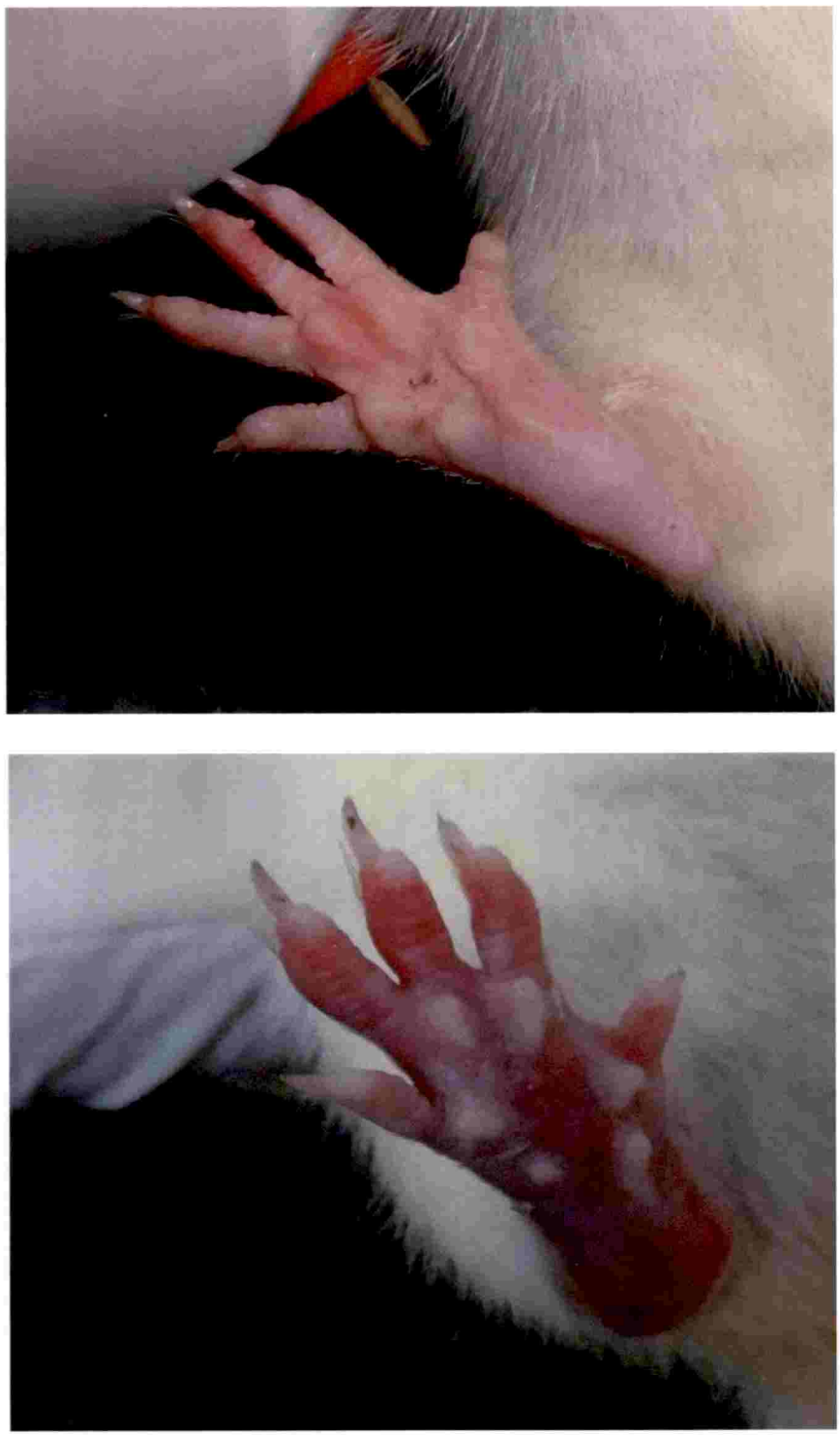

Plate 6. Hind paw of Wistar rats before and after inflammation

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4.3 ANTIINFLAMMATORY STUDIES

4.3 *IN VITRO* ANTIINFLAMMATORY STUDY

The inhibition of hypotonicity induced HRBC membrane lysis was taken as a measure of the antiinflammatory activity. Ethanolic extracts of *C. laxum* at different concentrations (50, 100, 200 µg/mL) showed a significant stabilization of the HRBC membrane. Ethanolic extract of *C. laxum* at the concentration of 200 µg/mL offered maximum HRBC membrane protection. It showed the maximum inhibition of 55.69 % at 200 µg/mL. The EC_{50} value of extract was found to be 162.47 µg/mL. The results were shown in Table 8.

Table 8. *In vitro* anti-inflammatory activity of *C. laxum* R. Br.

Concentration (µg/mL)	**(%protection) ECL**	**EC_{50} value (µg/mL)**	**(%protection) Standard**	**EC_{50} value (µg/mL)**
Control	-	162.47	-	84.32
50	33.67 ± 0.66		41.65 ± 1.03	
100	40.34 ± 0.51		55.20 ± 0.78	
200	55.69 ± 0.74		72.93 ± 0.72	

Values are expressed as mean ± SD, for n=6.

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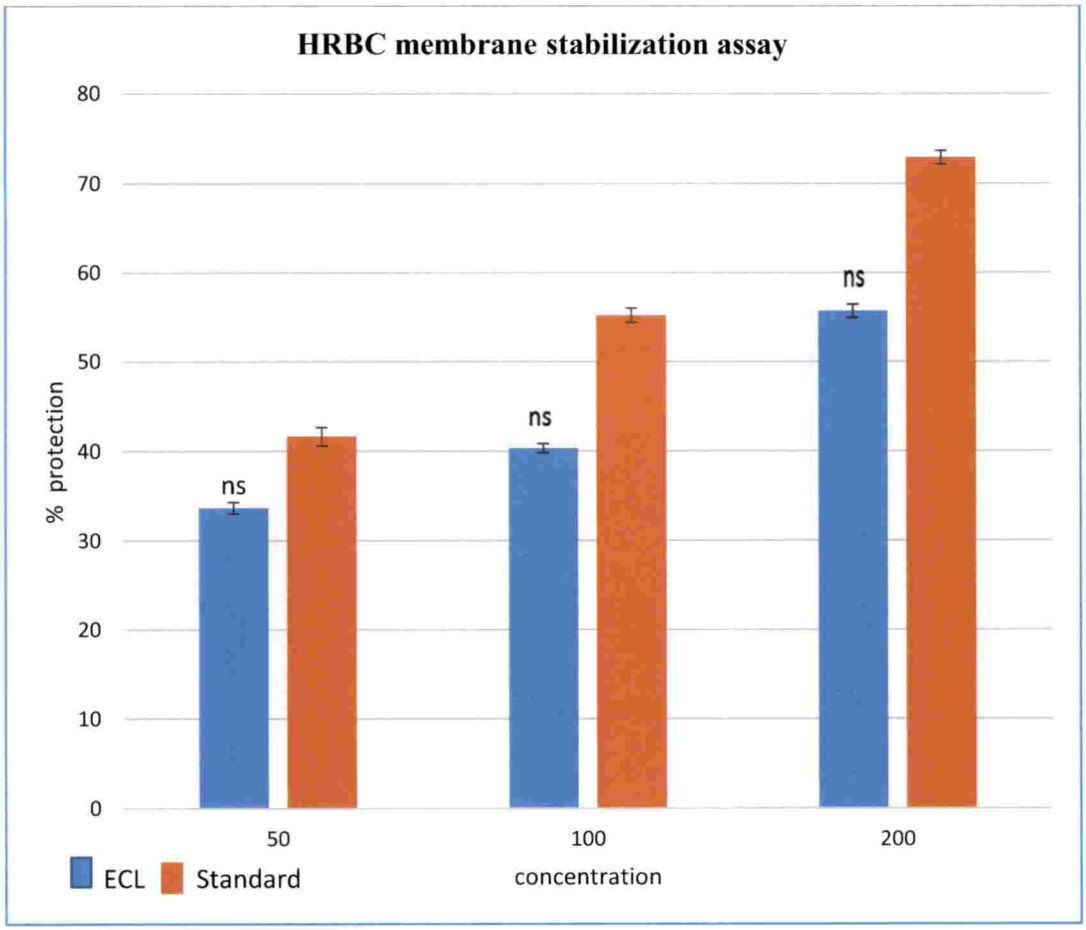

Figure 4. *In-vitro* antiinflammatory activity *C. laxum* R. Br

Values are expressed as mean ± SD, for n=6, one way ANOVA followed by Duncan's multiple comparison test, ns- no significant difference compared with standard.

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4.4 *IN VIVO* ANTIINFLAMMATORY STUDY

4.4.1 Carrageenan induced paw oedema in Wistar rats

The effect of ethanolic extract of *C. laxum* on carrageenan induced paw oedema was examined in adult Wistar rats. The percentage of inhibition of paw oedema volume are shown in Table 9 and difference in paw oedema volume are shown graphically in Figure 5. The maximum percentage of inhibition of paw oedema (80.98%) was exhibited by a dose of ECL (450 mg/kg). The standard drug Indomethacin exerted a promising level of inhibition (87.61%) at a dose of 10 (mg/kg) body weight.

Table 9: Effect of ethanolic extract of *C. laxum* on Carrageenan induced paw oedema.

Sl. No.	Concentration (mg/kg)	Percentage of inhibition
1	Control	----
2	Indomethacin (10 mg/kg)	87.61 %
3	ECL (50 mg/kg)	33.34 %
4	ECL (150 mg/kg)	61.90 %*
5	ECL (450 mg/kg)	80.98 %*

Values are expressed as mean ± SD, for n=6, $*P \leq 0.05$ compared with standard

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4.4.2 Formalin induced paw oedema in Wistar rats

The effect of ethanolic extract of *C. laxum* on formalin induced paw oedema in rats were studied. The percentage inhibition of paw oedema volume on 1 st and 7 th day are shown in Table 10 and difference in paw oedema volume on 1 st and 7 th day are shown graphically in Figure 6. The degree of inflammation was found to be significantly reduced in the groups treated with 150 and 450 mg/kg ECL, where they showed maximum inhibitions of 60.34% and 72.41% respectively after 7 days of formalin treatment. The standard drug Indomethacin exhibited a percentage inhibition of 77.58% at a dose of 10 mg/kg. The results indicates the potential antiinflammatory activity of *C. laxum.*

Table 10: Effect of ethanolic extract of *C. laxum* on Formalin induced paw oedema.

Sl. No.	Group	% inhibition 1 st day	% inhibition 7 th day
1	Normal	----	----
2	Standard (Indomethacin)	31.11	77.58
3	ECL (50 mg/kg)	8.88	48.27
4	ECL (150 mg/kg)	17.77*	60.34*
5	ECL (450 mg/kg)	24.44*	72.41*

The difference in paw volume of animals treated with ECL compared with control group. Results are expressed as mean ± SD, for n=6, $*P \leq 0.05$ compared with standard

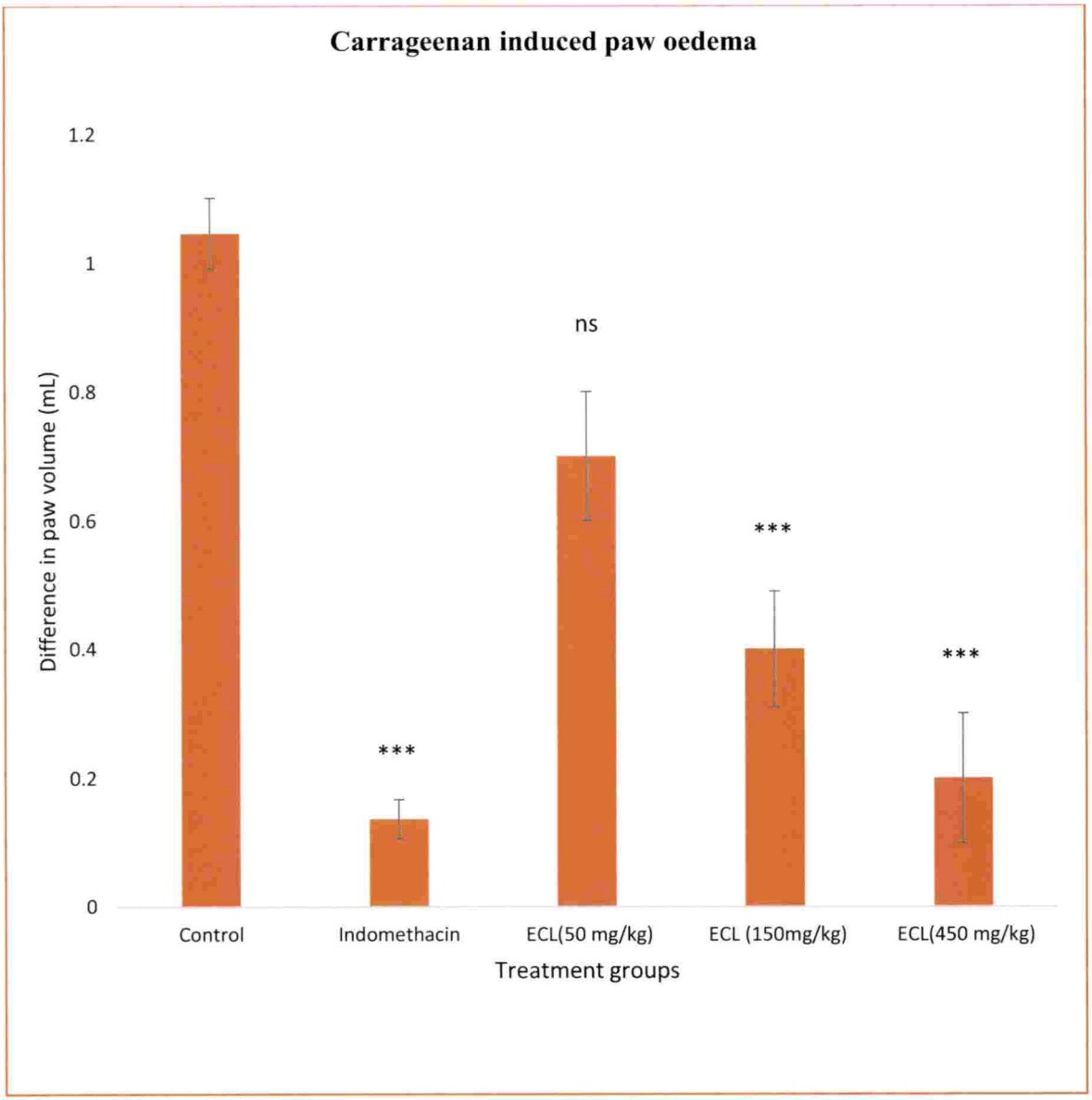

Figure 5. Effect of ethanolic extract of *C. laxum* on Carrageenan induced paw oedema in rats

Values are expressed as mean ± SD, for n=6, one way ANOVA followed by Duncan's multiple comparison test, ***$P \leq 0.05$ and ns- no significant difference compared with control group.

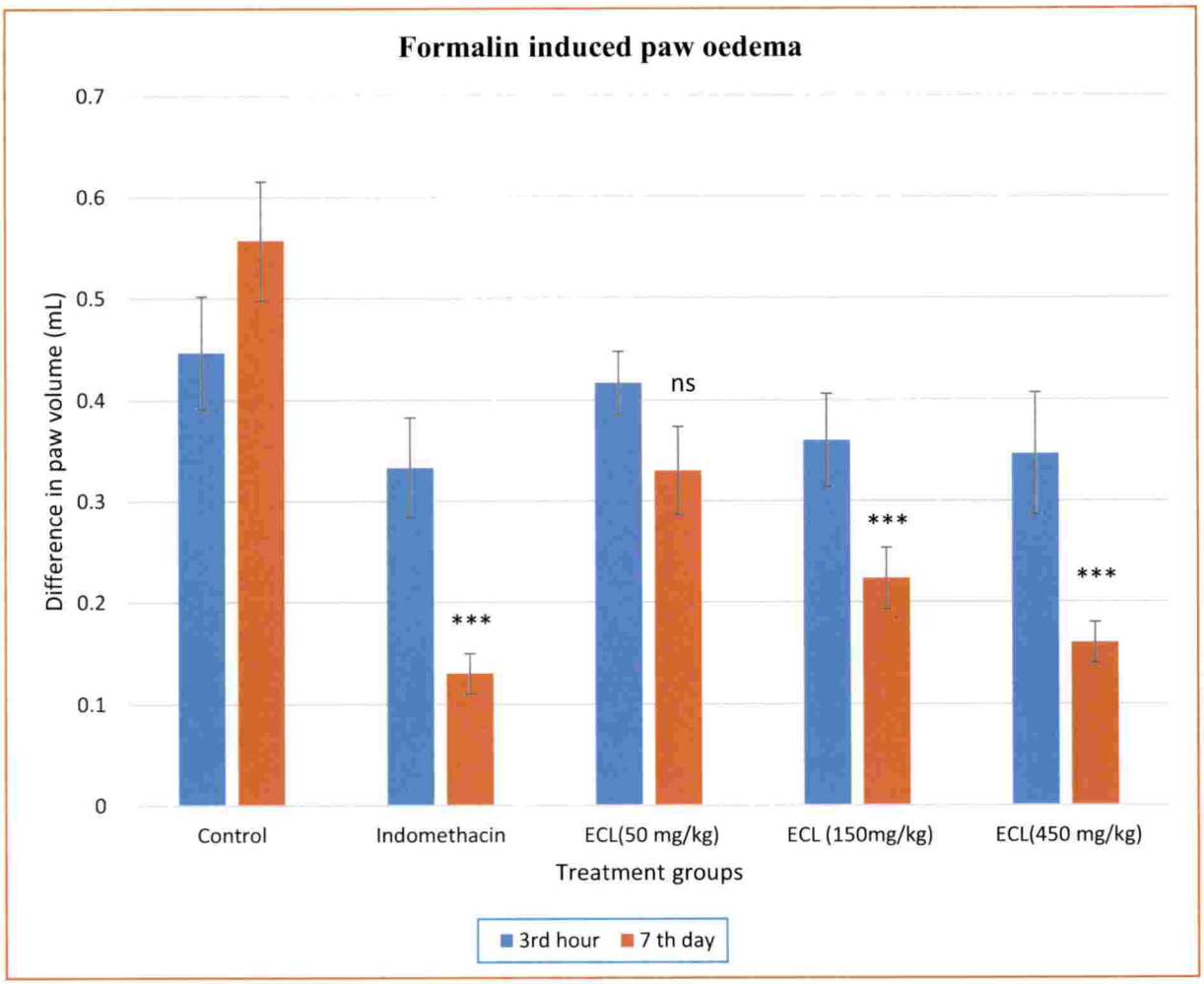

Figure 6. Effect of ethanolic extract of *C. laxum* on Formalin induced paw oedema in rats

Values are expressed as mean ± SD, for n=6, one way ANOVA followed by Duncan's multiple comparison test, ***$P \leq 0.05$ and ns- no significant difference compared with control group.

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4.5 *IN VITRO* ANTIOXIDANT STUDY

4.5.1 Hydroxyl radical scavenging assay

In hydroxyl radical scavenging assay, the hydroxyl radical is generated through the Fenton reaction, where hydrogen peroxide reacts with iron-EDTA complex to generate hydroxyl radical. The ethanolic extracts showed almost IC_{50} value of 36.62 µg/mL when compared with the standard ascorbic acid with IC_{50} value of 22.41µg/mL (Table 11 and Figure 7). The radical scavenging activity might be due to the presence of different phytochemicals including polyphenolics in the extracts.

Table 11. Effect of ethanolic extract of ECL on hydroxyl radical scavenging activity

Sl. No.	Conc. (µg/mL)	% scavenging of standard (530 nm)	IC_{50} value	Conc. (µg/mL)	% scavenging of ECL (530 nm)	IC_{50} value
1	25	67.34	22.41 µg/mL	50	51.32	36.62 µg/mL
2	50	75.66		100	59.55	
3	75	86.35		150	65.65	
4	100	93.64		200	72.27	

values are expressed as mean ± SD, for n=6.

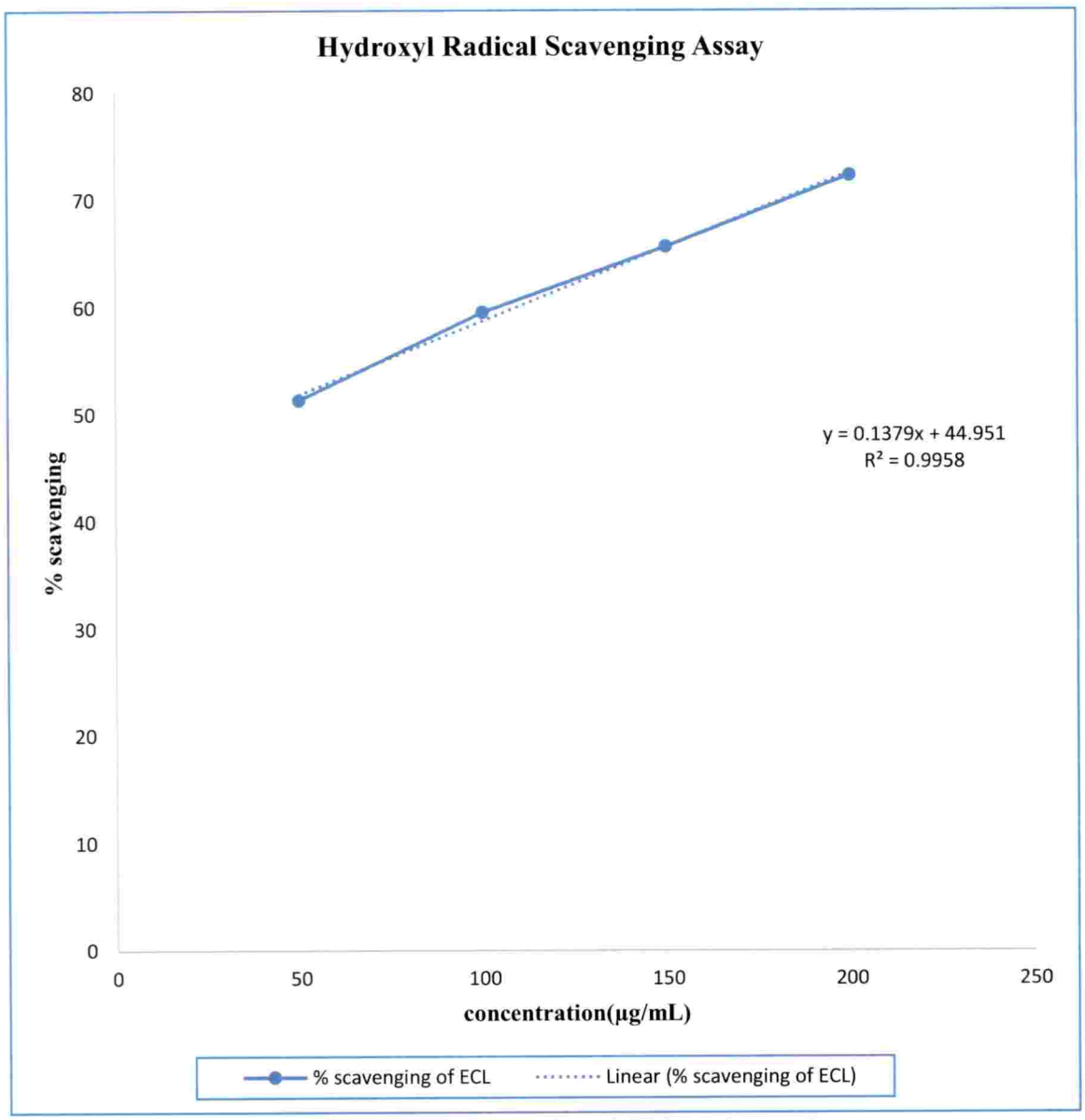

Figure 7: Hydroxyl radical scavenging activity of *Chlorophytum laxum*

Values are expressed as mean ± SD, for n=6.

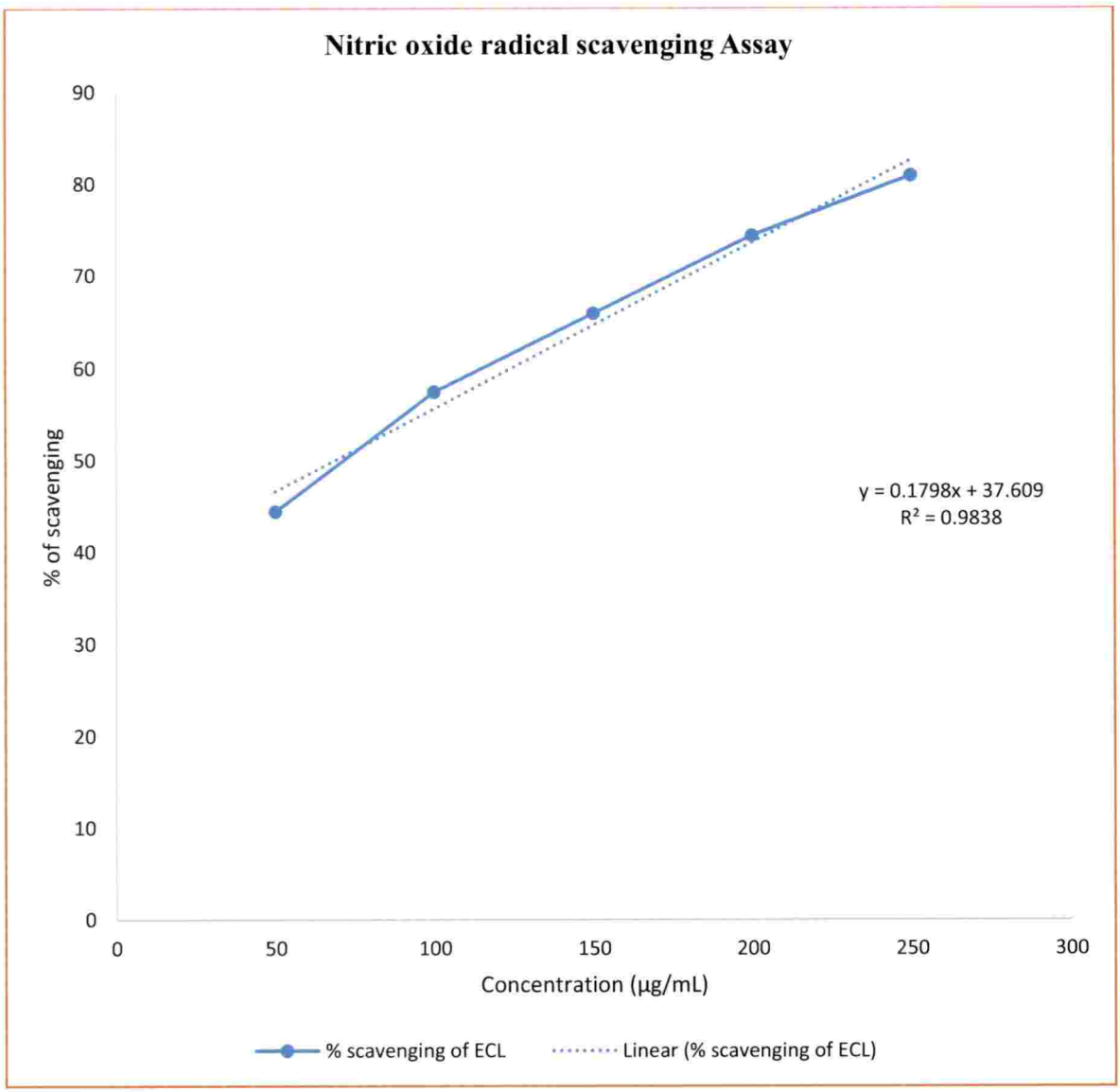

Figure 8: Nitric oxide radical scavenging activity of *Chlorophytum laxum*
Values are expressed as mean ± SD, for n=6.

4.5.2 Evaluation of Nitric oxide radical scavenging activity

The nitric oxide radical scavenging activity of ethanolic extract of *C. laxum* was shown in table 12. The results were compared with that of standard L-Ascorbic acid.

Table 12. Effect of ethanolic extract of ECL on nitric oxide radical scavenging activity

Sl. No.	Conc. (µg/mL)	% scavenging of standard (546 nm)	IC_{50} value	Conc. (µg/mL)	% scavenging of ECL (546 nm)	IC_{50} value
1	25	51.37	16.76 µg/mL	50	44.39	68.91 µg/mL
2	50	65.39		100	57.41	
3	75	75.49		150	65.87	
4	100	84.76		200	74.34	
5	125	93.36		250	80.87	

Values are expressed as mean ± SD, for n=6.

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4.5.3 Total Antioxidant Activity

The values are expressed as ascorbic acid equivalents (AAE). The total antioxidant activity of ethanolic extract of tubers of *C. laxum* was found to be 90.04 µg AAE/g of dry extract.

Table 13. Total antioxidant activity of *Chlorophytum laxum*

Conc. of Ascorbic acid (µg/mL)	Mean Absorbance (695 nm)
25	0.5658 ± 0.013
50	0.7759 ± 0.006
75	0.9942 ± 0.012
100	1.2745 ± 0.007
125	1.5029 ± 0.004
Sample Ethanolic extract (0.5 mg/mL)	0.7385 ± 0.011

Values are expressed as mean ± SD, for n=6.

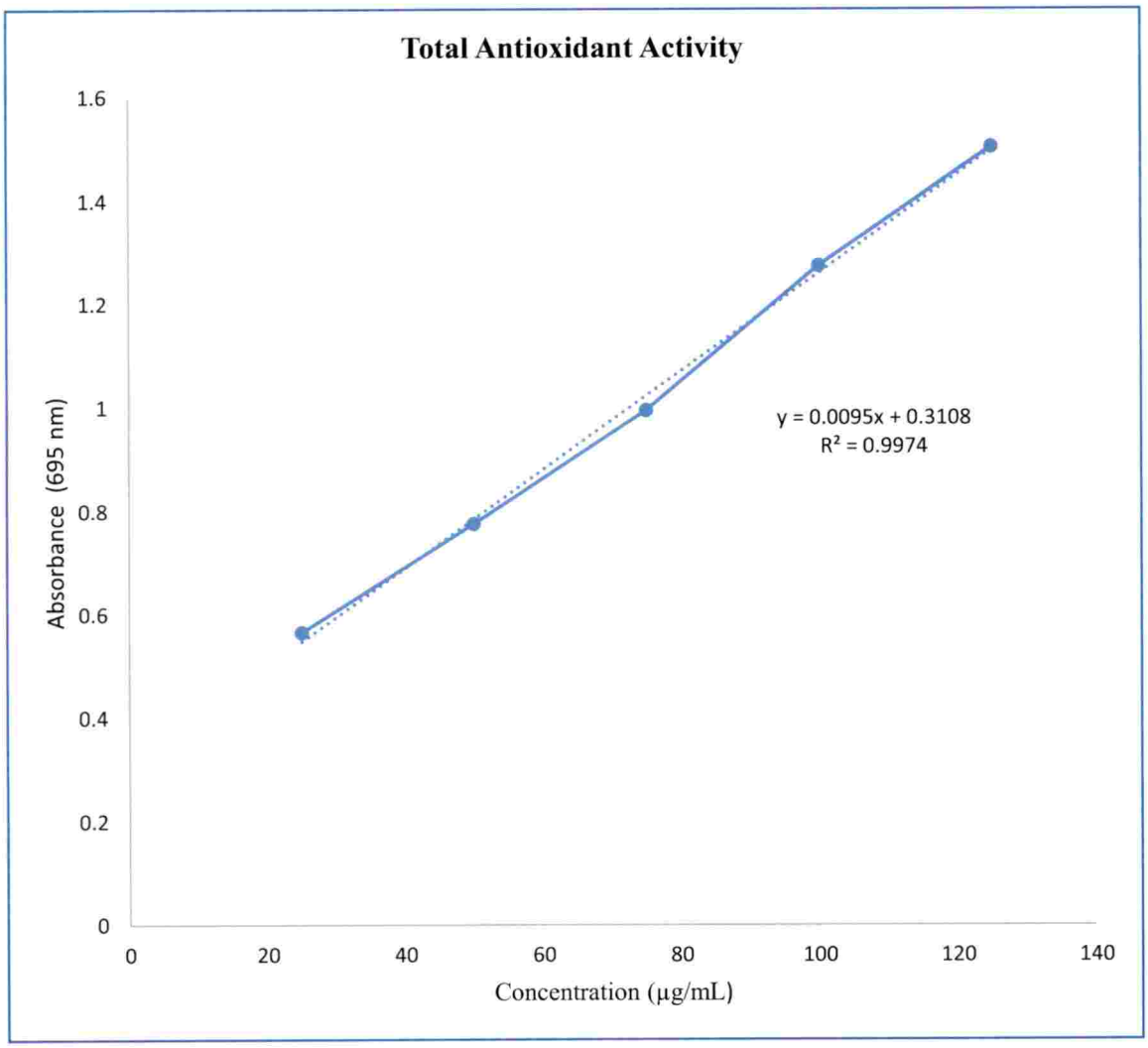

Figure 9. Total antioxidant capacity of *Chlorophytum laxum*
Values are expressed as mean ± SD, for n=6.

The total antioxidant activity of ethanolic extract of tubers of *C. laxum* was found to be 90.04 µg AAE/g of dry extract.

4.5.4 *In vitro* anti-lipid peroxidation studies

Chlorophytum laxum were tested for the ability to inhibit lipid peroxidation *in vitro*. The degree of lipid peroxidation in the liver tissue was determined in terms of malondialdehyde (MDA) produced in nmol/g wet liver. The IC_{50} values of ECL for *in vitro* anti-lipid peroxidation were found to be 135.67 µg/mL.

Table 14. Effect of ethanolic extract of ECL on anti-lipid peroxidation

Sl. No.	Samples (µg/mL)	MDA (n mol/g wet liver)	% of MDA Inhibition	IC_{50} (µg/mL)
1	Normal control	0.72 ± 0.02		
2	Toxin	2.88 ± 0.05		
3	$FeCl_2$ + AA + ECL 25	2.22 ± 0.03*	22.91	
4	$FeCl_2$ + AA + ECL 50	2.01 ± 0.08*	30.20	135.67
5	$FeCl_2$ + AA + ECL 100	1.64 ± 0.10*	43.05	
6	$FeCl_2$ + AA + ECL 200	1.02 ± 0.01*	64.58	

Values are expressed as mean ± SD, for n=6, *$P \leq 0.05$ significantly different.

DISCUSSION

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5. DISCUSSION

Chlorophytum is one of the medicinally important plant species as it possess properties like antidiabetic, antistress, immunomodulatory, anti-inflammatory, antioxidant, antimicrobial, aphrodisiac and anti-ageing (Mishra, 2012). *Chlorophytum laxum* R. Br. is one of the important medicinal plant used by tribal people in Karnataka, Maharashtra and Madhya Pradesh state. The plant is basically used for the treatment of piles and as astringent and the tuberous roots are being used as a well-known tonic and an aphrodisiac and also for the treatment of diarrhoea, dysentery and used as demulcent and galactogogue. The tubers of *Chlorophytum laxum* are used for treating insect bite and snake bites (Padal *et al.*, 2012). To meet the objective of the present study, antioxidant and anti-inflammatory properties of the *Chlorophytum laxum* has been investigated to substantiate the tribal claim.

5.1 ORAL ACUTE TOXICITY STUDY

The intention of toxicity studies is to elucidate the toxic profile of a herbal drug. The acute toxicity studies are done in experimental animal models such as mice or rats. The acute toxicity study was carried out by orally administering 4 doses of ECL extract (5, 50, 300 and 2000 mg/kg) in mice which showed no symptoms of toxicity or change in behavioural or physiological pattern even at the highest dose (2000 mg/kg). Hence the plant extract could be considered as safe for oral administration at this range. A similar study conducted by Jaijoy and his colleagues revealed that the water extract from the fruits of *Piper chaba* was found to be non-toxic even at the highest dose (2000 mg/kg) in Sprague-Dawley rats (Jaijoy *et al.*, 2011).

5.2 PRELIMINARY PHYTOCHEMICAL SCREENING

5.2.1 Standard phytochemical test (Quantitative phytochemical analysis)

Phytochemical analysis is one of the important steps in the detection of bioactive principles present in a particular medicinal plant, which may lead to novel drug discovery. Phytochemicals are biologically active naturally occurring secondary metabolites of plant, which provide protection against diseases (Devasagayam *et al.*, 2004). In the present study, some of the phytoconstituents identified correlate with bioactivities of the plant. The preliminary phytochemical screening of the plant was performed using standard methods. The medicinal value of the plants lies in their biologically active phytochemical constituents such as flavonoids, alkaloids, tannins and other phenolics substances, which produce a significant physiological action on the human body.

A preliminary phytochemical evaluation result of *C. laxum* has revealed the presence of carbohydrates, phenols, alkaloids, proteins, steroids, tannin, saponins and glycosides. Antimicrobial, anti-inflammatory, analgesic, anticancer activities has been reported for glycosides and tannins (Dembitsky, 2006). Saponins has shown anticancer activity, analgesic, antimicrobial activity and antiinflammatory activity (Moharram and El-Shenawy, 2007). Hence the phytochemicals present in the plant may be responsible for its medicinal properties.

5.2.2 Total phenolic content (Quantitative phytochemical analysis)

Phenolics comprises the largest group of phytochemical compounds that are responsible for the antioxidant property of plants (Ji *et al.,* 2011). Phenolic compounds are ubiquitous secondary metabolites in plants which are known to have antioxidant activity (Tepe *et al.*, 2006). The results obtained in this study showed a considerable level of phenolic content. Ethanolic extract was found to be more effective than aqueous extract. From the experiment, the content of total phenols in the ethanolic

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extract of *C. laxum* was expressed as Gallic acid equivalents per gram of dry extract and is 5.12 mg GAE/g of extract.

5.3 ANTIINFLAMMATORY STUDIES

Inflammation is a common protective reaction towards tissue injury which involves a complex array of mediator release, enzyme activation, fluid extravasations, cell migration, tissue breakdown and repair (Vane *et al.*, 1995). This complex process is normally associated with pain and involves occurrences suchlike increase of protein denaturation, vascular permeability and membrane alterations (Umapathy *et al.*, 2010). The complex inflammatory reactions involve the release of a wide variety of inflammatory mediators i.e. prostaglandins, thromboxanes and leukotrienes. When the cells get injured they release histamine, prostaglandins and kinins. These collectively causes increase in vasodilation (widening of blood capillaries) and permeability of the blood capillaries.

In vitro as well as *in vivo* methods are used in determining antiinflammatory activity of plant extracts. For the present study, Carrageenan and Formalin induced hind paw oedema models were used for *in vivo* anti-inflammatory study. HRBC membrane stabilization assay was used for *in vitro* anti-inflammatory study. Indomethacin, a non-steroidal anti-inflammatory drug (NSAID) was utilized as the standard for antiinflammatory studies.

5.3.1 *In vitro* antiinflammatory study

Since the erythrocyte membrane is analogous to the lysosomal membrane, the stabilization of HRBC membrane by hypotonicity induced membrane lysis have been used as a measure to investigate the *in vitro* anti-inflammatory activity (Shenoy *et al.*, 2010) and its stabilization entails that the extract could stabilize lysosomal membranes. The hemolytic effect of hypotonic solution results in excessive fluid accumulation in the cells that cause cell membrane to rupture. Stabilization of the lysosomal membrane

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is important in regulating the inflammatory response thereby preventing the release of lysosomal constituents of activated neutrophil, like proteases and bacterial enzymes, which causes tissue inflammation and damage over extra cellular release (Rajendran and Lakshmi, 2008).

The results demonstrated that ECL at the concentration of 200 µg/mL significantly protect the hypotonicity induced haemolysis of RBC. At the highest concentration (200 µg/mL), ECL showed maximum inhibition of 55.69 %, as compared with the standard Diclofenac sodium which showed 72.93 % inhibition of RBC haemolysis. The EC_{50} value of extract was found to be 162.47 µg/mL as compared with the standard having EC_{50} value of 84.32 µg/mL.

5.3.2 Carrageenan induced hind paw oedema

Carrageenan, is a mucopolysaccharide derived from Irish Sea moss, *Chondrus*. The inflammation induced by carrageenan is acute, non-systemic effects, non-immune as well as high degree of reproducibility (Hafeez *et al.*, 2013). The oedema development in the rat's paw after the carrageenan injection is due to the release of serotonin, histamine and prostaglandin like compounds (Vinegar *et al.*, 1969).

The ethanolic extract of *C. laxum* showed inhibition of carrageenan induced paw oedema in a dose dependent manner and the maximum inhibition was observed at 450 mg/kg (80.98%), which showed a significant similarity with the standard drug Indomethacin (87.61%). The inhibitory effect of extract may be due to the inhibition of inflammatory mediators such as prostaglandins, histamines, cytokines etc., either in an independent way or in a synergistic approach by the secondary metabolites in the plant extract.

5.3.3 Formalin induced hind paw oedema

Formalin induced paw edema in rats is one among the most appropriate method to evaluate the acute inflammation and it is a biphasic event. Formalin induction leads to alterations in connective tissue metabolism which is one of the major biochemical events during the inflammatory process. These changes are effected in the variation of relative composition of different components of connective tissue namely glycoprotein, mucopolysaccharides, hexosamine and hydroxy proline, sialic acid (Houck and Jacob, 1960).

In case of ECL administration, the maximum reduction in paw volume was observed at the dose 450 mg/kg after the 7^{th} day (72.41%), which was significantly comparable to that of the standard drug Indomethacin (77.58%). The results suggest a possible inhibition of inflammatory mediators in the inflammation cascade by the phytochemicals present in *C. laxum*.

5.4 *IN VITRO* ANTIOXIDANT ACTIVITY

A large number antioxidants are produced by plants which controls the oxidative stress caused by sunbeams and oxygen, they can be represented as a source of new compounds having antioxidant activity (Richards *et al.,* 1991). Antioxidants plays a major role in protecting the living forms from the toxic effects of reactive chemicals by inhibiting the formation of free radicals. Antioxidants impede the oxidative processes by chelating the free catalytic metals, scavenging the free radicals and work as electron donors (Gulcin *et al.,* 2005). The therapeutic effects of several medicinal plants are usually attributed to their antioxidant properties of its phytochemicals. Plant based antioxidants are preferred to the synthetic ones because of their multiple mechanisms of action and non-toxic nature.

In the present investigation, the *in vitro* antioxidant activity of *C. laxum* were assessed based on the scavenging activity of the stable hydroxyl radical, nitric oxide radical, antilipid peroxidation assay and total antioxidant capacity.

5.4.1 Nitric oxide radical scavenging activity

Nitric oxide is an essential bioregulatory molecule, which possess numerous physiological effects such as blood pressure control, transduction of neural signals, platelet function, antitumor and antimicrobial activity (Jagetia, 2004). Nitric oxide on reaction with superoxide and oxygen radicals shows toxic property where the byproducts are capable to cause severe cellular injury (Vriesman, 1997).

Nitrite production is reduced by the tested extracts of *C. laxum.* Nitric oxide radical scavenging activity of the extracts was found to be increased with increasing concentrations. The highest activity was observed with 250 µg/mL (80.87 %) and the IC_{50} value was found to be 68.91 µg/mL. This might be because of the antioxidant compounds in the extract that competes with oxygen molecules to react with the nitric oxide molecules, thus inhibiting nitrite generation.

5.4.2 Hydroxyl radical scavenging assay

Hydroxyl radical scavenging is an important antioxidant activity due to high reactivity of OH radical which reacts with a large number of molecules found in living cells, suchlike amino acids, sugars, nucleotides and lipids. Hydroxyl radical is an eminently reactive free radical produced by biological systems and have been concerned as a highly destructive species in free radical pathology that are able to cause damage in every molecular entities found in living cells (Yasuda *et al.*, 2000). The highly reactive hydroxyl radical can cause oxidative damage to DNA, lipid and protein (Srikanth *et al.*, 2010).

In this study, *C. laxum* was found to scavenge radicals significantly and in higher concentration and may protect the DNA, protein and lipid from damage. The hydroxyl radical scavenging ability of the extract was determined by its ability to compete with deoxyribose for hydroxyl radicals. The ethanolic extracts showed an IC_{50} value of 36.62 µg/mL as compared to the standard ascorbic acid with IC_{50} value of 22.41 µg/mL. Maximum scavenging activity of hydroxyl radical was 72.27 % at 200 µg/mL concentration as compared with standard ascorbic acid which showed maximum scavenging activity of 93.64 % at 100 µg/mL concentration. Results of this experiment indicates that *C. laxum* might contain remarkable amount of reductants which may react with the free radicals to stabilize and terminate the free radical chain reaction.

5.4.3 Total Antioxidant Capacity

The reactive free radicals might be created in the cells and tissues from internal (namely diseases, inflammation or metabolism) or external sources (such as food, irradiation, pollution, drugs), or as a result of decreased protective ability (Rice-Evans *et al.*, 1991). Total antioxidant capacity is the measure of the amount of free radicals that are scavenged by a test solution, utilized to evaluate the antioxidant potential of the biological samples (Pinchuk *et al.*, 2012). The total antioxidant activity of ethanolic extract of tubers of *C. laxum* was found to be 90.04 µg AAE/g of dry extract.

5.4.4 Anti-lipid peroxidation study

Unsaturated lipids in the liver tissue are susceptible to peroxidation when they are exposed to reactive oxygen species (Chun *et al.*, 1998). The liver tissue is incubated in the presence of an ROS generating system, ascorbate/ $FeCl_2$, and the effect on tissue homogenate examined by quantifying malondialdehyde (MDA), which is the product of peroxidation of lipid. Lipid peroxidation in biological systems, generates numerous degradation products, namely malondialdehyde, which is founded as an important basis of cell damage and cell membrane destruction (Dotan *et al.*, 2004). Thus decrease in

the MDA level in tissue homogenate treated with increasing concentration of plant extract signifies the role of extract as a potent antioxidant.

In the present study, *C. laxum* was found to decrease MDA production in liver of rats treated with $FeCl_2$–ascorbic acid mixture, showing its antilipid peroxidant effects. The highest % of inhibition was observed with 64.58 % at 200 µg/mL and the IC_{50} value was observed as 135.67 µg/mL. Lipid peroxidation inhibition may be due to the presence of phenolic compounds, which has been shown to be correlated to the antioxidant activity of natural plant products.

SUMMARY

6. SUMMARY

The thesis entitled 'Assessment of antiinflammatory and antioxidant properties of *Chlorophytum laxum* R. Br.' was carried out in the Ethnomedicine and Ethnopharmacology Division of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram during the academic year 2017-2018. The objective of the study was to scientifically evaluate antiinflammatory and antioxidant properties of an ethnomedicinal plant *Chlorophytum laxum* R. Br. (Neeruvatti).

Chlorophytum laxum R. Br. (Neeruvatti) of Liliaceae family is an important medicinal plant used by Kani tribes of Kerala. The tubers of *Chlorophytum laxum* R. Br. were collected from the hills of Western Ghats and maintained at JNTBGRI to conduct the pharmacological studies. Extraction procedures of the plant were carried out to prepare the drugs of different doses for the study. Acute oral toxicity studies were conducted in mice and antiinflammatory studies were conducted in rats.

In preliminary phytochemical investigation, standard phytochemical screening and estimation of total phenolic content of *Chlorophytum laxum* were carried out. The preliminary phytochemical screening of tubers of *Chlorophytum laxum* has shown the presence of secondary metabolites like carbohydrates, phenols, alkaloids, proteins, steroids, tannins, saponins and glycosides that may be responsible for its medicinal properties. The total phenolic content of *C. laxum* was estimated to be 5.12 mg GAE/g of extract.

Toxicity studies of *Chlorophytum laxum* tuber extract were investigated in Swiss albino mice for 14 days with the oral administration of 4 doses (5, 50, 300 and 2000 mg/kg body weight) and no symptoms of toxicity were seen in the animals even up to the highest dose.

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The antiinflammatory potential of tuber extract was investigated *in vivo* by carrageenan induced paw oedema and formalin induced paw oedema in wistar rats and *in vitro* by HRBC membrane stabilization assay. The ECL extract were administered at doses (50, 150 and 450 mg/kg) of body weight orally in adult wistar rats and the highest percentage inhibition of paw oedema in the right hind limb was shown by ECL 450 mg/kg in both the methods. ECL at higher concentration protect significantly the hypotonicity induced haemolysis of RBC by in HRBC membrane stability analysis. These reveals the antiinflammatory potential of *C. laxum.*

The antioxidant effect of ethanolic extract of *C. laxum* was evaluated by hydroxyl radical scavenging, nitric oxide radical scavenging, antilipid peroxidation assay and total antioxidant capacity. The antioxidant potential of *C. laxum* was comparable with the standard, which indicates the potential activity of the tuber extract.

The present study thus substantiate the medicinal properties of *C. laxum* which possess antiinflammatory and antioxidant potential. Further studies in future are to be needed to explore the exact mechanism behind the action of *Chlorophytum laxum* R. Br. in analyzing their bioactivities.

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APPENDICES

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8. APPENDICES

APPENDIX I

Wagner's reagent

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

APPENDIX II

Mayer's reagent

Mercuric chloride	1.36 g
Potassium iodide	5.00 g
Water	100 ml

APPENDIX III

Hager's reagent

Picric acid	1.00 g
Water	100 ml

APPENDIX IV

Dragendorff's reagent

Bismuth nitrate	0.5 g
Conc. Hydrochloric acid	10 ml
Potassium iodide	4 g

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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 0.5 ml
- Acetone 0.2 ml
- Distilled water 100 ml

APPENDIX VI

Griess reagent

Napthylethylenediamine dihydrochloride 0.1%

Sulfanilamide 1% (5% concentrated phosphoric acid)

ABSTRACT

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ASSESSMENT OF ANTIINFLAMMATORY AND ANTIOXIDANT PROPERTIES OF *Chlorophytum laxum* R. Br.

Submitted by

ARUNDHATHY G B

(2013-09-115)

Abstract of Thesis

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9. ABSTRACT

The thesis entitled 'Assessment of antiinflammatory and antioxidant properties of *Chlorophytum laxum* R. Br.' was carried out in the Ethnomedicine and Ethnopharmacology Division of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram during the academic year 2017-2018. The objective of the study was to scientifically evaluate antiinflammatory and antioxidant properties of an ethnomedicinal plant *Chlorophytum laxum* R. Br. (Neeruvatti).

Chlorophytum laxum R. Br. (Neeruvatti), herbaceous plant of family Liliaceae is one of the important medicinal plant seen in grasslands. The tubers of *Chlorophytum laxum* R. Br. were collected from the hills of Western Ghats and maintained at JNTBGRI to conduct the pharmacological studies. Extraction procedures were carried to prepare the drugs of different doses for the study. Acute oral toxicity studies in mice and antiinflammatory studies in rats, were done as pharmacological analysis.

The preliminary phytochemical investigation, tubers of *Chlorophytum laxum* has shown the presence of secondary metabolites like carbohydrates, phenols, alkaloids, proteins, steroids, tannin, saponins and glycosides that may be responsible for its medicinal properties. The content of total phenols in the ethanolic extract of *Chlorophytum laxum* expressed as gallic acid equivalents per gram of dry extract is 5.12 mg GA/g of extract.

Toxicity studies of tuber extract were investigated in Swiss albino mice for 14 days by the administration of 4 doses 5, 50, 300 and 2000 mg/kg body weight and no symptoms of toxicity were seen in the animals even up to the highest dose.

In detailed pharmacological studies, antiinflammatory potential of tuber extract was investigated *in vivo* by carrageenan induced paw oedema and formalin induced

paw oedema and *in vitro* by HRBC membrane stabilization assay. The extract were administered at doses of 50, 150 and 450 mg/kg body weight orally in adult wistar rats and the maximum percentage inhibition of paw oedema in the right hind limb was shown by ECL 450 mg/kg in both the methods. ECL at higher concentration protect significantly the hypotonicity induced haemolysis of HRBC by *in vitro* antiinflammatory analysis.

The antioxidant effect of ethanolic extract of *C. laxum* showed IC_{50} of 36.62 µg/mL in hydroxyl radical scavenging, 68.91 µg/mL in nitric oxide radical scavenging and 135.67 µg/mL in antilipid peroxidation assay. Total antioxidant capacity of ethanolic extract of tubers of *C. laxum* was found to be 90.04 µg AAE/g of dry extract. The antioxidant potential of *C. laxum* was compared with a standard and the results obtained gives the significant effect and almost equal effect.

The results of current study will help to develop a monograph of the drug for reference. These results substantiate the traditional claim of the plant for its medicinal use.