SCIENTIFIC VALIDATION OF ANTIINFLAMMATORY, ANTINOCICEPTIVE AND ANTIOXIDANT POTENTIAL OF *MALAVIRINJI* (*Actinodaphne bourdillonii Gamble*)

Submitted
By

ADARSH PRATHAP

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Thesis

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DEPARTMENT OF PLANT BIOTECHNOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM - 695 522
KERALA, INDIA

2017

DECLARATION

I hereby declare that this thesis entitled "**Scientific validation of antiinflammatory, antinociceptive and antioxidant potential of *malavirinji* (*Actinodaphne bourdillonii* Gamble)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title, of any other university or society.

Place: Palode
Date: 28-11-2017

Adarsh Prathap
(2012-09-124)

JNTBGRI

Jawaharlal Nehru Tropical Botanic Garden and Research Institute

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

CERTIFICATE

This is to certify that this thesis entitled **"Scientific validation of antiinflammatory, antinociceptive and antioxidant potential of *malavirinji* (*Actinodaphne bourdillonii* Gamble)"** is a record of research work done by **Mr. Adarsh Prathap** (2012-09-124) 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 him.

Dr. S. R. Suja
(Chairperson, Advisory Committee)
Scientist and HOD (i/c)
Ethnomedicine and Ethnopharmacology Division
JNTBGRI, Palode,
Thiruvananthapuram- 695 562

Place: Palode
Date: 28/11/2017

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

CERTIFICATE

We, the undersigned members of the advisory committee of Mr. Adarsh Prathap (2012-09-124), a candidate for the degree of B. Sc. – M. Sc. (Integrated) Biotechnology, agree that the thesis entitled **"Scientific validation of antiinflammatory, antinociceptive and antioxidant potential of *malavirinji* (*Actinodaphne bourdillonii* Gamble)"** may be submitted by Mr. Adarsh Prathap in partial fulfilment of the requirement for the degree.

28/11/2017

Dr. S. R. Suja
(Chairperson, Advisory Committee)
Scientist and HOD (i/c)
Ethnomedicine and
Ethnopharmacology Division
JNTBGRI, Palode
Thiruvananthapuram - 695 562

28/11/17

Dr. Swapna Alex
(Member, Advisory Committee)
Professor & Head
Department of Plant Biotechnology
College of Agriculture, Vellayani
Thiruvananthapuram – 695 522

28/11/17

Dr. Vinodkumar T. G. Nair
(Member, Advisory Committee)
Senior Scientist
Ethnomedicine and
Ethnopharmacology Division
JNTBGRI, Palode
Thiruvananthapuram - 695 562

28/11/17

Dr. K. B. Soni
(Member, Advisory Committee)
Professor & Course Director
B. Sc. – M. Sc. (Integrated) Biotechnology
Department of Plant Biotechnology
College of Agriculture, Vellayani
Thiruvananthapuram – 695 522

28/11/2017

Dr. K. Sujathan
(External Examiner)
Additional Professor
Division of Cancer Research
Regional Cancer Centre
Thiruvananthapuram

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ADARSH PRATHAP

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1. INTRODUCTION

The fabric of Indian ethos is intertwined with civilization, belief and tradition, if followed in right perspective can never remain a burden to advancement unless a society is fettered with wrongheadedness. Similar is the situation with the health care. In spite of implausible progresses in contemporary science, technology and allopathic medicine, we are inept to deliver quality health care to all.

Ethnomedicine is a system of medicine flourished before the epoch of modern medicine, founded on cultural views and practices. The understanding of certain plants, herbs, animals and minerals that have restorative and healing effects were diffused from one generation to another and it is the aftermath of bold investigation through trial and error method over hundreds of years (Vedavathy, 2013). Ethnomedicinal studies may constitute the starting point for the development of new drugs and useful substances. Mainstreaming of such medicinal knowledge is imperative for the wellbeing of people. Scientific evidence based amalgamation of traditional medicine in clinical practice will benefit to deliver worth health care to all.

Through the fast developing process of urbanisation and industrialisation we have lost a lot of our natural wealth. Some of them have even entered the endangered list and in a verge of extinction. Our fast concern is necessary to reserve this indigenous and precious information. In this context the contribution made by the traditional medicine in the modern system of medicine is worth nothing. For instance, *Rauvolfia serpentina* was used in India to treat people who had mental illness and snakebites (Reeta, 2013). The alkaloid isolated from this herb reserpine was responsible for the same curative actions (Mittal, 2012). Such is the importance of traditional used plants and medicines in context to the primary health care.

Inflammation is the body's immediate response to damage to its tissues and cells by harmful biological agents and harmful incentives such as chemicals, or injury. Mainly it is a tissue rescinding process that involves engagement of blood-derived products, such as plasma proteins, fluid and leukocytes into disconcerted tissue. Relocation is facilitated by the modifications in the native vasculature that lead to vasodilation, increased rate of vascular penetrability and blood flow. Inflammation safeguards the body from infective organisms and injurious agents and end with restorative and repair of affected area.

Nociception is an ability of body to sense damage or injury. Nociceptors or pain receptors are precisely intended to sense stimuli that may cause damage to the body. For instance, they may sense when there is physical damage to the body, or when they are bare to toxic substances or extreme temperatures.

Oxygen is one of the ultimate vital component for living, but is an extremely responsive atom that is adept of fetching share of potentially destructive molecules usually named "free radicals". They are proficient of destructing the fine cells of the body. Free radicals encompass unstable unpaired electrons and they arrest electrons from further constituents in order to disable themselves. This initially stabilizes the free radical but generates another in the process. Usually free radical development is controlled obviously by antioxidants. As soon as there is absence of these antioxidants, harm owed by free radicals can become amassed and incapacitating. Plants produce secondary metabolites which may contribute to this antioxidant and other pharmacological effects like analgesic, hepatoprotective, anticancer and anti-inflammatory actions.

Actinodaphne bourdillonii Gamble is a tree which belongs to Lauraceae family, ordinarily known as "Malavirinji" in Malayalam is a small sized tree which is endemic to the southern Western Ghats. The paste of fresh leaves of this tree is used externally for various treatments such as inflammation, reducing pain, bone setting etc., and dried leaf powder is medicated internally to reduce inflammation and pain by Kani tribes of Trivandrum District, Kerala.

Though, not any work has been reported on the following aspects of *A. bourdillonii*, the present study is to scientifically evaluate pharmacological properties such as anti-inflammatory, anti-nociceptive and antioxidant effects to investigate the scientific cogency of traditional practice using experimental animal models.

2. REVIEW OF LITERATURE

2.1 *Actinodaphne bourdillonii Gamble*

According to the Benthaman and Hooker Classification, the genus Actinodaphne belongs to the family Lauraceae, and comprise 70 species of evergreen trees and shrubs. This genus has been reported to produce isoquinoline alkaloids, lactones, lignans, and phenolic amides and also confirmed to have pharmacologically pertinent properties such as antioxidant, antimicrobial, hepatoprotective, anti-inflammatory and antibacterial activities (Salleh, 2016).

Actinodaphne bourdillonii Gamble is a tree locally named as "Malavirinji". It is endemic to the Western Ghats. The leaf-crushed paste of this tree is used externally for various treatments such as inflammation, reducing pain, bone setting etc., and dried leaf powder is taken internally to reduce inflammation and inflammatory pain by *Kani* tribes of Trivandrum District, Kerala. The detailed photos of the plant are given in the plate 1.

Systematic position

Kingdom : Plantae
Subkingdom : Tracheobionta
Super division: Spermatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Magnoliidae
Order : Laurales
Family : Lauraceae
Genus : *Actinodaphne*
Species : *bourdillonii*

Basic Information

Habit: Tree
Medicinal: Yes
Flowering & Fruiting: March-April
Habitat: Shola and evergreen forests
Distribution: Southern Western Ghats
Endemic to: Southern Western Ghats
Flower colour(s): Yellow, Green
Monocot/Dicot: Dicotyledonous Plants
IUCN status: NE
Localities: Thiruvananthapuram, Kollam, Idukki, Palakkad and some regions of Karnataka and Tamilnadu.

Plate 1a: *Actinodaphne bourdillonii* Gamble tree in its habit (Palode forest Range)

Plate 1b: Young leaflets of *Actinodaphne bourdillonii* Gamble (Palode forest Range)

2.1.1 Plant Description (Sasidharan, 2004)

Habit: Trees up to 10 m tall.

Trunk & Bark: Bark smooth, brownish, lenticellate; blaze light orange.

Branches and Branchlets: Young branchlets terete, fulvous tomentose.

Leaves : Leaves simple, alternate, spiral, subverticilate; petiole 0.8-1.2 cm long, flat, densely fulvous tomentose; lamina 11-24 x 2-6.3 cm, narrowly long elliptic-oblanceolate, apex narrowly acute to acuminate; base narrowly cuneate, margin entire, chartaceous, densely tomentose on midrib and nerves beneath, glabrous above except midrib; midrib raised above; secondary nerves 7-14 pairs, very oblique; tertiary nerves, closely horizontally percurrent.

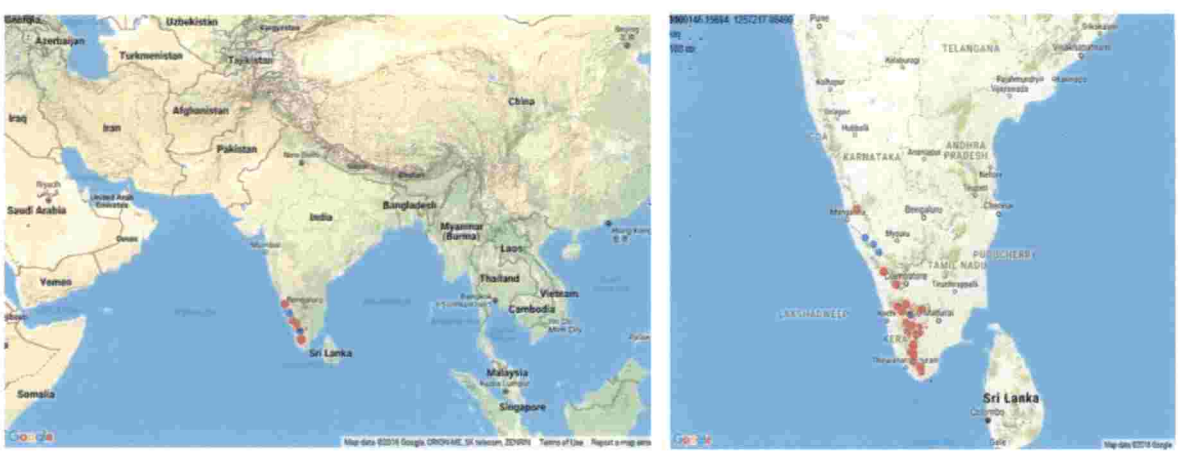
Inflorescence / Flower: Flowers unisexual, dioecious, in umbels on short peduncle.

Fruit and Seed: Berry, black when ripe, seated on cup shaped fruiting perianth

Ecology: Understorey trees in wet evergreen forests between 900 and 2400 m.

Distribution: *Actinodaphne bourdillonii* is endemic to the southern Western Ghats (Volga *et al.,* 2013). (Fig 1) demonstrations the distribution of *A. bourdillonii* Gamble.

Fig 1: Distribution of *Actinodaphne bourdillonii* Gamble



2.2 MEDICINAL PLANTS

The plants which having curative activity or applies positive pharmacological effects on animals and humans are generally designated as medicinal plants. It indeed synthesizes and accrue secondary metabolites like alkaloids, resins, terpenes, flavonoids, saponins, sterols, glycosides, tannins, volatile oils etc., in diverse parts of the plant, and these secondary metabolites are produced for the existence of plant from environmental stressors (IUCN, 2011).

Extraction is a critical phase in the parting of therapeutically active compounds from plant tissue using selective solvents through standard protocols. Different extraction methods were developed for herbal medicine.(Fig 2) The products obtained from plants can be semisolid state or oily (after removing the solvent) or dry powder form and they are used orally or externally (Tiwari *et al*., 2011).

Fig 2: Current extraction techniques for herbal medicine (Pan *et al,* 2013).

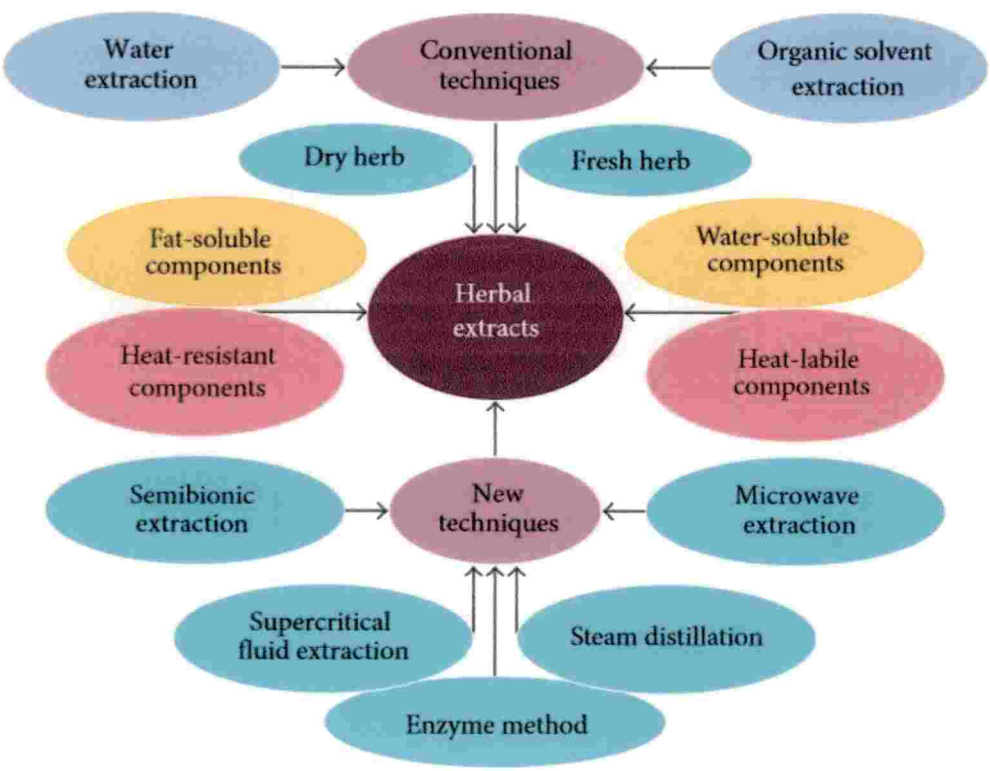
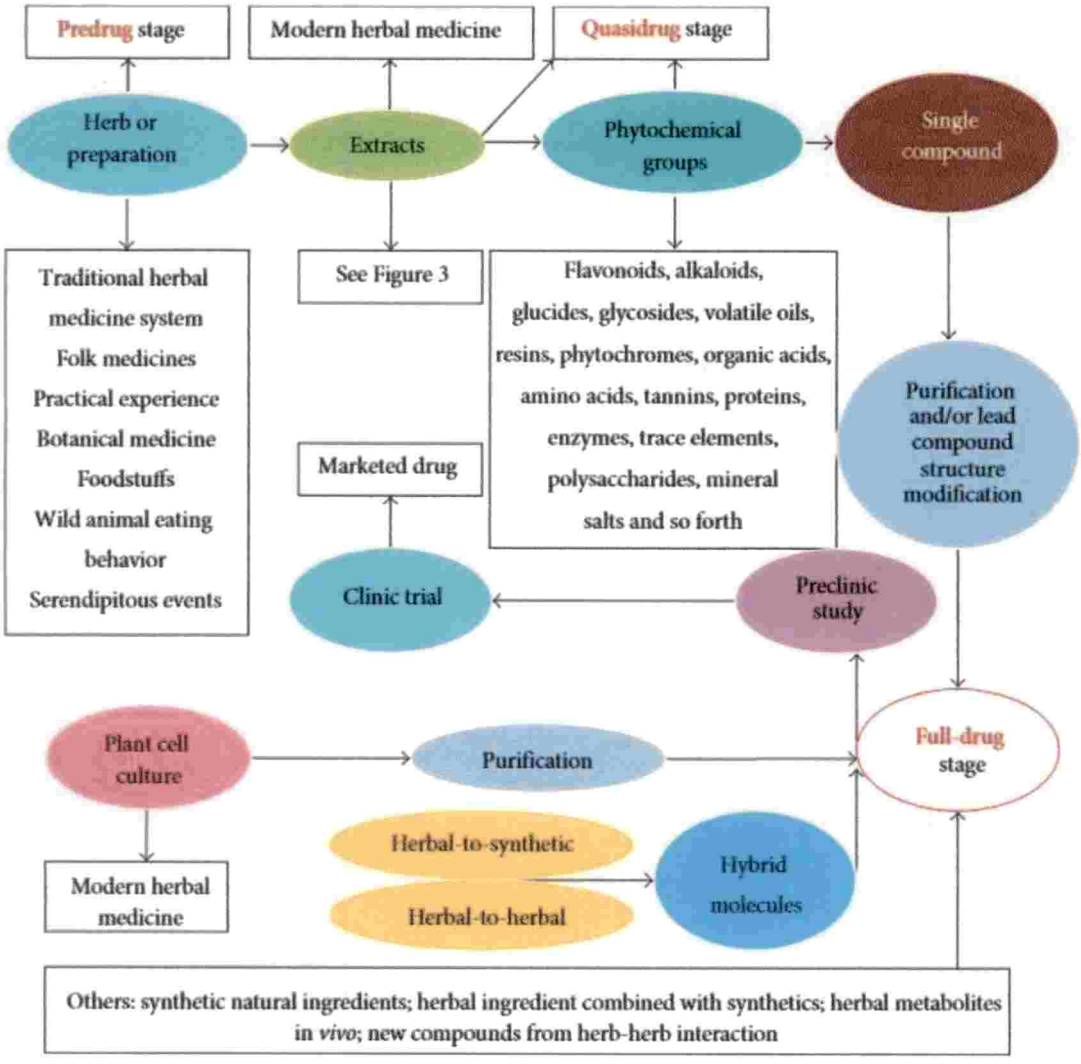


Fig 3: Current approaches for drug discovery from herbal medicine (Pan *et al,* 2013).



2.3 ETHNOBOTANY AND ETHNOMEDICINE

Ethnomedicine stands for the study of traditional medical practices chiefly swotting about the healing herbs which is concerned with the cultural interpretation of health condition, sickness and diseases and also addresses the health care seeking process and healing practices. Scientific authentication of this conventional healing approaches can persuade progress of healthcare. Research activities and attention in the arena of Ethnomedicine have been enormously improved throughout in the last decade

The practise of treatment using medicinal plants was overlooked by many biomedical practitioners for many reasons like chemical elements, toxicity and dosages of the plants used in ethnomedicine are not evidently demarcated. Healthier interest in the field of ethnomedicine enhanced the usage of herbs and become one of the most fruitful criteria used by the pharmaceutical research world in finding novel therapeutic agents for various areas of biomedicine (Cox and Balick, 1994). In recent times, (Schmidt *et al.*, 2009) experimentally confirmed that plants offer immense scope for researchers involved in authentication and validation of traditional claims for the development of novel drugs

Since curiosity in ethnomedicine have been snowballing world over, ethnopharmacological studies have gained reputation to discover the traditional knowledge predominantly in developing countries (Joshi and Joshi, 2000). Among the topmost 150 registered drugs used in the United States of America (USA), 57% comprise at least one key active compound which resulted from plants sources (Grifo and Rosenthal, 1997).

2.4 ETHNOPHARMACOLOGY

Ethnopharmacology is a mixture of perceptions, chiefly those of pharmacology, botany, pharmacognosy and anthropology. Hand-outs are also made by historians, agronomists, clinicians and biochemists. In this spirit, ethnopharmacology is the study of indigenous medical systems that connects the ethnography of health and healing with the physiologic relevance of its medical practices (Elisabetsky, 2009).

2.5 PHYTOCHEMISTRY AND PHYTOCHEMICALS

Phytochemicals are organically active naturally arising chemical complexes which are originate in plants, it delivers well-being for humans (Hasler and Blumberg, 1999). Their main action is to protect the plants from disease and damage caused by abiotic and biotic

stresses and these chemicals also contribute to the plant's aroma, colour and flavour. These plant chemicals i.e. phytochemicals, are produced to protect plant cells from environmental hazards such as drought, stress, pollution, pathogenic attack and UV exposure (Mathai, 2000). Currently they have an imperative role in maintaining the well-being of humans without any diseases so it ultimately enhances the quality of life. More than 4,000 phytochemicals have been recorded (Khan, 2016) and are classified by protective function, chemical characteristics and physical characteristics and approximately 150 of these phytochemicals have been investigated in detail (Meagher and Thomson., 1999).

The medicinal possessions in all medicinal plants arise from numerous bioactive constituents existing within the herb. Two classes of phytochemicals exists namely primary and secondary constituents (Krishnaiah *et. al*, 2009). Primary constituents include, sugars, proteins and amino acids among others with the secondary constituents consisting of tannins, phenols, saponins and others (Hamburger and Hostettmann, 1991). Documentation of phytochemicals in medicinal plants is the preliminary steps in the route of discovering novel plant-based drugs (Waweru, 2017).

Preliminary phytochemical screening of leaves of *Actinodaphne bourdillonii* Gamble which revealed that it is a rich source of mineral elements for instance, calcium, potassium, magnesium, iron, zinc, copper and sodium. The phytochemical study also shown the existence of alkaloids, tannins, saponins, cardiac glycosides, phlobatannins and terpenoids in the methanolic extract of leaves of *A. bourdillonii* (Deepa and Selvakumar, 2014). They may be responsible for the pharmacological activities like antioxidant, analgesic and anti-inflammatory effects.

Phytochemical constituents in the bark of *Ficus racemosa* was investigated by (Poongothai *et al.*, 2011). Result of phytochemical screening confirms the presence alkaloids, flavonoids, sugars, proteins, glycosides and sterols in the different extracts.

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

Actinodaphne hookeri leaves showed strong antioxidant properties, supporting the ethno medical use given to this plant (Prajapati *et al.*, 2009). *A. hookeri* leaves have potent antioxidant and hepatoprotective action against CCl4 – induced hepatic damage in rats (Akki *et al.*, 2014). Infusion of leaves of *A. hookeri* act as urinary tract disinfectant, antidiabetic, spasmolytic (Khere, 2007).

2.6 OXIDANTS

Oxidative stress plays an important role in the development of chronic and degenerative diseases such as aging, autoimmune disorders, cancer, arthritis, neurodegenerative and cardiovascular diseases (Lien *et al.*, 2008). Human body has numerous mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through dietary supplements. Endogenous and exogenous antioxidants act as "free radical scavengers" by preventing and repairing damages caused by ROS and RNS and boost the immune defence and lower the risk of cancer and other degenerative diseases (Singh, 2004).

2.7 ANTI-OXIDANTS

Antioxidants are the substances that have the capability to inhibits or delay oxidative damage caused by the free radicals in the body. Antioxidants control excessive free radicals via physical defences, antioxidant defences, preventative mechanisms and repair mechanisms. Our body generates various kinds of endogenous antioxidants to counteract free radicals produced and safeguard body from many disease leads by the tissue injury. Exogenous antioxidants are externally supplied in to the body through diet and other natural sources such as especially from the medicinal plants also plays important role in neutralizing the surplus free radicals produced, to prevent diseases and to defend the cells in the body against their toxic effects of free radicals (Birben *et al.*).

2.7.1 Antioxidant Process

When an antioxidant destroys a free radical at a time after the reaction antioxidant itself gets oxidized. So, antioxidant must be frequently restored in the body from internally as well as externally sources. Through the chain-breaking process, a radical discharges an electron, a second radical is formed in this response. The latter one applies the

similar action on alternative molecule and lasts until the free radical formed is alleviated by a chain-breaking antioxidant (carotenoids, vitamin E, C, etc.).

2.8 TOXICITY

Toxicology is the scientific learning of adversarial properties that happen in alive creatures triggered by chemical compounds. It comprises environmental agents and chemical compounds found in nature, as well as pharmaceutical compounds that are manufactured for health wariness. These substances could produce toxic effects in living organisms including disturbance in development patterns, uneasiness, illness and death.

Toxicity was categorized in to acute, sub chronic and chronic toxicity. Acute toxicity includes injurious effects in an organism over a single or short-term exposure. Sub chronic toxicity is the ability of a toxic substance to cause effects. Chronic toxicity is the proficiency of an element or fusion of substances to cause destructive effects over a long period, typically upon tedious exposure, occasionally lasting for the whole life of the exposed creature.

2.8.1 Objectives of toxicity testing of drugs originated from plants

The purpose of toxicology study of traditional medicine is to recognize contrary effects and to define dosage restrictions of contact level where potential effects happen. Dual noteworthy features which are engaged in the safety evaluation of every herbal drug are the nature and significance of the adverse effect. In addition, the maximum exposure level of the herbal drug and their effect are analysed.

Scrutiny of toxicity can disclose some intimidations that might be linked with the usage of medicinal plants, exclusively in subtle inhabitants. An equally important objective of toxicity investigation is the detection of toxic effect of plant extracts or compounds derived thereof in the early (pre-clinical) and late (clinical) stages during the drug discovery and development from plant sources. This will ease the documentation of various toxicants which can be discarded or altered during the process and create an opportunity for extensive assessment of harmless, promising alternatives for the toxic substances (Gamaniel, 2000). Modifications like structural or chemical group adjustments and dosage reduction may improve their tolerability.

2.9 NOCICEPTION

Nociceptive is also known as pain and it is experienced by everyone due to suffering of any kind of injury or localized inflammatory process. This pain is due to initiation of small diameter afferent nerve fibres that are sometimes called "nociceptive" nerve fibres. Nociception is a sub modality of somatic sensation that has been defined as an "emotional, unpleasant sensory and complex constellation of cognitive experiences that provoked by real or perceived tissue damage and manifested by psychological, behavioural reactions and certain autonomic" (Dublin and Patapautian, 2010).

Benefit of these kind of hostile sensations that helps us to recognize and diagnose different ailments depending up on the information of diverse reasons of pain to a discrete extent. Sensitivity to harmful provocations are important for the survival and well-being of living beings. Nociceptive stimulus voyages over redundant trails, guaranteeing the stimulus informs the individual to recover instantly. In extreme cases, there are patients lacking the ability to perceive pain due to hereditary neuropathies often maintain unrealized infections, self-mutilate, and have curtailed life spans (Axelrod and Hilz, 2003).

The high threshold physical and noxious chemical stimuli are detected by specialized peripheral sensory neurons called nociceptors. These sensory receptors can sense indications from injured tissue. They transduce a variety of noxious stimuli into receptor potentials, which in turn and initiate the action potential in pain nerve fibres.

The ethanol and aqueous extracts of *Pterocephalus hookeri* considerably improved the hot plate pain threshold and reduced acetic acid induced writhing response in mice. The ethanol and aqueous extracts remarkably inhibited the increase in vascular permeability induced by acetic acid and ear oedema induced by xylene which proved the analgesic activity. (Zhang, 2009)

2.9.1 Factors that Activate Nociceptors

Nociceptors counter when a stimulus reasons injury to tissue. The destruction of tissue fallouts in a discharge of a variety of substances from lysed cells as well as from new substances synthesized at the site of the injury. Some of these materials trigger the TRP channels which in turn initiate action potentials. These comprises substances like globulin and protein kinases, arachidonic acid, histamine, nerve growth factor (NGF), substance p (SP)

calcitonin gene-related peptide (CGRP), potassium - K^+. serotonin (5-HT), acetylcholine (ach), muscle spasm and lactic acid.

2.10 INFLAMMATION

Inflammation is a physiological response to a variety of stimuli such as infection and tissue injury. Injuries may be triggered in many ways such as physically (e.g., by temperature), mechanically (e.g., by compression or external bodies), by microorganisms (e.g., bacteria, virus, and parasites), chemically (e.g., by toxins, acidity and alkalinity) and by internal processes (e.g., uraemia). Inflammation frees the body from foreign matter and dispose the damaged cells and initiates wound healing.

Inflammation is controlled by mast cells that are in close proximity to autonomic nerves. This cells are the constituents of connective tissues having large granules that contain bradykinin, heparin, histamine and serotonin. These substances are released from the mast cell in response to injury and infection and it controls numerous processes involved during swelling.

There is another important pathway for triggering inflammation and is known as the arachidonic acid cascade which is mostly controlled by eicosanoids. Eicosanoids are local hormones that made from 20-carbon essential fatty acids (AA, DGLA and EFA), they are short-lived and can affect many aspects of the physiological function at the cellular level. Eicosanoids include all the thromboxanes, leukotrienes and prostaglandins. Depending on genetic as well as other factors that transform or controls the production of these inflammatory mediators such as prostaglandins, thromboxanes, and leukotrienes in response of any stimulus. Eicosanoids can initiate, regulate, and terminate all local inflammatory responses (Wichers and Maes, 2004).

Prolonged usage of antiinflammatory drugs are accompanying with serious hostile effects. Thus, the hunt for a novel and harmless painkilling and antiinflammatory drugs from herbs are ongoing globally.

Ethanolic extract of *Commiphora myrrha* inhibited the development of paw swelling induced by formalin significantly, which supports the traditional application of this herb in treating various diseases associated with inflammatory pain. (Su *et. al.*, 2010)

2.10.1 Types of Inflammation

i. Chronic Inflammation

Chronic inflammation ensues deprived of going through acute phase. The combination of fibroblasts, extracellular matrix and novel blood vessels is termed as granulation tissue. In the last portion of acute inflammation mononuclear predominance can be seen.

ii. Acute Inflammation

Acute inflammation is the early response of a tissue to an injury. This inflammation is nonspecific and can be evoked by any type of injury. It is the initial mark of defence beside an injury. Acute inflammation is characteristically of small interval, happening before the immune retort becomes recognized, and it is meant chiefly at eliminating the harmful agent.

2.11 PHARMACOLOGICAL REVIEWS

The GC-MS examination of the methanolic extract of leaves of *Actinodaphne bourdillonii* Gamble showed the occurrence of eighteen biologically vital compounds which indicated that it is a valuable tree with abundant medicinal properties and many bioactive principles (Deepa and Selvakumar, 2014)

Actinodaphne angustifolia leaves were used as anthelmintic and styptic, cardiovascular system stimulant. (Quattrocchi, 2016)

Roots and leaves of *Actinodaphne cupularis* Gamble were used for curing Hong Kong foot, burn and piles. Leaves infusion to promote digestion, lowering blood lipids, help clear the fats from the system, also a remedy for stomach-ache (Quattrocchi, 2016)

Dried *Actinodaphne lancifolia* (*Litsea coreana*) leaves are used to make 'Eagle Tea. Eagle tea is a strong antioxidant tea used for relief from heatstroke, detoxification and detumescence for hundreds of years in China and it also have anticancer, antidiabetic and antioxidant properties (Hao *et al.*, 2012). The root of *A. lancifolia* is used for curing of stomach-ache, arthritis and oedema in Chinese medicine. Isolancifolide is a compound extracted and isolated from *Actinodaphne lancifolia,* which is a traditional oriental medicine induces

apoptosis of HL-60 cells through both death receptor and mitochondria pathways, in caspase-8-dependent and -independent manners, suggesting that isolancifolide may be useful in anticancer strategies. (Salleh and Ahmad , 2016).

Antioxidant and anti-inflammatory actions of the essential oils of *Actinodaphne macrophylla* and *A. pruinosa* were examined by GC and GC-MS. The essential oil of the leaves of *A. macrophylla* was described by the occurrence of 31 components (78.2%), thru germacrene B and globulol as the major constituents. A whole of 28 constituents (71.6%) were categorized in the leaf oil of *A. pruinosa*. The antioxidant action was analysed by DPPH radical scavenging and total phenolic content, while anti-inflammatory action by lipoxygenase assay. The essential oil of A. pruinosa verified noteworthy action on DPPH, phenolic content and in the lipoxygenase assays. (Salleh and Ahmad, 2016).

The bark and leaves of *Actinodaphne pilosa* were applied for rheumatism, treating tuberculosis, relieving swelling, dispelling statis, relieving cough, and strokes. The bark of *Actinodaphne obovata* was used to treat fractures (Quattrocchi, 2016)

Prajapati *et al.* (2009) stated that leaves of *Actinodaphne hookeri* (Lauraceae) showed a persuasive antioxidant property and this plant was also given traditionally to treat diabetes and urinary disorders. This plant is a useful antioxidant and a free radical scavenging agent.

Deepa and Selvakumar (2015) reported that the phytochemicals existing in the methanolic extracts of leaves of *A. bourdillonii* possesses strong antifungal activity. In-vitro antifungal evaluation studies was carried out using agar well diffusion method against four fungal strains *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Candida tropicalis*.

Umesh *et al.* (2013) reported that phytochemical screening of methanolic and chloroform leaf extracts of Blumea lacera revealed the presence of various primary and secondary metabolites such as carbohydrates, alkaloids, phytosterols, steroids, phenolic compounds, tannins, flavonoids, cumarine, amino acids, terpenoids and saponins.

Santhoshkumar *et al.* (2014) reported that phytochemical evaluation of leaves of Boerhavia diffusa revealed the presence of alkaloids, flavonoids, phenols, tannins,

carbohydrates, saponins, glycosides, and proteins. The study showed antiinflammatory effects in both carrageenan induced inflammation and cotton pellet induced granuloma.

Ranjan *et al.* (2013) informed that aqueous and methanolic extracts of the leaves of Datura metel exposed to *In vitro* screening models such as DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity, total phenolic content, total antioxidant capacity and total antiinflammatory activity by HRBC (human red blood cell) membrane stabilization possess considerable antiinflammatory and antioxidant activity.

Sachin *et al.* (2014) investigated antiinflammatory activity of leaves of *Alternanthera brasiliana* using carrageenan induced paw oedema (acute), cotton pellet granuloma (sub-acute) and formaldehyde induced arthritis (chronic) method. The analgesic action of hydro-ethanolic extract of *Alternanthera brasiliana* leaves (HEAB) was studied by means of formalin induced paw licking. HEAB did not show any significant effect in early phase of formalin paw licking while it significantly decreased the paw licking in late phase at given doses which showed its significant antiinflammatory activity.

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

The antioxidant activity of *Actinodaphne .madraspatana* was assessed by many antioxidant assays, like DPPH radical scavenging, total antioxidant, hydrogen peroxide scavenging, reducing power and hydroxyl radical scavenging. Standard drug was ascorbic acid. The result revealed that the ethanolic extract of leaves of *A. madraspatana* has a potential effect as an antioxidant in all the tested in-vitro methods. (Saravanan *et al.*, 2016)

Ragesh *et.al.* (2017) reported that the aqueous extracts *T. akara* root possess considerably higher phenol and flavonoid content. The ethanolic fraction of *T. akara* root showed 86 % inhibition against DPPH radicals at 320 µg/ml concentration. Hexane and chloroform fractions presented comparatively less DPPH radical activity. In nitric oxide radical scavenging assay, all the extracts excluding hexane fraction showed effective activity against the free radicals and in superoxide radical scavenging assay the maximum activity was exhibited by the ethanolic fraction when compared to the standard ascorbic acid. The

antioxidant potential and free radical scavenging effects *of T. akara* may be attributed to the high polyphenolic content.

Anti-inflammatory and analgesic properties of the ethanolic extract of the rhizome of *Drynaria quercifolia* and its phytochemical profile was evaluated by (Anuja *et al.*, 2010). *Drynaria quercifolia* significantly lessened acute and delayed phases of formalin-induced pain and acetic acid-induced writhing episodes in mice. The analgesia was comparable to that produced by sodium salicylate and aspirin respectively. Phytochemical analysis gave positive tests for catechin, coumarins, flavonoids, phenolics, saponin, steroids, tannins, and triterpenes. The total phenolics in *Drynaria quercifolia* was 244 mg/g and naringin content was 0.048%. The results suggest the presence of potent anti-inflammatory and analgesic principles in *Drynaria quercifolia* that justifies its use for alleviating painful inflammatory conditions.

Anti-inflammatory and analgesic activities of ethanolic root extract of *Adhatoda vasica* Linn. (Acanthaceae) was evaluated by (Wahid *et al.*, 2010). The anti-inflammatory potential of ethanolic extract has been determined by using carrageenan-induced paw oedema assay, formalin-induced paw oedema in Wistar albino rats. The analgesic activity was tested by using acetic acid-induced writhing response, hot plate method and tail flick method in albino rats. The administration of extract at doses of 200 and 400 mg/kg, by orally it has significantly inhibited inflammation on both carrageenan and formalin-induced inflammation and also significantly inhibited antinociceptive effect in the acetic acid-induced writhing, formalin-induced pain licking and hot-plate-induced pain.

Sowemimo *et al,* (2013) showed that extract of *B. maderaspatensis* in carrageenan-induced test caused a significant inhibition (84.5%, 90 min) of paw edema at a dose of 75 mg/kg while the xylene-induced test caused a significant inhibition (62.65%) at 50 mg/kg. The histamine-induced test showed significant inhibition (90.9%, 90 min) while serotonin-induced test showed moderate inhibition (54.10%, 180 min). These results collectively demonstrate that the ethanol extract of *B. maderaspatensis* possesses anti-inflammatory and anti-nociceptive properties, and this supports the ethnopharmacological use of the plant in the treatment of inflammation.

3. MATERIALS AND METHODS

The present study entitled "Scientific validation of antiinflammatory, antinociceptive, and antioxidant potential of 'malavirinji' (*Actinodaphne bourdillonii* Gamble)" was carried out at the Ethnomedicine and ethnopharmacology division of Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, during 2016-2017 academic year.

3.1 MATERIALS

3.1.1 Sample collection

The leaves of *Actinodaphne bourdillonii Gamble* were collected from JNTBGRI premises and various other localities of Thiruvananthapuram and Pathanamthitta districts and was taxonomically identified by DR. E. S. Santhosh Kumar, Technical Officer, JNTBGRI. The specimens of the plant material were deposited in the herbarium collection of the JNTBGRI (TBGT 86896/dated 31/7/2017).

3.1.2 Preparation of extract

The plant leaves were detached and wash away with distilled water and then dried in shade for nearly 14 days. Using mixer grinder (Butterfly mixer grinder) the dried leaves were pulverised to become fine powder and lay open to soxhlet extraction for 24 hours with 99% ethanol. The mixture was vanished to aridness by means of a rotary flash evaporator (Buchi, rotavapor R – 210) and kept in freezer. The crude extract was used for examination.

3.1.2 Experimental animals

Wistar rats (150 to 220g) and Swiss Albino Mice (20-25 g), either of sex, were acquired from animal house of Jawaharlal Nehru Tropical Botanical Garden Research Institute, Palode. They were grouped and housed in Poly-acrylic cages (six animals per cage) and maintained under standard laboratory environments (temperature 24° C, relative humidity of 55-65% and 12-hour dark- light cycles). They were fed thrice in a day with water and branded rat feed (Lipton India Ltd, Mumbai, India). Every animal experimentations were done conferring to the NIH recommendations and after receiving the sanction from Institutes Animal Ethics Committee (B/13/12/2016/EM & EP-09-12)

3.1.3 Chemicals and Equipment's

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

3.2 PHYTOCHEMICAL TESTS

3.2.1 Preliminary Phytochemical Screening

The preliminary phytochemical screening tests were determined by the method described by (Harborne., 1998) and test were conducted in drug concentration 5 mg/mL.

1. Detection of Alkaloids

A) Wagner's Test

The 1mL of the extract was taken in the test tube and few drops of Wagner's reagent was added. The formation of a reddish brown precipitate indicates the occurrence of alkaloids.

B) Hager's Test

1mL of the extract was taken in a test tube and few drops of Hager's reagent was added. The formation of yellow colour shows the occurrence of alkaloids.

C) Meyer's Test

1mL of the extract was taken in a test tube and few drops of Meyer's reagent was added. The formation of yellow or cream precipitate indicates the presence.

2. Detection of Flavonoids

A) Shinoda Test

1mL of the extract was taken in a test tube. Add a pinch of Magnesium turnings and add 1-2 drops of concentrated HC into it. The formation of pink colour indicates the occurrence of flavonoids.

B) Lead Acetate Test

1mL of the extract was taken in a test tube and added a few drops of 10 % 1M lead acetate solution. The formation of yellow precipitate indicates the occurrence of flavonoids.

C) Alkaline Test

1mL of the extract was taken in a test tube and added few drops diluted HCl. Observe the yellow colour which turns colourless of adding few drops of diluted HCl.

3. Detection of Phenols

Lead Acetate Test

1mL of the extract was taken in a test tube and then added 3 mL of distilled water, few drops of 1 % lead acetate was added, observe the yellowish cream precipitate.

4. Detection of Tannins

Braymer's Test

1mL of the extract was taken in a test tube and 1mL water and 2-3 drop ferric chloride was added. The formation of green precipitate shows the presence of tannins

5. Detection of Saponins

Froth Test

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

Foam Test

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

6. Detection of Steroids

Salkowski test

1mL of the extract was taken in a test tube and added 1mL of chloroform and sulphuric acid along the sides of test tube. Observe the formation of the reddish brown ring at the junction which indicates the occurrence of steroids.

7. **Detection of Protein**

Xanthoproteic test

1mL of the extract was taken in a test tube and added 1mL sulphuric acid. The formation of white precipitate shows the presence of protein.

Biuret test

1mL of the extract was taken in a test tube and added with 10% sodium hydroxide and is heated. After heating add 0.7% of copper sulphate. Formation of purplish violet colour was observed which indicates the occurrence of protein.

8. **Detection of Carbohydrates**

Fehling's Test

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

9. **Detection of Glycosides**

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

10. **Detection of Phlobatanins**

1mL of the extract was taken in a test tube and then added 1mL of dil. HCl and heat. Formation of red precipitate indicates the presence of Phlobatanin.

3.4 ANTI-OXIDANT STUDIES

3.4.1 Total Phenolic Content

Total contents of phenolics were determined by the method of (Singleton *et al.*, 1999) with slight modification. Plant extract solution (0.2mL) is added to 2.5mLof Folin–Ciocalteu phenol reagent (1:50 v/v) was added and vigorously shaken. After 10 minutes' incubation, 2 mL of 7.5% w/v Sodium carbonate was added and the mixture was shaken repeated thoroughly. After 30 min of incubation, the absorbance of the mixture was measure

at 743 nm using a spectrophotometer. The concentration of polyphenols in the samples derived from a standard curve of Gallic acid ranging from 5 to 50µg/mL. The contents of total phenolics were expressed as milligrammes of Gallic acid equivalents per gram of dry extract.

3.4.2 Total Flavonoid Content

Total contents of flavonoids were determined by the modified method of (Zhishen *et al.*, 1999). Plant extract 0.5mL were added to 1.5mL of 95% methanol, 0.1mL 10% Aluminium chloride (w/v), 0.1mL 1M potassium acetate and 2.8mL DD H_2O, mixed, and vigorously shaken for 30 min. Then the absorbance was measured at 415 nm using a spectrophotometer. The contents of total flavonoids were expressed as milligrams of Quercetin equivalent (QE)/ g dry extract.

3.4.3 DPPH free radical scavenging assay

The DPPH radical (1, 1-diphenil-2- picrylhydrazyl) was determined by the method described by (Blois, 1958). A stock solution of ethanolic leaf extract of PR and L-Ascorbic acid as standard was prepared in the concentration of 1 mg/mL. Varying concentration from 50 to 250 µg/mL of plant extract and L-Ascorbic acid was taken and transferred to tubes. Add 200 µL by methanol and 2mL DPPH (0.02mM) and incubated for 20 minutes at dark at room temperature and absorbance was measured at 517 nm by spectrophotometer (Agilent Technologies). The annihilation activity of free radicals was calculated in percentage of scavenging according to the following formula given below:

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

3.4.4 Nitric Oxide Scavenging Activity

The nitric oxide radical scavenging activity of ethanolic leaf extract of PR was investigated according to the method of (Marconii *et al.*, 1994). L- ascorbic acid was used as standard. A stock solution of ethanolic leaf extract of PR and L-Ascorbic acid as standard was prepared in the concentration of 1 mg/mL. 1mL of leaf extract and standard solution at different concentrations (50 to 250 µg/mL) was added into the different test tubes. 1 mL of SNP solution (10mM) was added and the tubes were incubated at 29°C for 2.5 hrs. 1mL of the incubated solution was taken and diluted with 1mL Griess reagent [1% sulfanilamide in 2% H_3PO_4 and

0.1% N-(1-Naphthyl) ethylenediamine dihydrochloride]. The absorbance was measured in 532nm by spectrophotometer (Agilent Technologies). Percentage inhibition is calculated according to the following formula,

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

3.4.5 Hydroxyl Radical Scavenging Activity

Hydroxyl Radical Scavenging Activity was investigated by using the colorimetric deoxyribose method (Elizabeth *et al.*, 1990). The reaction mixture contained in a final volume of 5mL, 100µL 2-deoxyribose (2.8mM); KH_2PO_4.KOH buffer (20mM pH 7.4); 100µL $FeCl_3$ (100µM); 100µL EDTA (100µM); 100µL H_2O_2 (1.0mM); 100µL Ascorbic acid (100µM); and concentrations (50 to 250µg/mL) of the leaf extract and standard compound. After incubation for one hour at 37^0C, 0.5mL of the reaction mixture was added to 1.5mL of 2.8% of TCA, then 1.5mL of 1% aqueous TBA was added and the mixture was incubated at 100^0C for 15 minutes to develop the colour. After cooling, the absorbance was measured at 530 nm against an appropriate blank solution. Quercetin was used as a positive control. Percentage of scavenging was evaluated by comparing the test and the blank solution.

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

3.4.6 Ferric reducing antioxidant potential (FRAP) assay

The capability of plant extract to reduce the ferric ion was measured using the altered version of the method defined by (Benzie and Strain, 1996). 200 µL of plant extract/serial fractions (mg/mL), was added to 3 mL of FRAP reagent (10 part 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM- 2,4,6-tripyridyl-s-triazine (TPTZ) solution and one part 20 mM- FeCl3 6H2O solution) and the reaction mixture was incubated in a water bath at 37°C for 30 min. The absorbance was measured at 593 nm. The antioxidant capacity of the plant extracts was calculated from the calibration curve of Trolox and expressed as µmol Trolox equivalent/g of extract.

3.4.7 Estimation of superoxide radical scavenging activity

The effect of superoxide radical scavenging activity was analysed by the Nitroblue tetrazolium reduction method defined by Fu *et al.*, (2010). 1 mL of NBT solution (156 µM NBT in 100 mM phosphate buffer, pH 7.4), 1 mL of NADH solution (468 µM NADH in 100 mM phosphate buffer, pH 7.4) and 0.4 mL of different concentrations of the plant extract/serial fractions were mixed. The reaction was started by adding 100 µL of phenazine methosulphate (PMS) solution (60 µM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560 nm was measured against blank samples, containing all the reagents except the PMS. All readings were taken in triplicate and Trolox was used as the standard. Percentage of inhibition was calculated using the formula

[1 - (Absorbance of sample/absorbance of control)] × 100.

3.5 Oral Acute Toxicity Study

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

3.6 ANTI-INFLAMMATORY STUDIES

3.6.1 Carrageenan induces paw oedema

Anti-inflammatory activity of EAB will be investigated by the Carrageenan-induced paw oedema method described by (Winter *et al.*, 1962). Wistar rats were divided and categorized into five groups of six animals each. Group A, the control group will receive 1 mL Dist. H2O, Group B will receive 1 mL Indomethacin (10 mg/kg, in D. H2O, p.o), Groups C, D and E will receive various concentrations of the 1 mL of EAB (50, 150 and 450 mg/kg, p.o).

After 30 min animals of all the groups were injected with 0.1 mL of 1% carrageenan in physiological saline in the right-hand foot under the plantar aponeurosis. The hind paw volume was calculated using plethysmometer just before and 3h after carrageenan injection (Plate 2). The change in the paw volumes will be recorded, which indicates the degree of inflammation. The Percent inhibition of induced paw oedema was calculated using the formula given below:

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

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

Plate 2: Measurement of paw volume using Plethysmometer

3.6.2 Formalin-induced paw oedema

Anti-inflammatory potential of EAB was studied by the formalin- induced paw oedema method (Chau, 1989) with slight modification. Wistar rats were categorized into five groups of six animals each. Group A, the control group will receive 1 mL Dist. H2O, Group B will receive 1 mL Indomethacin (10 mg/kg, in D. H2O, p.o), Groups C, D and E will receive various concentrations of the 1 mL of EAB (50, 150 and 450 mg/kg, p.o). After 30 min animals of all the groups were injected with 0.1mL of 2% of formalin per 100g in the right-hand foot under the plantar aponeurosis. The drug was administered once a day for 7 days. On the first and seventh day, after 1hr of drug administration paw volume was measured using a

plethysmometer. The difference in paw thickness before and after induction of inflammation was calculated and presented as a mean increase in paw volume (mm). The difference in the paw volumes will be recorded, which indicates the degree of inflammation. The percent inhibition of paw oedema was calculated using the formula given below:

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

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

3.7 ANTINOCICEPTIVE STUDIES

3.7.1 Acetic acid-induced writhing test

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

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

3.7.2 Eddy's Hot plate test

The thermal pain sensitivity was investigated to measure latency to paw licking by the method of (Eddy and Leimbach, 1953) hot plate. Swiss Albino mice were separated and categorized into 5 groups of six animals each. Group A, the control group will receive 0.5 mL Dist. H_2O, Group B will receive 0.5 mL Aspirin (10 mg/kg, in D. H_2O, p.o), Groups C, D and E will receive various concentrations of the 0.5 mL of EAB (50, 150 and 450 mg/kg, p.o). The

temperature of the plate (Lyzer, Analgesometer type III) was fixed at 55 ± 10°C. The latency of nociceptive response such as licking of any of the hind legs or forelegs, jumping or tapping was recorded at 0, 15, 30, 60, 90, 120 min after drug administrations. The cut off time was put at 20 seconds to avoid tissue damage. When 30 sec elapsed without the animal presenting any of the response, it was removed from the plate. The prolongation of the latency times comparing the values before and after administration of the test compounds.

3.8 Statistical analysis

Results were expressed as mean ± standard error of mean and presented as graphs and tables. Data were analysed using a statistical programme SPSS/PC+, Version 20.0 (SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) utilized for comparison of significant differences among group investigated. Pair-fed comparisons between these groups was made by Duncan's. Results were considered significant at $p<0.05$.

4. RESULTS

4.1 Preliminary Phytochemical Screening

Several solvent extracts have displayed the existence of diverse compounds in (Table1).

Table 1: Preliminary phytochemical screening of leaf extract of *A. bourdillonii*

Sl. No	Compounds	Acetone	Chloroform	Ethanol	Ethyl Acetate	Hexane	Hydroethanol	Water
1	Phenols	+	+	+	+	+	+	-
2	Flavonoids	-	+	+	-	-	+	+
3	Steroids	-	-	+	-	-	+	-
4	Alkaloids	+	-	+	-+	+	-	+
5	Carbohydrates	+	+	-	+	-	+	+
6	Tannins	+	+	+	+	-	-	-
7	Saponins	+	+	+	+	+	+	+
8	Glycosides	+	+	+	+	-	+	+
9	Phlobatannin	–	-	+	+	-	-	+
10	Proteins	+	-	-	-	-	-	-

+ present and – absent

4.2 Oral acute toxicity study

Acute toxicity study of the aqueous extract of *Actinodaphne bourdillonii Gamble* administered in mice orally. It doesn't show any mortality at the highest dose of 1600 mg / kg weight of the animal. And other parameters indicating toxicity of the drug such as Digestion, food intake, fatigue, behavioural pattern etc. of test animals observed and the result tabulated on the (Table 2).

Table 2: Cage side observation of acute oral toxicity studies of ethanolic extract of leaves of *A. bourdillonii*.

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

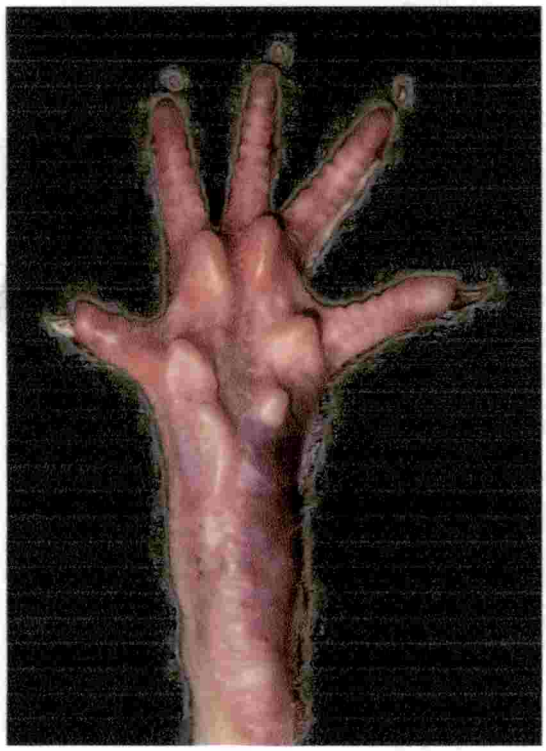
11	Condition of teeth	Normal	Normal	Normal	Normal
12	Breathing abnormalities	Nil	Nil	Nil	Nil
13	Gait	Normal	Normal	Normal	Normal

*Four animals per group (Groups of four)

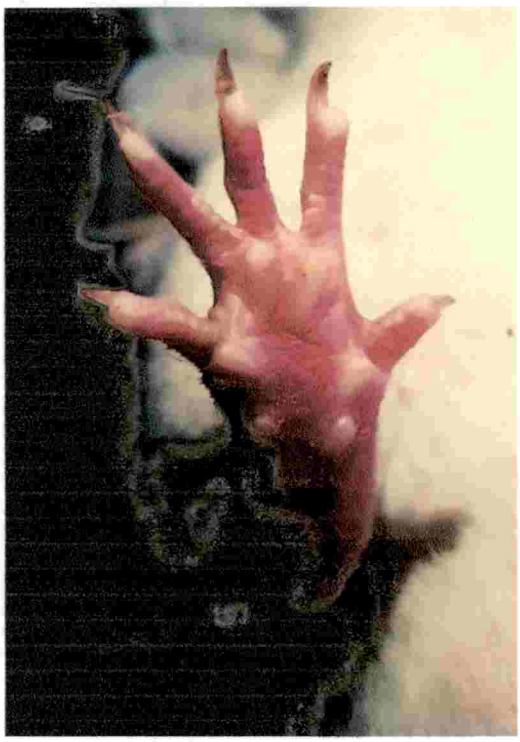
4.3 ANTIINFLAMMATORY STUDIES

Difference in the paw before and after the induction of inflammation are presented in the plate 3.

Plate 3: Difference in the paw before (a) and after (b) the induction of inflammation



(a)



(b)

4.3.1 Carrageenan Induced Paw Oedema

Carrageenan induced inflammation is a biphasic incident with early hyperaemia due to the release of histamine, serotonin and the delayed oedema due to the release of bradykinin and prostaglandin. Effect of ethanolic extract of leaves *of A. bourdillonii* on carrageenan induced paw oedema in rats are presented in the (Fig 4). At a dose of 450 mg/kg, given the maximum percentage of inhibition in rat's paw oedema obtained was 83.63%. Indomethacin exerted a major protective role by inducing a protection of 85.69% at a dose of 10mg/kg body weight.

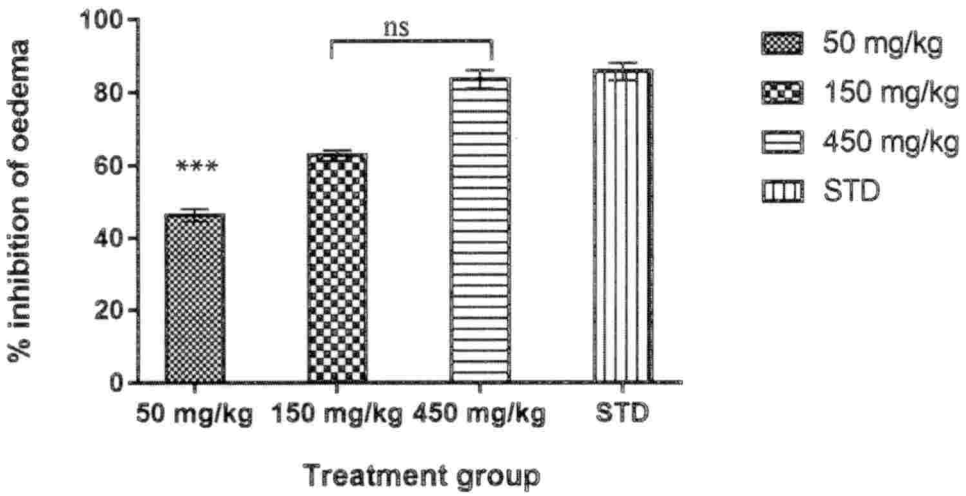
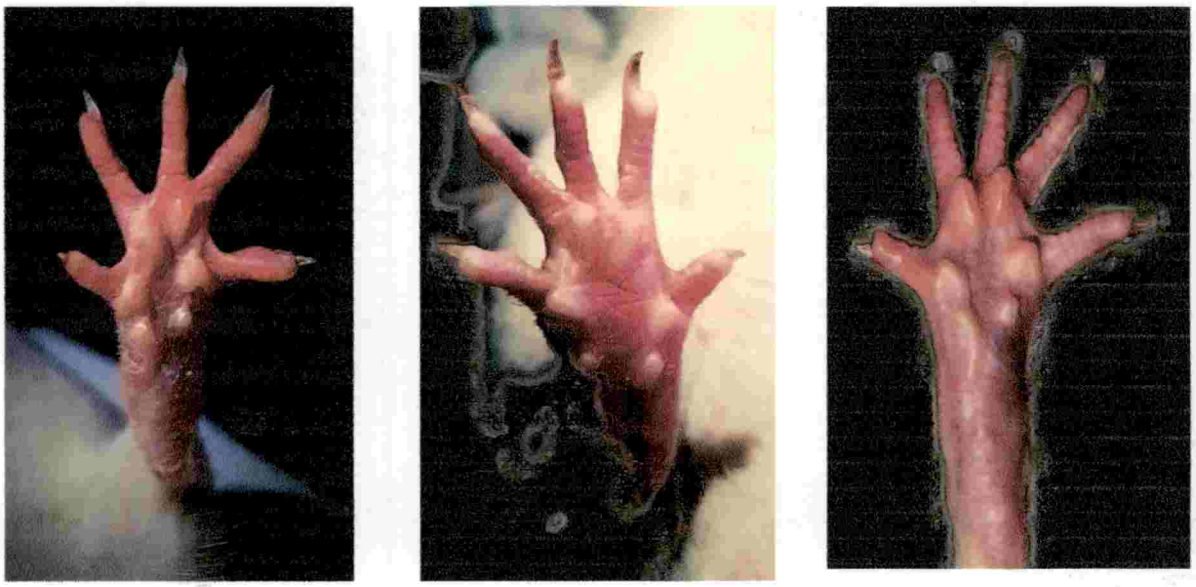


Fig 4: Effect of EAB on carragenan induced paw oedema in Wistar rats.

Values are expressed as mean±SD, n=6, one-way ANOVA followed by Dunnett's multiple comparison test, $P<0.01$, ns= no significant difference and ***= significant difference when compared to standard.

Plate 4: Carrageenan induced antiinflammatory studies



(a) **(b)** **(c)**

(a) =Normal control, (b) = carrageenan control, (c) = EAB 450 mg/kg

4.3.2 Formalin Induced Hind Paw Oedema

Effect of ethanolic leaf extract of *A. bourdillonii* on formalin induced paw oedema in rats are presented in the (Fig 5). At a dose of 450 mg/kg of the ethanolic leaves extract *A. bourdillonii* reduced the inflammation in rat compared to other doses and the percentage of inhibition obtained was 70.23% and 78.36% at day1 (early phase) and day7 (late phase) respectively. While Indomethacin exerted a major protective effect by inducing a protection of 73.22 and 80.23% at a dose of 10mg/kg body weight.

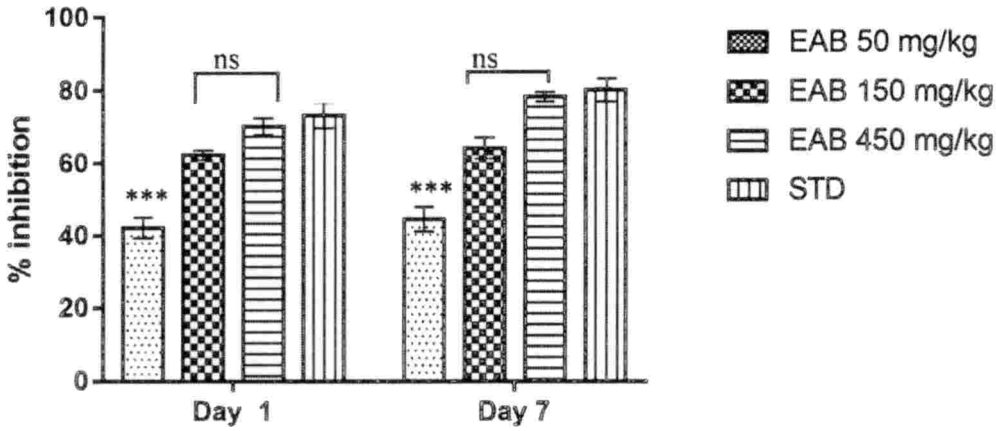


Fig 5 : Effect of EAB on formalin induced paw oedema.

Values are expressed as mean±SD, n=6, one-way ANOVA followed by Dunnett's multiple comparison test, P<0.01, ns= no significant difference and ***= significant difference when compared to standard control.

4.4 ANTI NOCICEPTIVE STUDIES

4.4.1 Acetic Acid Induced Writhing

Effect of ethanolic extract of leaves *of A. bourdillonii* on acetic acid induced writhing was investigated on mice and the values were presented in the (Fig 6). The maximum percentage of inhibition of constrictions was 87.76% observed at 450 mg/kg drug dose which is almost comparable to the standard drug Acetyl salicylic acid (10 mg/kg) with 88.73% inhibition. At this dose the mean number of the writhes was significantly lower than that of control group. Even though EAB 150 mg/kg dose exhibited 76.22% of writhing inhibition in animals, EAB 50mg/kg showed an inhibition of 58.62%.

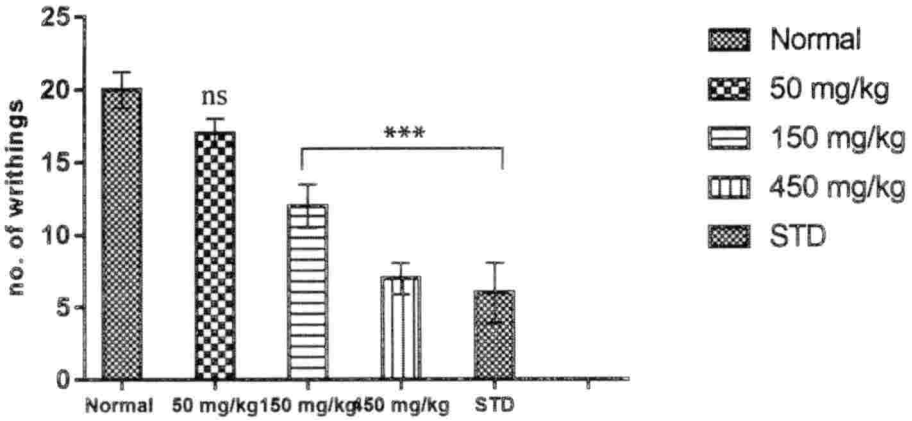


Fig 6: Acetic acid Induced writhing.

Values are expressed as mean±SD, n=6, one-way ANOVA followed by Dunnett's multiple comparison test, P<0.01, ns= no significant difference and ***= significant difference when compared to standard control.

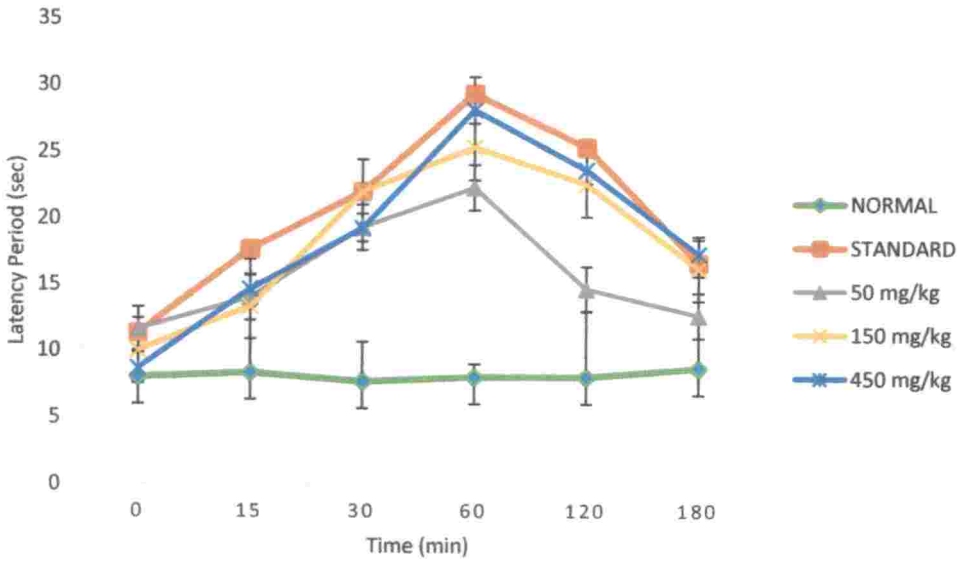
Table 3: Acetic acid induced writhing – percentage of inhibition analysis

Group	% inhibition
Normal	-
Standard	88.73
EAB 50mg/kg	58.62
EAB 150mg/kg	76.22
EAB 450mg/kg	87.76

4.4.2 Eddy's Hot plate method

Effect of ethanolic leaf extract of *A. bourdillonii* on Eddy's hot plate method are presented in the (Fig 7). At a dose of EAB 450 mg/kg given the maximum the percentage of inhibition obtained was 71.55% and the mean number of paw licking was meaningfully lower than that of other groups including control and standard group. Acetyl salicylic acid exerted a major protective role by inducing a protection of 72.72% at a dose of 10mg/kg body weight.

Fig 7: Eddy's hotplate analysis



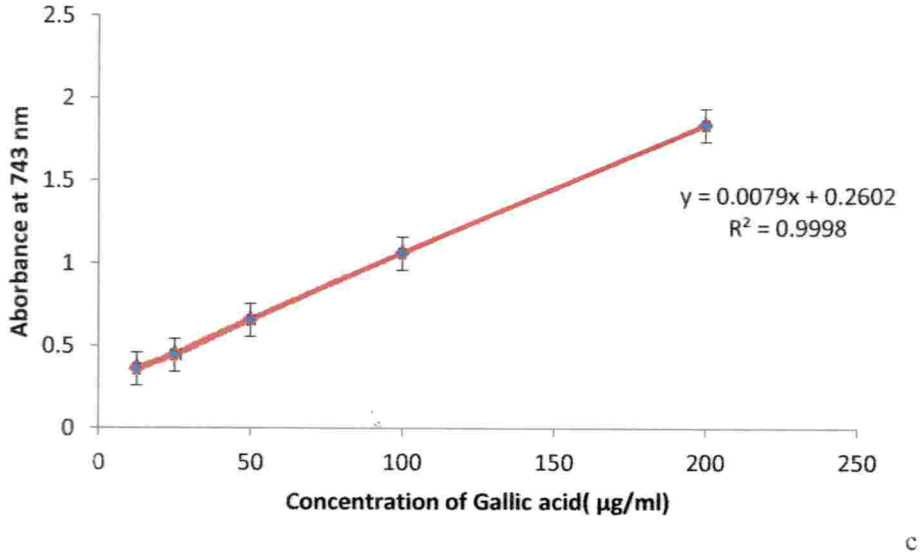
4.5 ANTIOXIDANT STUDIES

In vitro Antioxidant studies

Comparative in vitro antioxidant studies of ethanolic extract of *A. bourdillonii* were done using standard procedures and the outcomes are given below.

4.5.1 Total phenolic content

The total phenolic content of ethanolic extract of leaf of *A. bourdillonii* was found to be 51.84 µg GAE / mg mass of extract (Table 4) and the graph were plotted in fig 1.



c

Fig 8: Standard graph of Total phenolics.

Table 4: Determination of total phenolic content in *A. bourdillonii*leaf extract

Conc. of Gallic acid (µg/mL)	**Mean Absorbance (743 nm)**
12.5	0.362 ± 0.006
25	0.446 ± 0.004
50	0.659 ± 0.006
100	1.064 ± 0.004
200	1.839 ± 0.002
Sample:	
Ethanolic extract EAB (500µg/mL)	0.465 ± 0.0456

The TPC of ethanol extract of *A. bourdillonii* = **51.84µg** GAE / mg dry extract.

4.5.2 Total flavonoid Content

The total flavonoid content present in the extraction/fraction is calculated from the standard curve of quercetin and expressed as Quercetin equivalents (QE) in µg/mg of dry extract. From the standard calibration graph, the total Flavonoid content of the plant ethanolic leaf extract of *A. bourdillonii* was found that in 500µl sample contains 30.77 µg QE / mg mass of extract (Table 4) and the graph were plotted in (Fig 9)

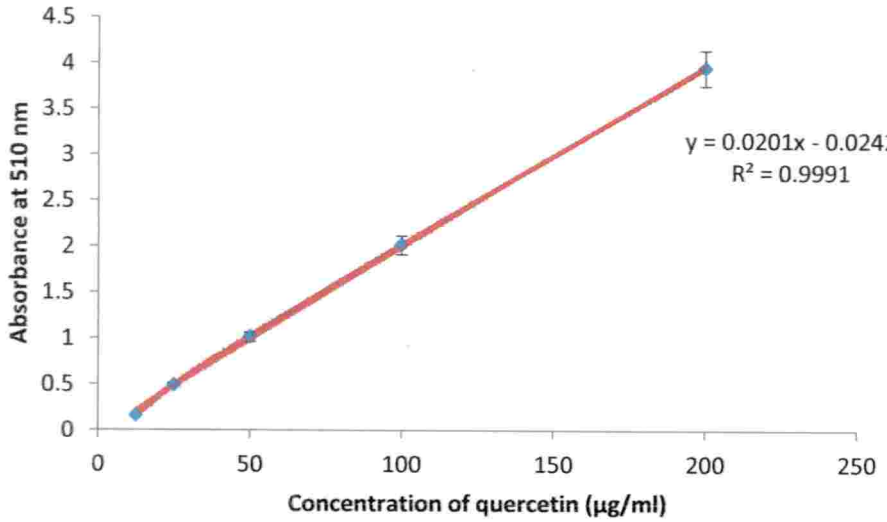


Fig 9: Standard graph of Total flavonoids.

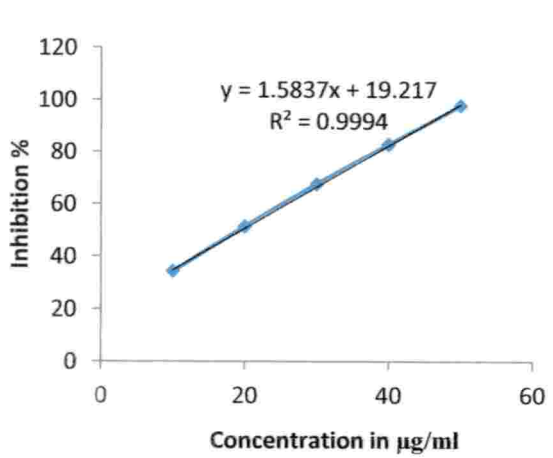
Table 5: Determination of total flavonoid content in *A. bourdillonii* leaf extract

Conc. Of Quercetin (µg/mL)	**Mean Absorbance (415 nm)**
12.5	0.1651 ± 0.01
25	0.4912 ± 0.002
50	1.0256 ± 0.001732
100	2.0234± 0.001528
200	3.9645± 0.00347
Sample:	
Ethanolic Extract EAB (500 µg/mL)	0.285 ± 0.007802

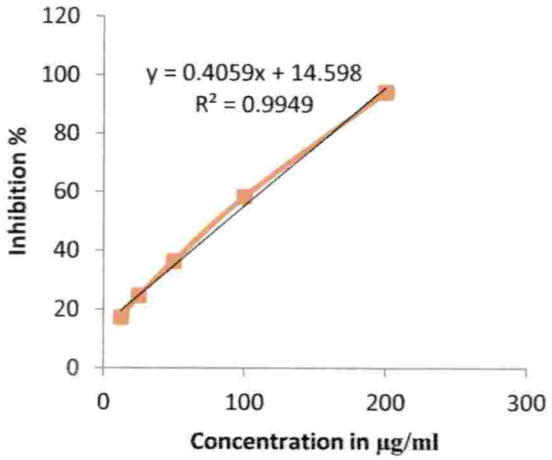
The TFC of ethanol extract = **30.77** µg QE / mg dry extract

4.5.3 DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of the ethanolic leaf extract of *A. bourdillonii* was shown in table. The results were compared with that of L-Ascorbate. IC_{50} value was determined from the calibration curve (Fig 10). The lower IC_{50} value reflects to higher antioxidant activity of plant extract.



(a)



(b)

Fig 10: Effect of L-Ascorbic acid (a) and EAB (b) on DPPH Radial scavenging activity

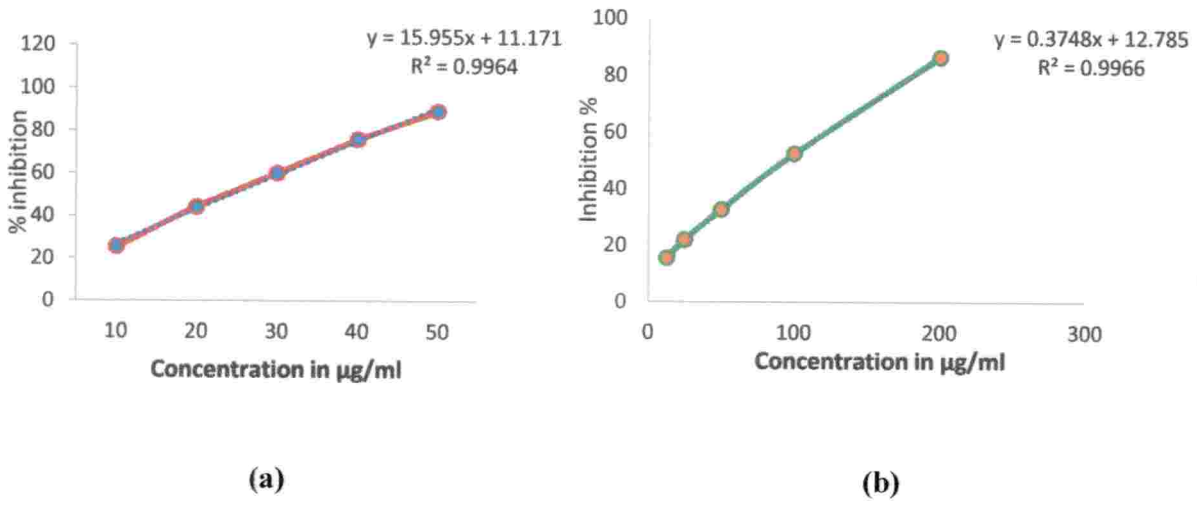
Table 6: Effect of L-Ascorbic acid and ethanolic extract of leaves *of A. bourdillonii* on DPPH Radical Scavenging Activity.

Sl. No	Conc. of ascorbic acid (AA- µg/mL)	Percentage of Scavenging	IC_{50} Value	Conc. of EAB	Percentage of Scavenging	IC_{50} Value
1	10 µg/mL	35.7 %	**19.05 µg/mL**	12.5 µg/mL	17.18 %	**87.23 µg/mL**
2	20 µg/mL	52.23 %		25 µg/mL	24.56 %	
3	30 µg/mL	68.52%		50 µg/mL	36.23 %	
4	40 µg/mL	82.74 %		100 µg/mL	58.18 %	
5	50 µg/mL	96.79 %		200 µg/mL	94.13 %	

Values are the mean ± SD, n = 6.

4.5.4 Nitric oxide (NO) scavenging activity

The Nitric oxide radical scavenging action of the ethanolic extract of leaves of *A. bourdillonii* was given away in Fig 11. The results were compared with that of L-Ascorbate. IC_{50} value was determined from the calibration curve (Table 7).



(a) **(b)**

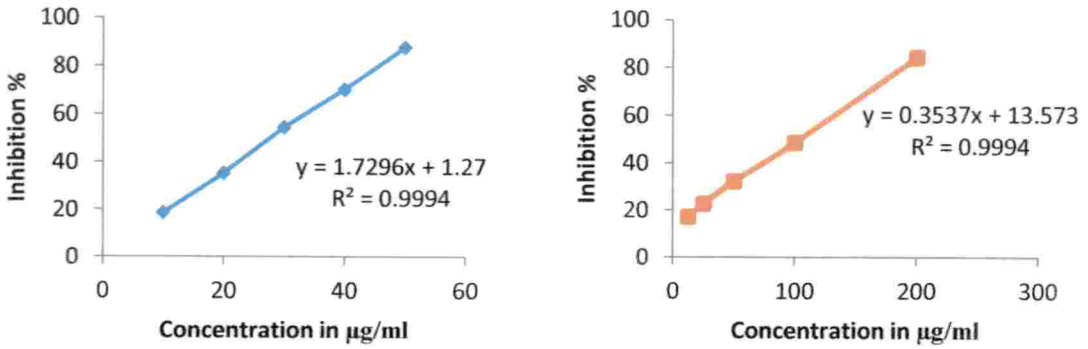
Fig 11: Effect of L-Ascorbic acid (a) and EAB (b) on Nitric Oxide Scavenging Assay

Table 7: Effect of L-Ascorbic acid and ethanolic leaf extract of *A. bourdillonii* on Nitric Oxide Scavenging Activity

Sl. No	Conc. of ascorbic acid (AA- µg/mL)	Percentage of Scavenging	IC_{50} Value	Concentration of EAB	Percentage of Scavenging	IC_{50} Value
1	10 µg/mL	25.46%	**24.33 µg/mL**	EAB12.5µg/mL	15.56%	**99.29 µg/mL**
2	20 µg/mL	44.33%		EAB 25 µg/mL	21.98%	
3	30 µg/mL	60.09%		EAB 50 µg/Ml	32.67%	
4	40 µg/mL	76.10%		EAB 100 µg/mL	52.44%	
5	50 µg/mL	89.30%		EAB 200 µg/mL	86.52%	

4.5.5 Hydroxyl radical scavenging assay

The Hydroxyl radical scavenging activity of the ethanolic extract of leaves of *A. bourdillonii* was shown in Table 8. The results were compared with that of L-Ascorbate. IC_{50} value was determined from the calibration curve (Fig 12)



(a) **(b)**

Fig 12: Effect of L-Ascorbic acid (a) and EAB (b) on Hydroxyl radical scavenging assay.

Table 8: Effect of L-Ascorbate and ethanolic leaf extract of *A. bourdillonii* on Hydroxyl radical scavenging activity.

Sl. No	Conc. of ascorbic acid (AA-µg/mL)	Percentage of Scavenging (517nm)	IC_{50} Value	Concentration of EAB	Percentage of Scavenging (517nm)	IC_{50} Value
1	10 µg/mL	18.52 %	**28.17 µg/mL**	12.5 µg/mL	17.15%	**102.98 µg/mL**
2	20 µg/mL	35.22 %		25 µg/mL	22.65%	
3	30 µg/mL	54.34 %		50 µg/mL	32.25%	
4	40 µg/mL	70.22%		100 µg/mL	48.63%	
5	50 µg/mL	87.5 %		200 µg/mL	84.35%	

Values are the mean ± SD, n = 6.

4.5.6 Total antioxidant activity

Total antioxidant action of the extracts was estimated via phosphomolybedenum process and the results were quantitatively expressed as of ascorbic acid equivalents/g of dry weight. Total antioxidant activity was calculated from the calibration curve of ascorbic acid. Total antioxidant activity of EAB was found to be 151.35 ug of ascorbic acid equivalent/mg of dry extract respectively.

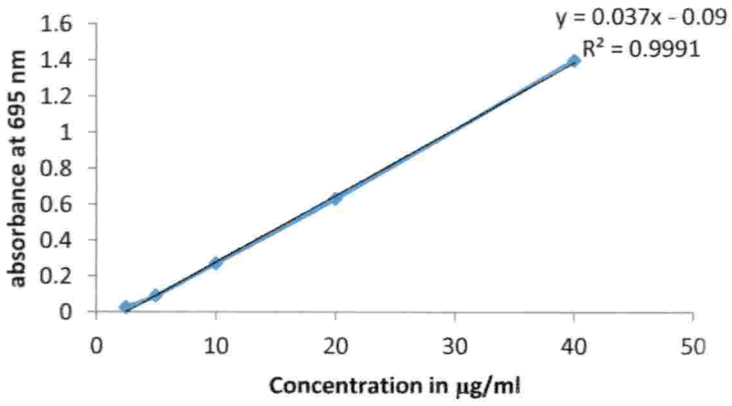
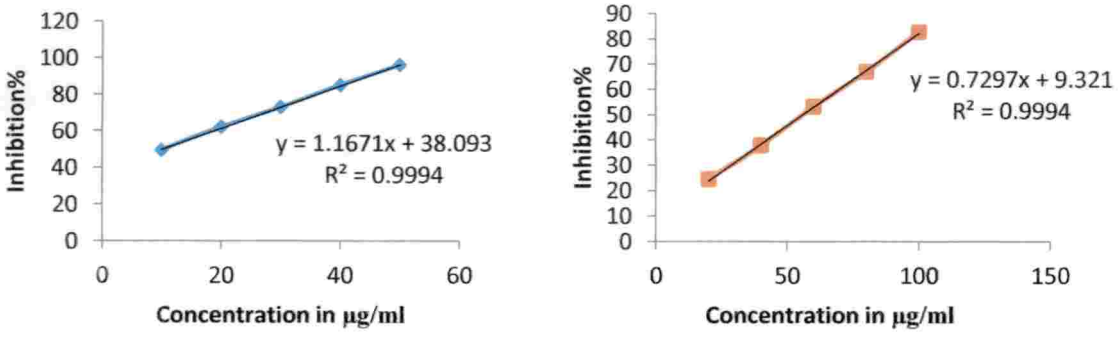


Fig 13: Total antioxidant activity of Ethnolic extract of *A. bourdillonii.*

4.4.7 Superoxide radical scavenging activity

The superoxide radical scavenging activity of EAB was determined by the nitroblue tetrazolium reduction method and represented in the (Fig 14). EAB showed superoxide radical scavenging of 75% at 450 mg/kg and the EC50 value was found to be 55.11 µg/mg compared to the standard Ascorbic acid used, which showed an EC50 value of 10.12 µg/mg.



(a) **(b)**

Fig 14: Superoxide radical scavenging activity of Ascorbic acid (a) and EAB (b)

4.5.8 Ferric reducing antioxidant potential (FRAP) assay

The ability of plant extract to reduce the ferric ion was determined from the standard graph drawn from known concentrations of Trolox (Fig 15) and expressed as 95.17µg of trolox equivalent/mg of EAB.

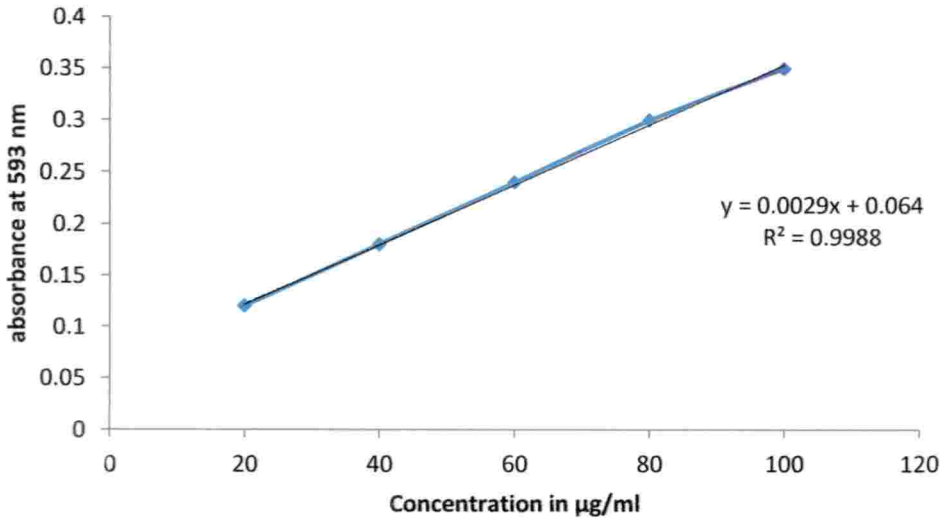


Fig 15: Ferric reducing antioxidant potential (Frap) assay Standard Curve.

5. DISCUSSION

Preliminary Phytochemical Screening

Traditional ethnic treatment through wild medicinal plants has always steered researchers to quest for novel medications to improve healthy life for human beings and animals. Phytochemical assessment of herb is vital to learning the pharmacological actions. Modern practices are used by WHO to safeguard quality of therapeutic herbal products. (Shinde, 2009) Due to the importance in the above context, such preliminary phytochemical screening of plants is the prerequisite in order to discover and develop novel therapeutic agents with enhanced effectiveness. (Rocha, 2010)

Preliminary phytochemical screening of leaves of *Actinodaphne bourdillonii* Gamble which revealed that it is a rich source of mineral elements which contains, calcium (40.8 ppm), potassium (33.05 ppm), magnesium (22.61 ppm), iron (5.206 ppm), zinc (1.966 ppm), copper (2.099 ppm) and Na (8 ppm) (Deepa and Selvakumar, 2014). Calcium is indispensable for growing and preserving well teeth and bones and helps in blood clotting, oxygen transport, contraction of muscles, nerve conduction, enzyme activity and cellular secretion of fluids. (Dosunmu, 1997) which may be the basis for the traditional use of *A. bourdillonii* for bone repairing.

Considerable amount of these macro elements present in *A. bourdillonii* demonstrates its efficiency in curing various mineral deficiency related disorders. The phytochemical study also shown the existence of alkaloids, tannins, saponins, cardiac glycosides, phlobatannins and terpenoids in the methanolic extract of leaves of *A. bourdillonii.* (Deepa and Selvakumar, 2014). They may be responsible for the pharmacological activities like antioxidant, analgesic and anti-inflammatory effects.

ANTI-OXIDANT STUDIES

Antioxidants can act as highly effective free radical scavengers due to their redox potential, which can play an important role in adsorbing and neutralizing free radicals produced in our body by quenching singlet and triplet oxygen or decomposing peroxides (Lobo, *et al.*, 2010).

The occurrence of antioxidants such as phenolics, flavonoids, tannins and proanthocyanidins in plants may deliver defence counter to a number of diseases; for example, ingestion of natural antioxidants has been contrariwise related with morbidity and mortality from degenerative conditions. Phenolic compounds are one of the influential chain breaking antioxidants (Adithya, 2012). Phenolics constitute is one of the major groups of compounds acting as main antioxidants or free radical terminators hence it was reasonable to detect their amount in the crude extracts (Padmanabhan and Jangle., 2012). The content of total phenols in ethanolic extract of *Actinodaphne bourdillonii*, expressed as gallic acid equivalents (GA) per / mg of dry extract is 51.84µg GAE extract.

Flavonoids are also effective free radical scavengers for most of the oxidizing molecules such as singlet oxygen and several other free radicals produced during various diseases (Bravo, 1998). Flavonoids inhibit formation of reactive oxygen, chelate trace elements and scavenging reactive species that is involved in free-radical production and up-regulate antioxidant defence in the body (Agati, *et al.*, 2012). The concentrations of flavonoids in ethanolic leaf extracts of *Actinodaphne bourdillonii* is 30.77 µg QE / mg mass of extract.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity is one of the most extensively used technique for screening the antioxidant potential of plant extract. In this study, Antioxidant activity was determined in the ethanolic extract of leaves of *Actinodaphne bourdillonii* (EAB) showed a better antioxidant potential in higher concentration of 450 µg/mL by DPPH radical scavenging method and in some of the concentrations of EAB showed a significant percentage of scavenging when compared to standard ascorbic acid. IC 50 value found to be 19.05 µg/mL and 87.23 µg/mL for ascorbic acid and ethanolic extract of *Actinodaphne bourdillonii* respectively.

Nitric oxide scavenging activity

Nitric oxide (NO) is a significant chemical intermediary which is produced by macrophages, neurons, endothelial cells, etc. and is involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases (Lalitha *et al.*, 2013). Antioxidant activity of ethanolic extract of leaves of *Actinodaphne bourdillonii* (EAB) showed a better result and it is due to the scavenging of nitric oxide.

At EAB 450 µg/mL showed similar antioxidant potential with standard L-Ascorbic acid (AA). IC 50 value found to be 24.33 µg/mL and 99.29 µg/mL for ascorbic acid and ethanolic extract of *Actinodaphne bourdillonii* respectively.

Hydroxyl radical (OH) scavenging activity

During Fenton's reaction hydroxyl free radicals is formed in the presence of reduced transition metals such as Fe2+ and H_2O_2, those are supreme reactive species of all the reduced forms of dioxygen is thought to initiate cell damage *in vivo* (Duan *et al.*, 2007). The hydroxyl radical scavenging potential of ethanolic extracts of *Actinodaphne bourdillonii* leaves were increased with increasing concentration.

The hydroxyl radical scavenging activity of ethanolic extract of *A. bourdillonii* was observed in the range of 17.15%-84.35% from 12.5 to 200 µg/mL concentrations. At 200 µg/mL showed maximum percentage of scavenging for both L-ascorbic acid and ethanolic leaf extracts and IC 50 value found to be 28.17 µg/mL and 102.98 µg/mL for L-ascorbic acid and ethanolic extract of *Actinodaphne bourdillonii* respectively.

Total antioxidant activity

Total antioxidant capacity of the extracts was estimated by phosphomolybedenum method and the results were quantitatively expressed as of ascorbic acid equivalents/g of dry weight. Total antioxidant activity was calculated from the calibration curve of ascorbic acid. Total antioxidant activity of EAB was found to be 151.35 ug of ascorbic acid equivalent/mg of dry extract respectively.

Superoxide radical scavenging activity

The superoxide radical scavenging activity of EAB was determined by the nitroblue tetrazolium reduction method. EAB showed superoxide radical scavenging of 75% at 450 mg/kg and the EC50 value was found to be 55.11 µg/mg compared to the standard used Ascorbic acid which showed an EC50 value of 10.12 µg/mg.

Ferric reducing antioxidant potential (FRAP) assay

The ability of plant extract to reduce the ferric ion was determined from the standard graph drawn from known concentrations of Trolox and expressed as 95.17µg of trolox equivalent/mg of EAB.

Oral Acute toxicity study

Oral acute toxicity study is an essential method for revealing of toxicity by rising dose till appearance of toxicity signs. The use of animals in safety and toxicological evaluations in acute systemic toxicity testing is still in practice. This will help to understand the effect of drug in a biological system. There is no behavioural changes happened during the observations made in different time intervals after the administration of EAB of higher concentrations.

ANTI NOCICEPTION STUDIES

Acetic acid induced writhing

The writhing response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. Local peritoneal receptors were involved during this response. This technique is mainly done to evaluate superficially performing painkillers and in this model, peak analgesic action of aspirin was detected. Simvastatin likewise showed noteworthy painkilling effect however fewer than aspirin (Barsante, *et al.*, 2005).

Tumour necrosis factor-a (TNF- a), Bradykinin (BK), Chemokine CXCL and the Interleukin-1b (IL-1b) persuade about the identical amount of nociception and pre-treatment with atorvastatin concentrated each of these hyper nociceptive conditions remains unchanged (Garzo'n, *et al.*, 2006). Ethanolic leaf extracts of *Actinodaphne bourdillonii* (EAB) was significantly inhibited writhing response induced by acetic acid has showed maximum inhibition of 87.76 % at EAB 450mg/kg. So EAB also have the potential to inhibit these mediators involved during the nociception.

Eddy's Hotplate Test

The hot plate method is employed to study the thermal stimuli, the paw licking in test animal in hot plate response is due to complex supraspinal integrated behaviour (Chapman, *et al.*, 1983). The nociceptors are sensitized by sensory nerves. The participation of endogenous substances such as PGs may be minimized. In centrally acting analgesic techniques, the drug in EAB 50 mg/kg, EAB 150 mg/kg and EAB 450 mg/kg was found to be significantly effective when compared to the standard drug. EAB 450 mg/kg was found to be more effective in this analgesic study for evaluating centrally acting drugs and also shows more

inhibition than the standard drug (10mg/kg of Aspirin). These neuronal fibres are sensitive to both narcotics and non-steroidal antiinflammatory drugs (Collier, *et al.*, 1968).

Ethanolic extract of *Actinodaphne bourdillonii* showed anti nociceptive activity at the maximum dose (450 mg/kg), it could assuage pain in all times of paw licking test.

ANTI-INFLAMMATORY STUDIES

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

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

Carrageenan Induced hind paw oedema

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

The ethanolic leaf extracts of *Actinodaphne bourdillonii* considerably inhibited paw oedema induced by carrageenan after 3 hrs. It has showed a maximum inhibition of 83.63% at a dose of EAB 450mg/kg, this finding suggests a possible inhibition of cyclooxygenase synthesis by the ethanolic leaf extracts of EAB and the effect of EAB is similar to that of nonsteroidal anti-inflammatory drugs such as indomethacin, whose mechanism of action is by inhibiting the production of cyclooxygenase enzyme.

Formalin induced hind paw oedema

Formalin induced paw oedema is a sub-acute inflammation fallouts from cell damage, which incites the making of endogenous intermediaries. Histamine, serotonin, prostaglandins, and bradykinin are example of some endogenous mediator's produced during the inhibition of oedema induced by formalin in rats is one of the appropriate protocol to screen anti-inflammatory agents (Mishra *et al.*, 2010).

Indomethacin displayed more or less inhibition of oedema in early intermediate and later phases. Ethanolic extract of *Actinodaphne bourdillonii* showed a more or less significant inhibition of 70.23 % and 78.36% at EAB 450mg/kg in formalin induced oedema has shown significant inhibition in the early phases and also in later phases respectively when compared to Indomethacin showed a protection of 73.22% and 80.23% at a amount of 10mg/kg body weight.

6. SUMMARY

The investigational study entitled "Scientific validation of antiinflammatory, antinociceptive and antioxidant property of 'malavirinji' (Actinodaphne bourdillonii Gamble)" was conducted at the Ethnomedicine and Ethnopharmacology Division of Jawaharlal Nehru Tropical Botanical Garden (JNTBGRI), Palode, Thiruvananthapuram, during the year 2016 to 2017. Objective of the study was to scientifically evaluate the antiinflammatory, antinociceptive, antioxidant potential of leaves of an ethno medicinal plant *Actinodaphne bourdillonii Gamble*.

Actinodaphne bourdillonii Gamble leaves were obtained from the premises of the JNTBGRI, Palode, Thiruvananthapuram were subjected to pharmacological studies.

The preliminary phytochemical screening of leaves of *Actinodaphne bourdillonii* has shown the occurrence of secondary metabolites like phenols, saponins, flavonoids etc. and these phytochemicals, may play a vital role in medicine for healing different kinds of diseases.

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

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

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

The anti-nociception potential of the ethanolic leaf extract of *A. bourdillonii* investigated by Acetic acid writhing method and Eddy's Hot Plate method and in mice with

three different doses i.e. 50, 150, 450mg/kg of body weight. Treatment with ethanolic extract of leaves of *A. bourdillonii* at above doses significantly reduced the pain in test animals.

The present study revealed that *A. bourdillonii* exhibited satisfactory pharmacological effect in all the tests like anti-oxidant antiinflammatory and antinociceptive studies. From the detailed pharmacological studies, the effective dose of the drug was found to be 450 mg/kg for all experiments because it has shown the maximum percentage of inhibition.

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8. APPENDIX

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)

9. ABSTRACT

The study entitled "Scientific validation of antiinflammatory, antinociceptive and antioxidant potential of *malavirinji* (*Actinodaphne bourdillonii* Gamble)" was conducted at the Ethnomedicine and Ethnopharmacology Division of Jawaharlal Nehru Tropical Botanical Garden (JNTBGRI), Palode, Thiruvananthapuram, during the year 2016 to 2017. Objective of the study was to scientifically evaluate the antiinflammatory, antinociceptive, antioxidant potential of leaves of an ethnomedicinal plant *Actinodaphne bourdillonii* Gamble.

Phytochemical examination revealed the presence of various phytoconstituents like alkaloids, flavonoids, saponins, alkaloids, carbohydrates and phenols.

In *in vitro* antioxidant method the ethanolic extracts of leaf showed higher free radical scavenging activity compared to standards with IC_{50} of DPPH, NO Scavenging Activity, Hydroxyl Free Radical Scavenging Activity, Superoxide radical scavenging activity, Total antioxidant activity and Ferric reducing antioxidant potential (FRAP) assay.

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

In the detailed *in vitro* pharmacological studies for antiinflammatory and antinociception were conducted. Antiinflammatory activity was determined by Carrageenan (Acute inflammation) and formalin (sub chronic inflammation) induced paw oedema on hind limb in rats with three different doses 50, 150 and 450 mg/kg. At the dose of EAB 450mg/kg give 83.63% of inhibition in carrageenan induced paw oedema and in the formalin induced paw oedema (sub-acute) study a dose of EAB 150mg/kg gives maximum inhibition of 70.23% and 78.36% of inhibition in the first and seventh day respectively. Antinociception activity was determined by Eddy Hot Plate method and Acetic acid writhing method in mice with three different doses 50, 150 and 450 mg/kg. At the dose of EAB 450mg/kg gives maximum inhibition 87.76% of inhibition in Eddy's hot plate method and in the acetic acid induce writhing study a dose of EAB 450mg/kg gives maximum inhibition of 71.55% of inhibition.