EVALUATION OF ANTI-INFLAMMATORY AND ANTIOXIDANT POTENTIALS OF *Zingiber wightianum* Thwaites (MALAYINCHI), AN ETHNOMEDICINAL PLANT OF KERALA

Submitted by

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(2015-09-008)

THESIS

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requirement for the degree of

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Faculty of Agriculture

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DEPARTMENT OF PLANT BIOTECHNOLOGY

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KERALA, INDIA

2020

DECLARATION

I hereby declare that this thesis entitled "**Evaluation of anti-inflammatory and antioxidant potentials of *Zingiber wightianum* Thwaites (Malayinchi), an Ethnomedicinal plant of Kerala**" is a bonafied record of the research work carried out by me under the supervision and guidance of Dr. S. R. Suja, Senior Scientist and HOD, Ethnomedicine and Ethnopharmacology Division, KSCSTE – JNTBGRI, 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.

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This is to certify that this thesis entitled **"Evaluation of anti-inflammatory and antioxidant potential of *Zingiber wightianum* Thwaites, an ethnomedicinal plant of Kerala**" is a record of research work done by Ms. Meera T. S. (2015-09-008) 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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LIST OF ABBREVATIONS & SYMBOLS USED

%	Percent
°C	Degree Celsius
µg	Microgram
µL	Microlitre
µmol	Micromolar
AAE	Ascorbic Acid Equivalents
AAPH	2,2′-azobis (2-amidinopropane) hydrochloride
ABTS	2,2’-azinobis (3-ethylbenzthiazoline-6-sulphonic acid)
Acc No	Accession number
ANOVA	Analysis of variance
AZW	Aqueous extract of *Zingiber wightianum*
cm	Centimetre
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl 2- picrylhydrazyl
EC_{50}	Effective Concentration 50 percent
EDTA	Ethylene Diamine Tetra Acetic Acid Disodium salt
et al.	And other co-workers
Etc.	Etcetera
EZW	Ethanolic extract of *Zingiber wightianum*
FRAP	Ferric Reducing Antioxidant Power
g	Gram
GAE	Gallic Acid Equivalent
hr	Hour
H_2O_2	Hyrogen peroxide
HCl	Hydrochloric acid
HRBC	Human Red Blood Cell
HZW	Hydro ethanolic extract of *Zingiber wightianum*

IC_{50}	Inhibitory Concentration 50 percent
IL	Interleukin
$K_2S_2O_8$	Potassium persulfate
Kg	Kilogram
L	Litre
M	Molar
mg	Miliigram
Min	Minute
mL	Millilitre
mM	Millimolar
NFκB	Nuclear factor kappa B
nm	Nanometre
NO	Nitric oxide
NSAID	Non-Steroidal Anti-Inflammatory Drugs
O^{-2}	Superoxide radical
OECD	Organization of Economic Cooperation & Development
OH^-	Hydroxyl radical
pH	Potential of hydrogen
PMS	Phenazine methosulfate
RE	Rutin Equivalent
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
rpm	Rotation per minute
SD	Standard Deviation
STD	Standard
TAC	Total Antioxidant Capacity
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TFC	Total Flavonoid Content

TNF-α	Tumour Necrosis Factor Alpha
TPC	Total Phenolic Content
TPTZ	2,4,6-tripyridyl-s-triazine
UV	Ultra Violet
WHO	World Health Organization

1. INTRODUCTION

Medicinal plants have made a major contribution to people's primary healthcare worldwide. Increase in population, inadequate drug supplies, prohibitive treatment costs, side effects of new synthetic drugs, and the development of drug resistance to infectious diseases has led to increased use of plant materials as a medicinal source. The WHO has recently reported that 80 % of people depend on herbal medicines for some aspects of their primary health care needs worldwide. Roughly 21,000 plant species may potentially be used as medicinal plants according to the WHO (Tefera *et al*., 2019).

India is rich with a biodiversity of natural medicinal plants that have been used for centuries in traditional ayurvedic system to treat human illnesses. It is worth noting that typical Indian medicinal formulations are multi - element blends whose medical application is based on scientific knowledge and not on a theoretical understanding of the bioactive compounds in the mixture (Mohanraj *et al*., 2018).

Inflammation is one of the predominant events in the majority of both acute and chronic neurological diseases, and is a key cause of morbidity in the modern world. Response of an acute inflammation manifests itself as redness, heat, swelling, pain and function loss. Increased vascular permeability, accelerated blood supply and sensitization of nerve fibres, respectively, are correlated with swelling, redness and pain. In recent years, phytochemicals tends to be a significant source of medicines and are now being evaluated as candidate for anti-inflammatory drugs. Phytoconstituents assessment in animal models is a core component of the disease-specific anti-inflammatory drug discovery programme (Patil *et al*., 2019).

Clinical and medical literature widely addresses reactive oxygen species and antioxidants. Antioxidants are needed to prevent formation and to counteract the actions of reactive oxygen and nitrogen species that are formed in vivo and cause DNA, lipids, proteins, and other biomolecules to damage. Since natural antioxidant defenses such as superoxide dismutases, H_2O_2-removing enzymes, metal binding proteins, etc., are inadequate to prevent harm entirely, dietary antioxidants are quite important to avoid huge damage. Several dietary compounds were suggested as important antioxidants:

there is clear evidence that vitamins E and C play a key role and there is also increasing interest in the function of plant phenolics, especially flavonoids (Halliwell, 1996).

Zingiber wightianum Thwaites (commonly called as “Malayinchi”) belongs to the family, Zingiberaceae. In Kerala, the traditional healers of Wayanad uses rhizomes of *Zingiber wightianum* for the treatment of swelling and pain and also as a liver protectant. *Zingiber wightianum* is used as a potential bio-pesticide as it against the early fourth instar larvae of *Culex quinquefasciatus* and *Culex sitiens*.

The purpose of the present study is to scientifically evaluate anti-inflammatory and antioxidant potentials of *Zingiber wightianum* Thwaites (Malayinchi), an Ethnomedicinal plant of Kerala. It is the first study on *Zingiber wightianum* for its anti-inflammatory, antioxidant potential and preliminary phytochemical analysis to the best of our knowledge.

2. REVIEW OF LITERATURE

2.1 MEDICINAL PLANTS

People are using plants as a traditional medicine over thousand years. The oldest records etched on clay tablets in ancient Mesopotamian writing dated from about 2600 BC ; among the substances used were extracts of Myrrh, Cedrus, Licorice, Poppy and Cypress still used today in the therapy of diseases varying from common cold and coughs to inflammation and parasite infections. India has a rich history and a solid foundation for Ayurveda, the traditional herbal medicine system. Herbal plants have massive role in the prevention and treatment of human diseases. Plants were related to the growth of human civilization worldwide. Nevertheless, plants are considered a major sources of bioactive compounds which have numerous medicinal properties. Driven bioassay isolation and identification of the bioactive components is still needed and thorough work is also necessary to reveal the structural activity relationship of these active constituents. Medicinal plants are technically considered as a tool for the herbal drug discovery. The pharmacological properties of medicinal plants are seen as a possible future medical commodity for the administration of healthcare as in 21st century (Shakya, 2016).

Owing to their usage in ethno medicine, which cures diseases such as cold, fever, , medicinal plants are receiving a lot of attention lately and are now backed by solid science proof. The study on medicinal plants begins with solvent extraction procedures which plays a pivotal role in the results of extraction (e.g. yield and quality of phytochemicals) as well as the subsequent assays. Nowadays there are a broad number of techniques available for various extraction methods (Azwanida, 2015).

2.2 ETHNOBOTANY, ETHNOMEDICINE AND ETHNOPHARMACOLOGY

Ethnobotany is a discipline concerned with the connection of local people with plants and medicinal applications in the diagnosis of several diseases, social and economic support structures and advantages in our life (Singh *et al.,* 2015). This advance was in response to a repeated call for concept-inspired and scientific theory-driven research to improve the discipline's rigor. Despite changes, recent ethnobotanical

work has greatly overstated the usage of quantitative ethnobotanical measures and methodological methods borrowed from ecology, but also underscored the production and incorporation of a good theoretical foundation (Gaoue *et al.,* 2017).

Plants have always been a crucial resource for humans. Ethnobotany, located at the interface of natural and social sciences, is a discipline that addresses the relationships between humans and plants. Among the numerous applications of plants, those related to human health and well-being are the most diverse. Bioprospecting for new drugs with a botanical origin and for new food crops has classically been based on ethnobotanical information. Ethnobotanically directed bioprospecting has become more powerful than random assays for finding and identifying bioactive compounds from plants (Garnatje *et al.,* 2017).

The success of ethnomedicinal use of medicinal herbs has drawn the worldwide attention of scientists to further investigate its medicinal properties. Technological advances in drug design and discovery work have contributed to the development of metabolite-based synthetic drugs through bioinformatics and medicinal chemistry studies. In addition, new insights into the biosynthetic pathways of metabolites can be elucidated with the advent of genomics, transcriptomics, proteomics, and metabolomics, enabling researchers to predict the possible bioactive compounds that are responsible for the plant's medicinal properties. The compound’s extensive biological activities require further study through studies such as drug development, polypharmacology, and the use of nanotechnology to deliver drugs.

With the advancement of science, the ethnomedicinal uses of herbal plants can be explained by *in vitro* and *in vivo* studies to prove the plant extract’s activities, molecular progressions further allow scientists to look deeper into the biosynthetic pathways of bioactive compounds and get a clearer picture of the whole cycle, which in turn may promote the production of better and stronger drugs and combat diseases in the future (Eng-Chong *et al.*, 2012).

2.3 PHYTOCHEMICALS

Plants produce bioactive compounds, which is actually a survival adaptation, for example defending against herbivores, defending against environmental stress

(Awuchi, 2019). Medicinal plants are used as a reservoir of various forms of bioactive compounds with varying therapeutic properties. Therapeutical potential of medicinal plants has been well explored over an extremely long period of time. The broad range of medicinal plant derived therapeutic activity includes anti-inflammatory, antiviral, antitumor, antimalarial, and analgesic (Raina *et al.,* 2014). Many medicinal plants contain phenols and flavonoids which have strong antioxidant potential. The crude extracts of different parts of herbs were analysed for total antioxidant activity, total phenolic and flavonoid content in order to find their potential (Bajpai *et al.,* 2005).

The chemical components of medicinal plants have mainly antioxidant properties. The main groups of bioactive constituents responsible for the antioxidant action are flavones, isoflavones, phenols, flavonoids, anthocyanins, coumarins, lignans, catechins, and isocatechins. The antioxidant property of phenolic compounds varies with the aromatic ring structure, position of attachment, number of hydroxyl groups (Balasundram *et al.,* 2006). Many phytochemicals such as flavonoids, tannins, terpenoids, etc. are believed to be good antioxidants which minimize the incidence of cardiovascular diseases, brain damage and rheumatic disorders.

Analysis of Nishat *et al.*, (2012) showed that the *Alpinia nigra* rhizome contained total flavonoids (54.14 mg), total phenols (120.7 mg) and strong total alkaloids (215.0 mg) suggesting that *Alpinia nigra* is an outstanding source of antioxidants. They protect plants from disease and contribute to the colour, fragrance and taste of the plant. Generally speaking, plant chemicals that protect plant cells against environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called phytochemicals. These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial influence, detoxification of enzyme regulation, immune system activation, diminished platelet aggregation, and hormone metabolism and anticancer property regulation. There are more than thousand known phytochemicals and many unknown. It is common knowledge that plants develop such chemicals in order to defend themselves (María *et al.,* 2018).

In the history of world, diverse cultures and societies have acknowledged the potential use of ginger for the treatment and prevention of various diseases. In a wide range of applications, various studies associated with ginger and its extract support the potential effects. Different active medicinal compounds present in ginger were presumed to minimize the risk of various diseases such as cardiovascular, anti-tumour, anti-diabetic and also to display beneficial results against gastric problems. The compounds present in ginger such as gingerols, shogaol, and paradols are the useful ingredients that can prevent various cancers, apoptosis induction, angiogenesis and metastasis, and cell cycle progression inhibition. The antimicrobial and anti-inflammatory ability of Ginger is also well known in diverse studies. Overall, ginger is one of the most traditional medicinal herbs that has great beneficial impact in improving health issues.

Some beneficial ginger compounds that bear medicinal properties include ingenol, gingerdiols and zingerone. Gingerols are regarded as the most important of all these compounds because of their active pharmacological properties .Their ability to suppress granulomatous inflammation is likely due to polar compounds present in crude extract. (Funk *et al.,* 2016).

Ample clinical studies provide convincing proof of the possible usage of ginger as a medication for a number of immunomodulatory, anti-inflammatory and allergic conditions, such as acute respiratory distress syndrome, and chronic pain along with rheumatism, during which the pain and degree of swelling can be reduced by using ginger extracts (Tasneem *et al.*, 2018).

Cardiac glycosides, steroids, saponins, anthraquinones were identified in the methanolic leaves and stems extracts of *Etlingera coccinea* whereas cardiac glycosides and steroids were found in the extracts of rhizome of *E. coccinea.* Highest antioxidant potential were found in the extracts of leaves. It has even stronger antibacterial and antifungal properties than stem and rhizome. The high content of flavonoid and flavonol and the heavy antioxidant activity indicate that this plant has enormous opportunity in the food and pharmaceutical industries.

2.4. ZINGIBERACEAE

Zingiberaceae is one of largest families of the plant kingdom and it is widely distributed across the tropics, notably in south-east Asia. It is an essential resource that provide the human being with many valuable products such as spices, medications, colourants, etc. India has a vibrant collection of Zingeraceae members; about 20 genera and more than 200 species.

In the genus Alpinia, *A. galanga* is used for preparing "Rasnadi powder", which helps to relieve headache, dizziness, cough with sputum, sinusitis, etc. In Curcuma, *C. longa* is the most popular one, which has been studied in greater depths already. *C. aromatica* is used to cure skin diseases. *Kaempferia galanga* is used in the treatment of lung and stomach related diseases. Of late, it is being used in preparations of mouth washes and oral deodorants. *Zingiber officinale* has often been influential in the formulation of various traditional ayurvedic medicines (Kumar *et al.,* 2013).

Ginger is used as galactagogue and also used for treating asthma, inflammation, osteoarthritis, complications in menstrual cycle. Gingerol is the active pungent compound found abundantly in the fresh ginger. Upon dehydration, gingerol forms respective shogaols and it exert distinctive pungent flavour to the dried ginger. Gingerol and shogaols is accountable for the bioactivities of ginger. Owing to the vast uses of Zingiberaceae herbs in cosmetics, health care items and medicines, essential oils from ginger have drawn growing interest as well (Akbar, 2020).

Turmeric is a kind of herb from ginger family which is found abundantly in the tropical region of South and West Asia. Turmeric, which plays a key role in Asian cuisine such as Indian, Chinese, etc., is also used as a condiment and enhances the texture, colour and flavour. Turmeric is also believed to have been used in India and China for the treatment of diseases such as dermatological disorders, parasitic infections, anxiety and depression for centuries (Kocaadam *et al.,* 2017).

Zingiber zerumbet, a plant belonging to family Zingiberaceae, is used in the Malaysian herbal system. Carlos *et al*. (2019) suggests that the bioactive compound present in the *Zingiber zerumbet* called zerumbone is responsible for its analgesics and antinociceptive activities.

Curcuma aromatica belonged to genus Curcuma in family Zingiberaceae, is an aromatic medicinal herb. *C. aromatica* is distributed throughout the tropical and

subtropical regions in the world, and widely cultivated in Asian countries, especially in China, India and Japan. Since ancient times, *C. aromatica* has already been used as flavouring agent, yellow dye and traditional Chinese medicine. Medicinally, it possesses various pharmacological performances, such as antioxidative, antimicrobial, anti-inflammatory, anticancer and antiangiogenic activities (Xiang, 2017).

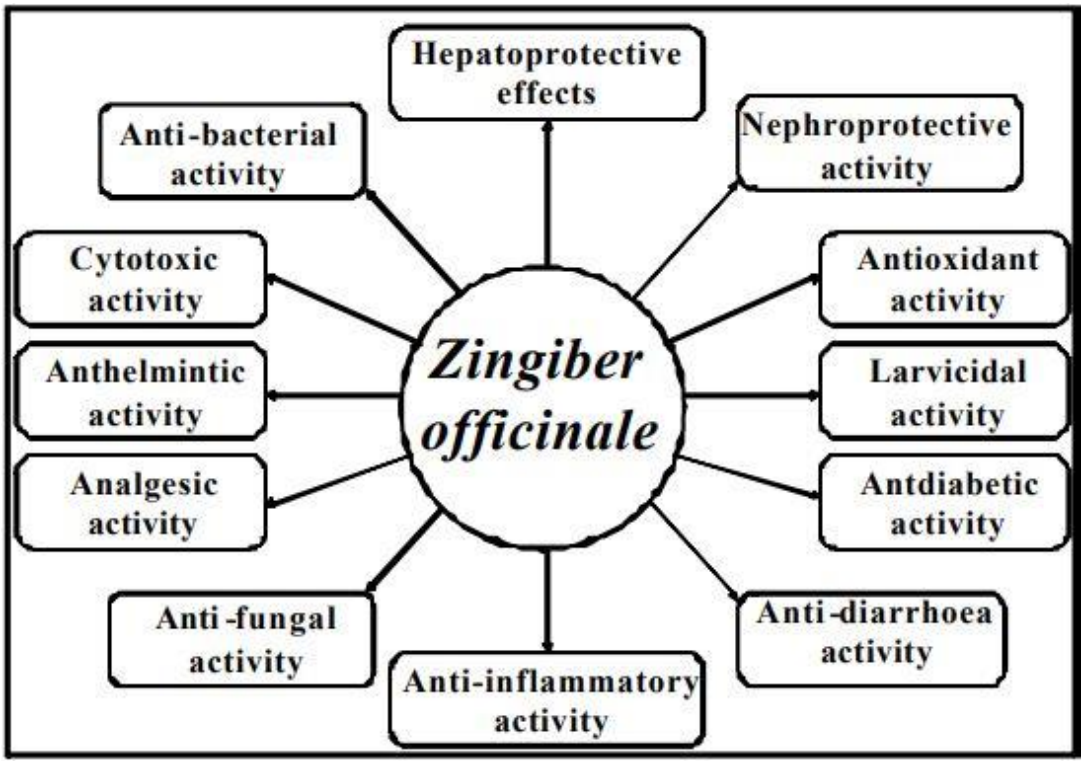

Figure 1. Various uses of *Zingiber officinale* (Kumar *et al.*, 2011)

The phytochemical content of the Z. officinale has been widely researched in the many studies. *Z. officinale* has key phytochemical classes including essential oils, phenolic compounds, flavonols, sugars, steroids, alkaloids, glycosides, saponins, terpenoids and tannins. Such phytochemicals plays a significant part in this plant's medicinal properties (Kumar *et al.,* 2011).

Alpinia calcarata Roscoe rhizomes are also used as a treatment for bronchitis, cough, respiratory problems, diabetics, asthma and arthritis in mainstream medicine systems in Sri Lanka. Medicines used for arthritis usually have antinociceptive and anti-inflammatory properties. Arambewela *et al*. (2005) have isolated 18 volatile constituents in essential oils of Sri Lankan grown *Alpinia calcarata* rhizomes, roots and leaves.

Kaempferia galanga Linn. (Zingiberaceae) is native to tropical Asia, where it is widely used in herbal medicine for inflammation, rheumatic disorders, asthma, dysentery, diarrhoea, and stomachache treatment (Koh, 2009). Despite these essential medicinal properties *Kaempferia galanga* is still largely unknown and unused. A variety of inquiries, moreover, were carried out in support of these traditional claims, namely: the *Kaempferia galanga* extracts shows nematicidal, insect repellent, and larvicidal behaviour. Certain reported properties include sedative, vasodilating, anti-microbial, anti-neoplastic, anti-allergic, antioxidative, analgesic and wound healing properties (Hong *et al.,* 2011). *Kaempferia galanga* essential oil from rhizome has also been known to have germicidal activities (Sahoo *et al.,* 2014).

In conventional medicine uses, small cardamom capsules have been used to control hypertension, tooth and gum lesions, cataracts, fatigue, vomiting, and heart, respiratory, and renal diseases. The flexible use of cardamom capsules has many other beneficial results, which are important in the light of conventional and current pharmaceutical insights (Ashokkumar *et al.,* 2019).

Hedychium coronarium is a perennial herb of the Zingiberaceae family known for its top quality essential oil. Essential oil is obtained from *Hedychium coronarium* rhizomes and bulbs. *Hedychium coronarium* is used in strongly classified beauty products. (Chan *et al.,* 2015). The pounded rhizome is used in Ayurvedic system to reduce fever and is used as excitant (Ray *et al.,* 2015). The parts of plant have various common medicinal applications. In Malaysia, leaves are boiled to cure indigestion and are eaten. To relieve stomach pain, leaves are taken with betel nut. Boiled leaves are used in Thailand for treatment of stiff and swollen joints. Rhizomes are eaten as stimulants and as carminants. A stem decoction is gargled over for tonsillitis. In Hawaii and Japan, flowers are consumed as vegetables, dressed as garlands, and used for fragrance. Rhizomes are used in Vietnam for treating acne, skin infections, headache and arthritic pain. In Brazil, leaves are eaten with a hot water infusion to combat hypertension (Zhong *et al.,* 2009).

In Venezuela, inflorescences of *A. purpurata* are boiled and the infusion of hot water is consumed to treat symptoms of sore throat. The rhizomes are eaten in India to stimulate appetite, taste and voice. They are also used for treating headache, rheumatism, sore throat and renal infection (Raj *et al.,* 2012).

2.5. ANTIOXIDANTS

The active oxygen species are created by several metabolic processes. The most prominent, harmful and damaging are the four main active oxygen species [superoxide radical O^{-}_{2}, hydrogen peroxide H_2O_2, hydroxyl radical OH^{-} and singlet oxygen O_2]. Among them, H_2O_2 and the hydroxyl radical are most active, toxic and destructive. Also high concentrations of salt impair the transport of cellular electrons within the various subcellular compartments and lead to the generation of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radicals and singlet oxygen superoxide (Mittler, 2002).

Excess ROS triggers phytotoxic reactions such as degradation of proteins, and DNA mutations. ROS interacts with proteins and induces many reactions that cause protein damage and leads to peptide cleavage and amino acid oxidation. DNA oxidation leads to breakage of DNA strands and ROS interaction with mRNA may lead to defective translations of proteins. Higher plants' ability to scavenge the active toxic oxygen also seems to be a very crucial component of their sensitivity to high stresses. In vitro studies have shown that many metabolites of plants guard against oxidative damage by free radicals and by inhibiting or quenching reactive oxygen species (Ali *et al.,* 2006).

ROS in living cells contains hydroxyl radicals, usually occurring through Fenton type reaction, nitric oxide derived peroxynitrite, hypochlorous acid acts as a substrate for many strong oxidizing species. ROS is produced primarily in organelles such as mitochondria, peroxisomes and endoplasmic reticulum and mainly via mitochondrial respiratory complexes during high production levels of ATP, fatty acid oxidation and detoxification through xenobiotic processes (Sarangarajan *et al.,* 2017).

Antioxidant compounds are chemicals commonly used to combat the oxidative stress produced by free radicals in the cell. The antioxidant compounds can come from natural sources including plants. Medicinal plants have antioxidant properties because of flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins, and isocatechins (Kumar *et al.,* 2011).

Antioxidant capacity of methanolic extracts from different plant parts of *Alpinia galanga*, *Curcuma longa* and *Etlingera elatior* exhibited varied results. Significant antioxidant activity were found in the polymeric tannin rhizome fraction of *A. galanga*, non-polymeric phenolic fraction of *C. longa* rhizomes and its extract, and in the polymeric tannin fraction of *E. elatior* leaves (Chan *et al.*, 2011).

Mahdavi *et al*. (2017) found that the *Etlingera sayapensis* leaf extracts had the strongest antioxidant activity accompanied by the stem and then the extracts from the rhizome. Polarity of the solvent used in solvent extraction specifically influences the antioxidant activity of the extracts, resulting in the maximum antioxidant activity in methanolic extracts while lowest antioxidant activity is found in ethyl acetate extracts respectively.

Studies by Sattar *et al*. (2013) found that *Zingiber officinale* and *Alpinia allughas* had antioxidant activity (percent inhibition) ranging from 26.8 to 68.3 and 14.3 to 58.5 in various solvents, respectively. Generally, the results suggest that both spices are excellent sources of phytochemicals that can be used for medications and/or dietary supplements.

In addition to enhancing scavenging efficiency in *Curcuma alismatifolia*, Taheri *et al* (2014) concluded that radiation exposure up to 20 Gy would improve the consistency and volume of bioactive compounds, including phenolic compounds and flavonoids.

Barbosa et al. (2019) found that methanolic extracts from *Hornstedtia conoidea* leaves had considerably higher antioxidant potential compared with rhizomes. In addition, methanolic extracts from *Hornstedtia conoidea* leaves have slightly higher phenolic content relative to rhizomes. This is an implication that most of the phenolic compounds in *Hornstedtia conoidea* are primarily responsible for its strong antioxidant activity.

High phenolic content was found in the freeze dried peels of ginger and turmeric rhizomes. In addition phenolic compunds like 6- gingerol and curcumin increases their antioxidant potential enormously. Therefore, the peels discarded from ginger and turmeric rhizomes may be an fascinating source of bioactive compounds to be

introduced as food additives / preservatives, beneficial components, nutritional supplements and nutraceuticals because they are not only easy and inexpensive to manufacture but also without any perceptible danger to human safety. During a specific product / service life cycle, food waste including peels may be refined into value-added goods in this manner (Tinello *et al.*, 2019).

Chloroform, ethyl acetate and n-butanol-soluble rhizome extract of *Kaempferia rotunda exhibited* strong DPPH radical scavenging activity (Lotulung *et al.*, 2008) whereas the crude rhizome extracts of *Kaempferia galanga* has also shown DPPH radical scavenging activity. The *Kaempferia galanga* has demonstrated substantial antimicrobial activity and thus *Kaempferia galanga* crude rhizome extracts may be a possible option against microbes (Narasinga *et al.*, 2014). It was evident that increased antioxidant activity in DPPH free radical-scavenging assay was seen with increasing concentrations of oil samples of *Kaempferia galanga*, as a lower IC_{50} value indicated higher antioxidant activity (Sahoo *et al.*, 2014).

Rachkeeree et al. (2020) studied 16 ginger plants (*Alpinia nigra, Amomum aculeatum, Amomum coriandriodorum, Amomum dealbatum, Amomum uliginosum, Curcuma aeruginasa, Curcuma amada, C. aromatic, C. candida , C. latifolia, C. longa, C. manga, Etlingera araneosa, E. elatior, E. linguiformis, Kaemferia rotunda*) and found that they are good sources of phenolic compounds and antioxidants. Highest antioxidant activity is shown among the members of Alpinia, Amomum and Curcuma.

2.6 ANTI-INFLAMMATION

Inflammation usually occurs when infectious microorganisms such as bacteria, viruses or fungi invade the body, specifically reside in tissues and/or circulate in the blood. Inflammation can also occur in response to processes such as tissue damage, cell death, cancer, ischemia, and degeneration. Inflammatory reaction protects the host from tissue damage and invasion of the microbes. As such, this response should be short-lived and failure can result in many immune-related diseases. The treatment of inflammatory diseases today mainly involves interrupting the synthesis or action of essential mediators that drive the response of the host to the injury. While the main cure for inflammatory disorders was given by steroids and antihistamines, they function for

the cure of inflammation-driven diseases such as asthma, rheumatoid arthritis, psoriatic arthritis (Tung *et al.*, 2008).

Reactive oxygen species (ROS) were associated with various health issues including inflammation. Natural antioxidants can soften this Issues (Ghareeb *et al.*, 2018). Dose – dependent *in vitro* experiments have depicted that, a wide variety of constituents of ginger suppressed the development of nitric oxide, inflammatory cytokines, prostaglandin synthase and arachidonate-5-lipoxygenase. The latter in turn hinders the production of prostaglandins and leukotrienes from cyclooxygenase (COX) and lipoxygenase (LOX) (Nicoll *et al.*, 2007).

Fig 2. Anti-inflammatory potential of *Zingiber officinale* (Ezzat *et al.*, 2017)

Habib *et al.* (2008) evaluated the anti-inflammatory activity of ginger extract (*Zingiber officinale*) on the expression of NFκB and TNF-α in liver cancer-induced rats. In rats with liver cancer, ginger extract significantly reduced elevated expression of NF_KB and TNF-α. Their analysis concluded that ginger acts as an anti-cancer and anti-inflammatory agent through the suppression of the pro-inflammatory TNF-α by inactivating NFκB.

Ezzat *et al*. (2017) evaluated *in vitro* anti-inflammatory potential of *Zingiber officinale* via protein denaturation inhibition assay, membrane stabilization assay, heat induced hemolysis, protease inhibition assay and anti-lipoxygenase activity. *In vivo* anti-inflammatory potential is evaluated through assessment of rat paw oedema inhibition, determination of PGE2 level, assessment of cytokine levels in paw oedema exudates, assessment of myeloperoxidase (MPO) activity and determination of oxidative stress parameters such as tissue nitrate/nitrite content (NOx) and total antioxidant capacity (TAC). Highest % of protein denaturation inhibition (66%) was exhibited by the 50% ethanolic extract. In the case of membrane stabilization, 70% ethanolic extract was more potent than the standard, while in protein inhibiton assay 80 and 90% ethanol extracts (500 µg/ml) showed the maximum inhibition activity of 56%. Aqueous extract appeared to be even more potent than the standard in the case of inhibition of lipoxygenase activity. Aqueous extract of *Z. officinale* (25, 50, 100 and 200 mg/ kg) resulted in significant improvement in the inhibition of carrageenan-induced oedema by about 30%, 46%, 56% and 59%, respectively. Animals receiving aqueous extract of *Z. officinale* (200 mg/kg) showed significant reduction of PGE2 production in the inflammatory exudates by 80%. Treatment of animals with aqueous extract of *Z. officinale* (200 mg/kg) is as good as indomethacin in reduction NO_x level in the inflammatory discharge and the overall antioxidant potential has been increased.

Ginger anti-inflammatory activity is mediated by inhibiting macrophage and neutrophils activation as well as negatively affecting monocyte and leukocyte migration. This was evidenced by the dose-dependent decrease in pro-inflammatory cytokines and chemokines and replenishment the total antioxidant capacity.

During inflammatory responses of various types several inflammatory mediators are synthesized and secreted. Usually, inflammatory substances are classified into two main categories: pro- and anti-inflammatory mediators. However, some mediators like interleukin (IL)-12 also possess both pro and anti-inflammatory properties (Azab *et al.*, 2016).

Recognizing the molecular targets of specific ginger constituents provides an ability to refine and standardize ginger products with respect to their effects on

particular inflammatory biomarkers. These procedures will be beneficial in laboratory animal and human studies (Grzanna *et al.,* 2005).

Young *et al.* (2005) evaluated the anti-inflammatory effect of gingerol, the pungent compound of ginger and found out, [6]-Gingerol (50 mg/kg–100 mg/kg) produced an inhibition of paw oedema induced by carrageenan. These results suggested that [6]-gingerol possessed anti-inflammatory activity.

Jia *et al*. (2011) proposed that the ginger oil (0.25-1.0 g/kg) generates substantial analgesic effects in mice against chemically and thermally induced nociceptive pain stimuli ($P < 0.05, 0.01$). Also the ginger oil (0.25-1.0 g / kg) greatly reduced adjuvant arthritis, paw oedema triggered by carrageenan, and capillary permeability or microvascular permeability in rats caused by inflammatory mediators.

Jagadish *et al*. (2016) reported that the *Kaempferia galanga* rhizome petroleum ether extract was proven to be efficient for both acute and chronic inflammation in animal model.

The *Alpinia zerumbet* leaf extract showed strong antioxidant activity *in vitro* and hindered both cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) *in vitro*, with greater affinity against COX-2 and it also disrupted the activity of 5-lipoxygenase (LOX).These results indicate that the *Alpinia zerumbet* leaf extract may be a candidate for the development of a medication for the treatment of inflammation and ROS associated disorders (Ghareeb *et al.,* 2018).

Sulaiman *et al*. (2010) examined the anti-inflammatory function of zerumbone, a natural cyclic sesquiterpene derived from *Zingiber zerumbet* Smith using carrageenan-induced paw oedema and the intraperitoneal administration of zerumbone at a dosage of 5, 10, 50 and 100 mg / kg demonstrated substantial dose-dependent inhibition of carrageenan-induced paw oedema. They found that zerumbone has an anti-inflammatory effect against both exudative and proliferative inflammatory processes, which explains the extensive use of *Zingiber zerumbet* to treat inflammatory disorders.

Paramita *et al.* (2019) showed *Curcuma aeruginosa* dose dependently the paw oedema induced by carrageenan. In the case of evaluation of erythrocyte membrane

stabilization activity, the EC_{50} value of *Curcuma aeruginosa* and the standard, Indomethacin was comparable. Hence *Curcuma aeruginosa* rhizome extracts have the ability to enhance the anti- inflammatory process and acts as a natural anti-inflammatory agent.

Hydro and ethanolic extract of *Alpinia calcarata* Roscoe (250, 500, 750 and 1000 mg / kg) demonstrated a significant inflammatory inhibition, especially at the 4^{th} hour following carrageenan injection. The anti-inflammatory activity stimulated by 500 mg / kg of ethanolic extract of *Alpinia calcarata* Roscoe was better compared to indomethacin, the reference drug. Suppression of the development of histamine and prostaglandin synthesis is the possible pathway through which *Alpinia calcarata* facilitates its anti-inflammatory effect (Arawwawala *et al.,* 2012).

Ghosh *et al*., (2011) evaluated the acute and chronic anti-inflammatory activities of root extract of *Alpinia galanga* in rodents. Acute anti-inflammatory effects of extract were evaluated using carrageenan induced rat paw oedema and chronic anti-inflammatory effects were evaluated using formaldehyde-induced rat paw oedema. Inhibition of inflammation was seen to be 32.22% in carrageenan-induced and 55.75% in formaldehyde-induced rat paw oedema. They concluded that *A. Galanga* has anti-inflammatory properties that can operate by suppressing the receptors in histamine and serotonin pathway. Also in treating inflammatory disorders, it may be used as an excellent alternative to non-steroidal anti-inflammatory drugs and corticosteroids.

Umar *et al*., (2012) assessed the anti-inflammatory ability of *Kaempferia galanga*. Among numerous extracts of *Kaempferia galanga* such as petroleum ether, chloroform, methanol and water, the chloroform extract had the highest anti-inflammatory activity (42.9% inhibition) relive to the control; It was therefore regarded as the most effective crude extract which suggests that *Kaempferia galanga* has substantial anti - inflammatory properties; therefore, the study aims to contribute to the validity of conventional use of *K. Galanga* in inflammatory therapies.

Samodra *et al*., (2019) compared the effectiveness of *Kaempferia galanga* rhizome extract with diclofenac sodium as an anti-inflammatory agent. Ethanol extract

of *Kaempferia galanga* rhizome extract in the 5th hour at doses of 45, 90 and 180 mg/kg showed significant differences in the positive control group. This showed that the ethanol extract of *K. galanga* rhizome had anti-inflammatory activity at all dose levels.

3. MATERIALS AND METHODS

The study entitled "Evaluation of anti-inflammatory and antioxidant potentials of *Zingiber wightianum* Thwaites (Malayinchi), an ethnomedicinal plant of Kerala." was carried out in the Ethnomedicine and Ethnopharmacology Division of Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, during the 2019-2020 academic year.

3.1 *Zingiber wightianum* Thwaites.

3.1.1 Plant Description

Rhizome thick 2- 2.5 cm, fleshy, with many fleshy roots. Leafy shoots 1.2 m high. Leaf almost sessile, ligule 506 mm long, membranous, bifid, lobes rounded, minutely pubescent; lamina 15-35 x 5-6 cm, oblong – lanceolate, tip acuminate, lower surface pubescent, more near on midrib, upper surface glabrous. Inflorescence produced separately on a leafless short stalk; peduncle 4-8 cm long, clothed with membranous sheaths; spike 9x6 cm, ovate or oblong. Bracts 3-5 x 1-1.2 cm, lanceolate, acuminate, greenish – red, pubescent. Bracteoles shorter than the bracts, obtuse, greenish- red, slightly pubescent. Calyx 2 cm long, obscurely 3- toothed, unilaterally split, green with red markings, sparsely pubescent. Corola tube 2.5 cm long, yellowish green, pubescent; lobes almost equal, 2.5 x 0.5-0.8 cm, acuminate, yellow orange, heavily marked deep purple- red; lateral staminodes 1x 0.5 cm, acute. Stamen shorter than lip, almost sessile; anther –thecae 1cm long; curved beak 8 mm long, dark purple-red. Epigynous glands 4 mm long, linear, free from each other. Ovary 5 mm long, slightly pubescent, fruit 2.5 cm long, oblong, red. Seeds black, aril white.

Cytology: 2 n = 22 (n = no: of set of chromosomes)

3.1.2 Propagation

Zingiber wightianum is propagated vegetatively through small sections of the rhizome, called sets. Sets are developed from a living rhizome by cutting a small 3–6 cm out. Each piece should have at least one living bud that would produce shoots.

3.1.3 Uses

Rhizomes of *Zingiber wightianum* is used as traditional medicine for swelling and pain

and also consumed as liver protectant. Leaves of *Zingiber wightianum* is also used as a fumigant to repel mosquitoes.

3.1.4 Global distribution

Zingiber wightianum is native to dense evergreen forests of Peninsular India and Sri Lanka. In South India, it is endangered. It is distributed in Palakkad, Pathanamthitta, Thiruvananthapuram, Kozhikkode, Thrissur, Ernakulam, Idukki, Wayanad.

Systematic position of *Zingiber wightianum*

Kingdom : Plantae

Phylum : Tracheophyta

Class : Monocotyledonae

Order : Zingiberales

Family : Zingiberaceae

Genus : Zingiber

Species : ***Zingiber wightianum***

Basic information

Habit : Herb

Medicinal : Yes

Habitat : Dense tropical forest

Flowering & fruiting : February- August

Distribution : Tropics

Localities : Palakkad, Pathanamthitta, Thiruvananthapuram, Kozhikkode, Thrissur, Ernakulam, Idukki, Wayanad.

Plate 1. *Zingiber wightianum* Thwaites (Malayinchi)

Plate 2. Inflorescence of *Zingiber wightianum* Thwaites

Plate 3. Herbarium specimen of *Zingiber wightianum* Thwaites

3.2 MATERIALS

3.2.1 Plant material

The rhizome of *Zingiber wightianum* was collected based on the traditional claim of local people of Kozhikode district, Kerala. The rhizomes of *Zingiber wightianum* was collected from the Western Ghats hills, identified, authenticated by taxonomist of JNTBGRI and herbarium of plant specimen was deposited at JNTBGRI Herbarium (Acc No: 39190, 39191).

3.2.2 Experimental animals

Swiss Albino Mice (20-30 g) and Wistar rats (100 to 200 g) (Plate 5) of both sexes were obtained from the Animal house of Jawaharlal Nehru Tropical Botanical Garden Research Institute, Palode. The experimental animals were divided into various classes and held in cages of polypropylene and held under normal environmental conditions with temperature 25 ± 2 °C, the relative moisture of 60 ± 10 %, room air changes 15 ± 3 times/hour and 12-hour dark- light cycles. Commercial rodent feed (Lipton India Ltd; Mumbai, India) and water were supplied for the animals. Animals were acclimatized for a week before an experiment was initiated. Both animal studies were performed according to NIH guidelines and approval of the Institute Animal Ethics Committee.

3.2.3 Drugs and chemicals

Carrageenan, Formalin (Formaldehyde), 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, Thiobarbituric 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 rhizomes was washed thoroughly under running tap water and then in distilled water to remove debris. For nearly 10-12 days, they were dried in shade, and made into fine powder. Cold extraction of 100 g of rhizome powder (using magnetic stirrer) was done with 100 % ethanol for 3 hrs. The extract was filtered using Whattman No.1 filter paper, and the residue was replenished for the next round of extraction with fresh solvent. The extraction stage was conducted 3 times to improve the yield of the extract. The collected filtrates were transferred to round bottom flask and were pooled together and concentrated to obtain ethanolic extract in a rotary vacuum evaporator (Buchi, rotavapor R – 215) at 40^{o}C. The extraction was performed sequentially for 50% ethanol and 100% water, and the collected filtrates were concentrated at 40^{o}C in a rotary vacuum evaporator (Buchi, rotavapor R – 215) to obtain 50 % hydro alcoholic and aqueous extracts. The collected filtrate was transferred to the round bottom flask and was concentrated using a rotary evaporator (Buchi, rotavapor R – 215) and kept in a desiccator at room temperature for drying. The extracts were used for conducting the preliminary phytochemical screening, antioxidant activities and anti-inflammatory activities.

3.3.2 Determination of extract yield

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

3.3.3 Determination of solubility

In each mortar and pestle, a pinch of extract was blended into a few drops of distilled water, 0.1 percent Tween-80, 0.5 percent Tween-80 and 1 percent Tween-80 respectively and the extent of the solubility was observed.

3.4 PRELIMINARY PHYTOCHEMICAL TEST

3.4.1 Standard phytochemical screening tests

Using standard procedures of Harborne (1998), the qualitative phyotochemical screening of *Zingiber wightianum* was performed. Phytochemical evaluation of *Zingiber wightianum* was conducted using the following standard procedures.

1. Detection of Alkaloids

a) Mayer's test

Few drops of Mayer's reagent was added to the test tube containing 1 mL of filtrate. Formation of yellow crystalline precipitate indicates the presence of alkaloids.

b) Wagner's test

To a few mL of filtrate, a few drops of Wagner's reagent was added. The reddish brown precipitate formation confirms the presence of alkaloids.

c) Hager's test

A few drops of Hager's reagent was added to 1 mL of filtrate. Yellow precipitate formation confirms the presence of alkaloids.

d) Dragendorff's test

2 mL of Dragendorff's reagent were added to the test tube containing few mL of filtrate. Alkaloids presence is substantiated by the development of brownish orange precipitate.

2. Flavonoids

a) Alkaline reagent test

To the test tube containing 1 mL of extract, diluted HCl was added drop by drop. Presence of flavonoid is indicated by the transition of yellow fluorescence to colourless on addition of HCl.

b) Shinoda test

To a few mL of alcohol, the extract was mixed and added a small piece of magnesium turnings and add a drop of concentrated hydrochloric acid. Crimson colour formation indicates flavonoid presence.

c) Lead Acetate test

Few drops of 10% 1M lead acetate were added to the tube containing few mL of extract. Presence of flavonoid is indicated by the formation of yellow precipitate.

3. Phenols

a) Lead acetate test

Dissolved 50 mg of extract in distilled water and 3 mL of 1% lead acetate solution were added to it. Yellowish precipitate formation showed the presence of phenolic compounds.

4. Tannins

a) Braymer's Test

1 mL of distilled water and 2-3 drops of ferric chloride was added to the test tube containing 1 mL of plant extract. Green precipitate development indicates the presence of tannins.

5. Saponins

a) Foam test

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

b) Froth test

To the test tube containing 5 mL water, 1 g sample was added and heated. Froth formation confirms the presence of saponins.

6. Carbohydrates

a) Fehling's test

Equal amount of Fehling's solution A and B was added to 1 mL of the extract and the suspension was boiled in a water bath. The presence of sugar is indicated by reddish precipitate formation.

7. Proteins

a) Xanthoproteic test

To a test tube containing 1 mL of Sulphuric acid, 1 mL of filtrate was added. Presence of proteins is indicated by the formation of white precipitate.

b) Biuret test

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

8. Steroids

a) Salkowski's test

The extract was mixed with chloroform. To that, a drop of concentrated sulphuric acid was added. Reddish brown ring development in the lower layer shows the presence of steroids.

9. Anthocyanin

1 mL 2N HCl and a few drops of Ammonia was added to the test tube of 1 mL extract. The presence of anthocyanin indicated a change in pinkish red to bluish violet colouration.

10. Glycosides

1 mL of chloroform and acetic acid was added to the test tube containing 1 mL of the extract. The violet colour formation that changes to the blue indicates the presence of glycosides.

b) Killer killiani test

1 mL of distilled water and sodium hydroxide solution was added to the test tube of 1 mL extract, yellow coloration implies the presence of glycosides.

11. Phlobatannins

1 mL of dil. HCl was added to the test tube containing 1 mL extract, and heated. The presence of phlobatannins is indicated by the formation of red precipitate.

3.4.2 Total phenolic content.

The total phenolic content (TPC) was determined according to the method of Sakat *et al.,* (2009) by using spectrophotometry. To the tubes containing 1.0 mL 10 % Folin-Ciocalteu's reagent, 0.2 mL of EZW (1mg/mL), HZW (1mg/mL) and AZW (1mg/mL) was transferred separately. After 10 min, 0.8 mL of sodium carbonate solution (7.5% w/v) were added to the mixture. The tubes were kept for 30 min at room temperature, and the absorbance was read at 743 nm. The results were derived from a gallic acid calibration curve (ranging from 0–250 µg/mL) and expressed in equivalents of gallic acid (GAE) per gram of dry extract weight.

3.4.2 Total flavonoid content

The total flavonoid content (TFC) was determined according to the method of Marinova *et al*., (2005) with slight modification. Rhizome extract in methanol was mixed with 0.1 mL of 10% aluminium chloride hexahydrate, 0.1ml of potassium acetate and 2.8 ml of deionised water. After incubation at room temperature for 30 mins, the

absorption of the reaction mixture was determined at 415 nm. Rutin served as the standard. The total flavonoid content was calculated from the standard curve and expressed as Rutin equivalents (RE) per gram of dry extract weight.

3.5 *IN VITRO* ANTIOXIDANT ACTIVITY

In vitro antioxidant activity of EZW, HZW and AZW were evaluated using standard procedures as mentioned below.

3.5.1 Estimation of Nitric oxide radical scavenging activity

Using the standard procedure of Marcocci *et al*., (1994) nitric oxide radical scavenging activitiy of *Zingiber wightianum* is measured. 0.1mL of 10 mM of sodium nitroprusside was mixed with 1 mL of various concentrations (50 to 250 µg/mL) of rhizome extract / serial fractions of phosphate buffer (pH 7.4). The test tubes was incubated for 2.5 hours at 25° C. 1 mL of the incubated solution was added to 1 mL of Griess reagent (2% O-phosphoric acid, 1% Sulphanilamide and 1% Napthylethylenediamine dihydrochloride). L-Ascorbic acid served as standard. The absorbance was read at 546 nm and using the following formula, percentage of inhibition was calculated:

$$\text{Percentage of inhibition} = [(A_c - A_t) / A_c]$$

Where, A_c – Absorbance of control

A_t – Absorbance of test

3.5.2. Hydroxyl radical scavenging assay

Hydroxyl radical (Elizabeth *et al*., 1990) assay is based on quantification of 2-deoxy ribose degradation by condensation with thiobarbituric acid. 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); various concentrations of extract / standard (Ascorbic acid) (50-200 µg/ml) were added. The reaction mixtures had been incubated for 1 hr at 37^{o}C. The reaction was stopped after incubation by adding 1.5 mL of 2.8% TCA followed by adding 1.5 mL of 1% TBA to each mixture.

Spectrophotometrically, the intensity of the colour formed was measured at 530 nm. The sample-free reaction mixture was utilized as control. Percentage inhibition was calculated as:

$$\text{Percentage of inhibition} = [(A_c - A_t) / A_c] \times 100$$

Where, A_c – Absorbance of control

A_t – Absorbance of test

3.5.3. Estimation of DPPH radical scavenging activity.

The effect of *Zingiber wightianum* on DPPH radicals were assayed using the standard method (Saeed *et al*., 2012). A methanolic solution of 2 mL of DPPH (0.025 g/L) was added to 200 µL of the various concentrations (12.5 µg/mL to 200 µg/mL) of plant extract/serial fractions and allowed to react for 30 min at room temperature in the dark condition. Then the absorbance was measured at 517 nm. Methanol was used as a blank and 200 µL of methanol was added to DPPH instead of plant extract in positive control tubes. Ascorbic acid is served as standard. The percentage inhibition of the samples were calculated using the formula:

$$\text{Percentage of inhibition} = [(A_c - A_t) / A_c] \times 100$$

Where, A_c – Absorbance of control

A_t – Absorbance of test

3.5.4. Estimation of Superoxide radical scavenging activity.

The effect of Superoxide radical scavenging activity was determined by the method of reducing Nitroblue tetrazolium described by Morakinyo *et al*., 2012. 0.4 mL of different concentrations of the extract /serial fractions were mixed with 1 mL of NADH solution (468 µM NADH in 100 mM phosphate buffer, pH 7.4) and 1 mL of NBT solution (156 µM NBT in 100 mM phosphate buffer, pH 7.4). The reaction was triggered with the addition of 100 µL of phenazine methosulphate solution to the mix. The reaction mixture was incubated for 5 min at 25° C, and the absorbance

was measured at 560 nm against blank containing all the reagents except PMS. Trolox served as standard. Inhibition percentage was determined using formula:

$$\text{Percentage of inhibition} = [(A_c - A_t) / A_c] \times 100$$

Where, A_c – Absorbance of control

A_t – Absorbance of test

3.5.5. Determination of ABTS radical scavenging activity.

The ABTS [2,2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid)] radical scavenging activity was performed by the method described by Morakinyo *et al*., 2012. The ABTS radical was developed by the reaction of the ABTS stock solution, 7 mM with 140 mM of potassium persulphate ($K_2S_2O_8$) leaving it in the dark for 16 hours before use at room temperature. This solution was diluted sufficiently with alcohol to have an absorbance of 0.70 ± 0.05 at 734 nm. To 3 mL of rhizome extract, 1 mL of the ABTS solution was added. After 6 min, absorbance was measured at 734 nm. Ascorbic acid served as a standard of reference.

$$\%\text{ of ABTS radical scavenging} = [(A_c - A_t) / A_c] \times 100$$

Where, A_c – Absorbance of control

A_t – Absorbance of test

3.5.6. Ferric Reducing Antioxidant Potential (FRAP) assay.

The ability of *Z. wightianum* to reduce the ferric ion was measured using the method described by Meryem *et al*., 2016. 200 µL of rhizome 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- $FeCl_3$ $6H_2O$ solution) and the reaction mixture was incubated in a water bath at 37˚C for 30 min. The absorbance was measured at 593 nm. Antioxidant potential was calculated from Trolox calibration curve and expressed as an equivalent of µmol Trolox / g extract.

3.5.7. Determination of Total antioxidant activity.

The total antioxidant activity of the *Z. wightianum* was measured by the Phosphomolybdenum method (Prieto *et al*., 1999). 200 µl of extract solution (mg / mL) was mixed with 2 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4mM ammonium molybdate) in the respective solvent. The mixture of reactions was incubated for 90 min at 95^{o}C. The blank solution contained 2 mL of the reagent solution. The absorbance was measured at 635 nm. Ascorbic acid was used as the standard. The total antioxidant capacity of the rhizome was determined from the Ascorbic acid calibration curve and expressed as µg Ascorbic acid equivalents (AAE) /g of dry extract.

3.6 ACUTE TOXICITY STUDIES

In Swiss albino mice, the acute toxic effect of EZW was evaluated according to the OECD guidelines (Suja *et al*., 2004). Before starting the study, all groups were fasted for 12 hours 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 two 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 8 hours and once a day for the next 14 days. Animals were weighed and observations for mortality, behavioural 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.

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

Groups	Treatment	Volume (ml) (Per orally)	No: of animals per group
1	Control	0.5	2
2	EZW 50(mg/kg)	0.5	2
3	EZW 150 (mg/kg)	0.5	2
4	EZW 450 (mg/kg)	0.5	2
5	EZW 1000 (mg/kg)	0.5	2
6	EZW 2000 (mg/kg)	0.5	2

3.7 ANTI-INFLAMMATORY STUDIES

3.7.1 *In vitro* anti-inflammatory study

a) HRBC membrane stabilization assay

Using standard procedures of Gandidasan *et al*., (1991), HRBC membrane stabilization was determined and it was used as a tool for determining the anti-inflammatory response of a drug. From a healthy person, blood was collected and mixed with an equal volume of sterilized Alseiver solution (0.8% sodium citrate, 0.05% citric acid, 2% dextrose and 0.42 % sodium chloride in water). At 3000 rpm, the blood was centrifuged and the packed cells were washed with 0.85 % isosaline (pH 7.4). Then by using isosaline, a 10% v/v suspension was made. Using distilled water, various extract concentrations (50, 100 and 150 µg/mL) were prepared. The assay mix consists of the drug, 0.5 ml HRBC suspension mixture, 2 ml 0.36 % hypo saline and 1 ml 0.15 M phosphate buffer (pH 7.4). Diclofenac sodium was used as the reference standard. For 30 minutes, sample mixes were incubated at 37^{o}C, and then centrifuged. The amount of haemoglobin in the supernatant solution was measured using spectrophotometer at 560 nm. The haemolysis percentage was determined by assuming that 100% of the haemolysis was occurred in the presence of distilled water. The HRBC membrane stabilization or percentage inhibition was calculated using the following equation,

$$\% \text{ Inhibition} = [(Ac - At) / Ac] \times 100$$

Where, Ac – Absorbance of control

At – Absorbance of test

b) Albumin denaturation assay

Albumin denaturation assay was determined according to the method of Williams *et al*., (2008). The reaction mixture (5 mL) comprised of 2 mL of varying concentrations (50,100,150, 200µg/mL) of extract, 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 0.2 mL of fresh egg albumin. Same volume of double-distilled water taken as the control. Then the mixtures were incubated for 15 minutes at 37 ° C in a water bath and then heated for five minutes at 55 ° C. The absorbance was measured at 660 nm after

cooling. Diclofenac sodium was used as the reference standard. By using the following formula the percentage inhibition of protein denaturation was calculated:

$$\% \text{ Denaturation inhibition} = (1 - V_t / V_c) \times 100\%$$

Where, V_t = absorbance of the test sample

V_c = absorbance of control.

3.7.2 *In vivo* anti-inflammatory studies

i. Carrageenan induced paw oedema study

Using Carrageenan - induced rat paw oedema (Amdekar et al., 2011) method, the anti-inflammatory activity of the ethanolic extract of *Zingiber wightianum* (EZW) was studied. The animals were divided into four groups, with two animals in each cages, and were fasted overnight. The pattern of drug administration for each group was included in Table 2. 30 min after administering EZW (50 and 100 mg/kg) or with distilled water (vehicle control) or 10 mg/kg reference drug (Indomethacin) in different groups of animals, 0.1 ml 1 % carrageenan (in saline) was injected into the right hind paw, subcutaneously under the plantar apo-neurosis for oedema elicitation. The volume of hind paw was measured by using a plethysmometer, by dipping the foot up to the anatomical hairline on lateral malleolus. Paw oedema was measured, before and hourly during the first and third hours after the carrageenan injection. The ability of EZW to suppress paw inflammation was expressed as a percentage inhibition of paw oedema and calculated using the following equation:

Oedema volume = (Oedema volume of animals after swelling – Oedema volume of animals before swelling)

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

Table 2. Pattern of Drug administration for Carrageenan induced paw oedema

Groups	Treatment	Volume (per orally) (mL)	No. of animals/ group
1	Control (Distilled water)	1	2
2	Indomethacin (10 mg/kg)	1	2
3	EZW (50mg /kg)	1	2
4	EZW (100mg /kg)	1	2

ii. Formalin induced paw oedema study

The anti-inflammatory activity of the ethanolic extract of *Zingiber wightianum* (EZW) was studied by the method, Formalin induced paw oedema in wistar rats (Shikha *et al*., 2010). The animals were divided into four groups, with two animals in each cages, and were fasted overnight. The pattern of drug administration for each group was included in Table 3. 30 min after administering EZW (50 and 100 mg/kg) or with distilled water (vehicle control) or 10 mg/kg reference drug (Aspirin) in different groups of animals, 0.1 ml 1 % formalin (in saline) was injected into the right hind paw, subcutaneously under the plantar apo-neurosis for oedema elicitation. The volume of hind paw was measured by using a plethysmometer by dipping the foot up to the anatomical hairline on lateral malleolus. Paw edema was measured in the rats receiving the EZW, distilled water and aspirin, using a plethysmometer, before and hourly during the first and third hours after the formalin injection. The ability of EZW to suppress paw inflammation was expressed as a percentage inhibition of paw oedema and calculated using the following equation:

Oedema volume = (Oedema volume of animals after swelling – Oedema volume of animals before swelling)

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

Table 3. Pattern of Drug administration for Formalin induced paw oedema

Groups	Treatment	Volume (per orally) (mL)	No. of animals/ group
1	Control (Distilled water)	1	2
2	Aspirin (10 mg/kg)	1	2
3	EZW(50mg /kg)	1	2
4	EZW (100mg /kg)	1	2

3.8 STATISTICAL ANALYSIS

The obtained results were presented as mean and standard deviation (SD) and displayed as graphs and tables. Results were evaluated using a statistical toolpad in Microsoft Excel 2016 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 paired comparisons between groups. The level of significance was set at ***$p<0.05$.

4. RESULTS

4.1. MORPHOLOGICAL CHARACTERISTICS

Zingiber wightianum Thwaites is a dense evergreen herbaceous perennial, which grows up to 1 m height. The mode of propagation of *Zingiber wightianum* is by the rhizomes. The morphological characteristics of plant were studied, and observations were tabulated (Table 4).

Table 4. Morphological characters of Malayinchi (Zingiber wightianum)

Sl No.	Morphological Characters	Observation
1	Habit	Herb
2	Mode of propagation	Rhizomes
3	Leaves	ligule 5-6 mm long, bifid; oblong- lanceolate, tip accuminate
4	Root	Thick rhizome, 2-2.5 cm, fleshy, with many fleshy roots
5	Venation	Parallel
6	Colour	Green on ventral side and light green on dorsal side
7	Leaflet width	Ranges from 5-6 cm
8	Leaflet length	Ranges from 15-35 cm
9	Odour	Pungent
10	Taste	Peppery

4.1.2 Extract yield

The percentage yield of ethanolic extract (EZW) was found to be 7.04, hydro ethanolic extract (HZW) is 13.16 and aqueous extract (AZW) is 9.02.

4.1.3 Extract miscibility

The extract was highly soluble in distilled water and this solvent was chosen as vehicle for the preparation of drugs.

4.2 PRELIMINARY PHYTOCHEMICAL TEST

4.2.1 Standard phytochemical screening tests

Qualitative phytochemical analysis confirmed the presence of phenols, saponins, flavonoids, glycosides, phlobatannins, anthocyanins and steroids in rhizome (Table 5).

Table 5. Phytochemical study of plant Malayinchi (Zingiber wightianum)

Sl No		Test	Result		
			EZW	**HZW**	**AZW**
1	**Alkaloids**	Mayer's test	-	-	-
		Wagner's test	-	-	-
		Hager's test	-	-	-
		Dragendroff's test	-	-	-
2	**Flavonoids**	Alkaline reagent test	+	+	+
		Shinoda test	+	+	+
		Lead acetate test	+	+	+
3	**Phenols**	Lead acetate test	+	+	+
4	**Tannins**	Braymer's test	-	-	-
5	**Saponins**	Foam test	+	+	+
		Froth test	+	+	+

6	**Carbohydrates**	Fehling's test	-	-	-
7	**Proteins**	Xanthoproteic test	-	-	-
		Biuret test	-	-	-
8	**Steroids**	Salkowski's test	+	+	+
9	**Anthocyanins**	Test for Anthocyanins	+	+	+
10	**Glycosides**	Killer killiani test	+	+	+
11	**Phlobatannin**	Test for Phlobotannin	+	+	+

(+ and – indicates the presence and absence of respective phytochemicals)

4.2.2 Total phenolic content

From the mixture of 1.0 mL 10 % Folin-Ciocalteu's reagent, 0.2 mL of EZW (1mg/mL), HZW (1mg/mL) and AZW (1mg/mL) and, 0.8 mL of sodium carbonate solution (7.5% w/v), the absorbance at 743 nm of EZW, HZW and AZW was found to be 0.1997, 0.1905 and 0.1735 respectively. Gallic acid was used as the standard and the standard curve was plotted. The absorbance of gallic acid at concentrations (mg/ml) 50, 100, 150 and 200 are 0.9569, 2.055, 2.9076 and 3.7410 respectively. Using the trendline obtained from plotting the graph using these values, an equation for deriving the values for EZW, HZW and AZW was obtained (Figure 1).

$$y = 0.0191x + 0.0106$$

From the equation, the total phenolic content (TPC) of EZW, HZW and AZW was found to be 10.3 mg GAE/g of extract, 9.9 mg GAE/g of extract and 9.1 mg GAE/g of extract and expressed in equivalents of gallic acid (GAE) per gram of dry extract weight.

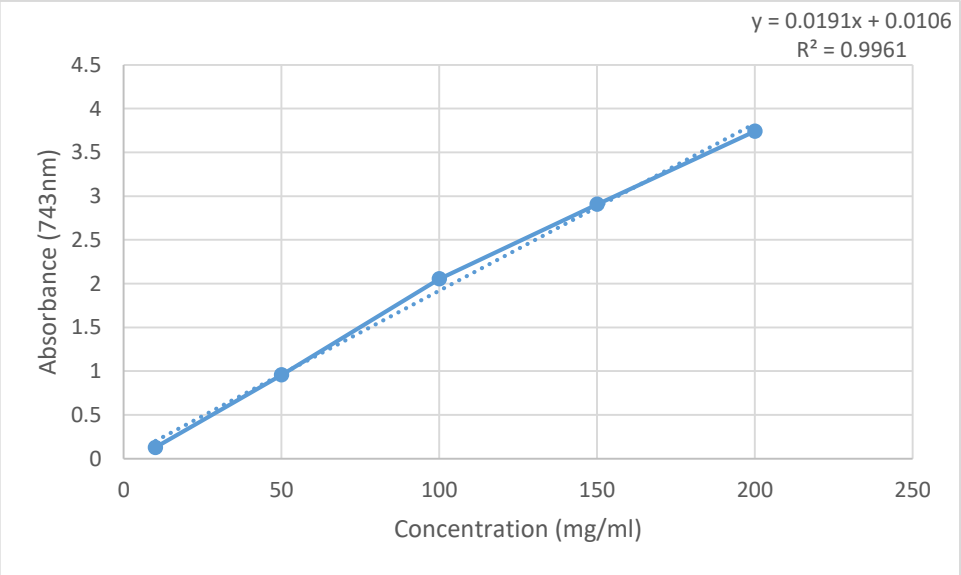

Figure 3. Standard curve of gallic acid

Values are expressed as mean ± SD, for n=3.

4.2.3 Total flavonoid content

From the mixture of methanolic rhizome extract, 0.1 mL of 10% aluminium chloride hexahydrate, 0.1ml of potassium acetate and 2.8 ml of deionised water, the absorbance of EZW, HZW and AZW at 415 nm was found to be 0.1197, 0.1086 and 0.1035 respectively.

Rutin was used as the standard and the standard curve was plotted. The absorbance of rutin at concentrations (mg/ml) 50, 100, 150 and 200 are 1.6048, 2.2517, 2.8862 and 3.3277 respectively. Using the trendline obtained from plotting the graph using these values, an equation for deriving the values for EZW, HZW and AZW was obtained (Figure 2).

$$y = 0.0161x + 0.4776$$

From the equation, the total flavonoid content (TfC) of EZW, HZW and AZW was found to be 7.5 mg RE/g of extract, 6.8 mg RE/g of extract and 6.5 mg RE/g of extract and expressed in equivalents of rutin (RE) per gram of dry extract weight.

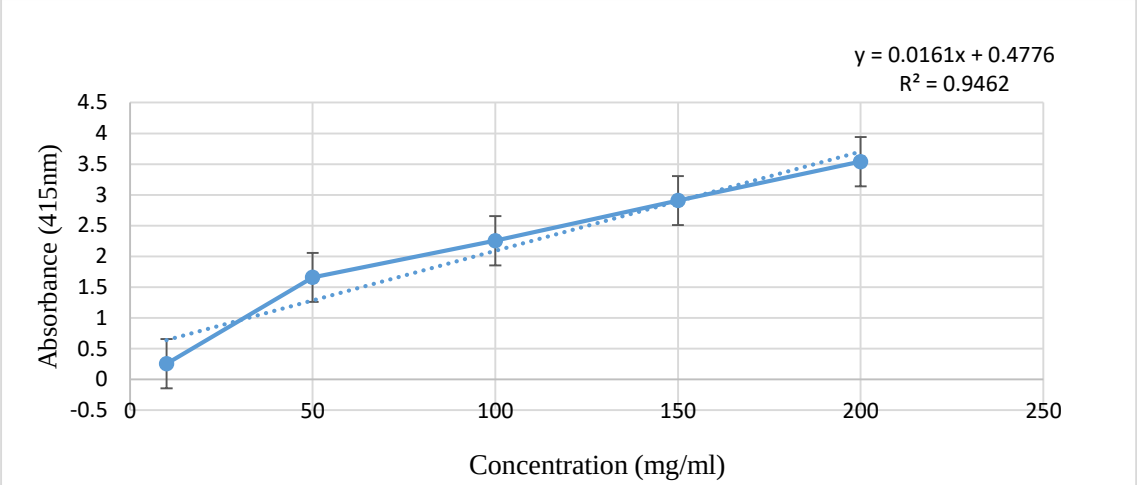

Figure 4. Standard curve of rutin.

Values are expressed as mean ± SD, for n=3.

4.3. *IN VITRO* ANTIOXIDANT STUDY

4.3.1 Hydroxyl radical scavenging assay

In hydroxyl radical scavenging assays, through Fenton reaction – OH radical is generated, where $FeCl_3$-EDTA complex reacts with hydrogen peroxide to produce hydroxyl radical. IC_{50} of ethanolic extract is 121.83 µg/mL whereas hydro ethanolic and aqueous extract showed IC_{50} value of 123.67 µg/mL and 132.66 µg/mL. Standard, ascorbic acid showed a highest scavenging activity (58.13 %) with IC_{50} of 20.08 µg/mL. The presence of phytochemicals such as phenols could be the reason for the hydroxyl radical scavenging activity.

Table 6. Effect of extracts of Z. wightianum on hydroxyl radical scavenging activity

Sl No	Conc. (µg/mL)	% scavenging of EZW (530 nm)	% scavenging of HZW (530 nm)	% scavenging of AZW (530 nm)	% scavenging of Ascorbic acid (530nm)
1	50	45.70 ± 0.50	44.57 ± 1.10	44.89 ± 1.80	60.65 ± 1.03
2	100	47.96 ± 1.06	48.04 ± 1.92	47.52 ± 0.75	64.68 ± 0.65
3	150	52.62 ± 0.63	52.17 ± 1.47	51.31 ± 0.95	70.93 ± 0.09

4	200	55.88 ± 1.15	54.26 ± 1.38	52.72 ± 1.09	80.44 ± 1.08
5	250	59.37 ± 1.35	54.79 ± 1.11	58.34 ± 0.91	88.47 ± 0.77
6	IC_{50}	121.83µg/mL	123.67µg/mL	132.66 µg/mL	20.80µg/mL

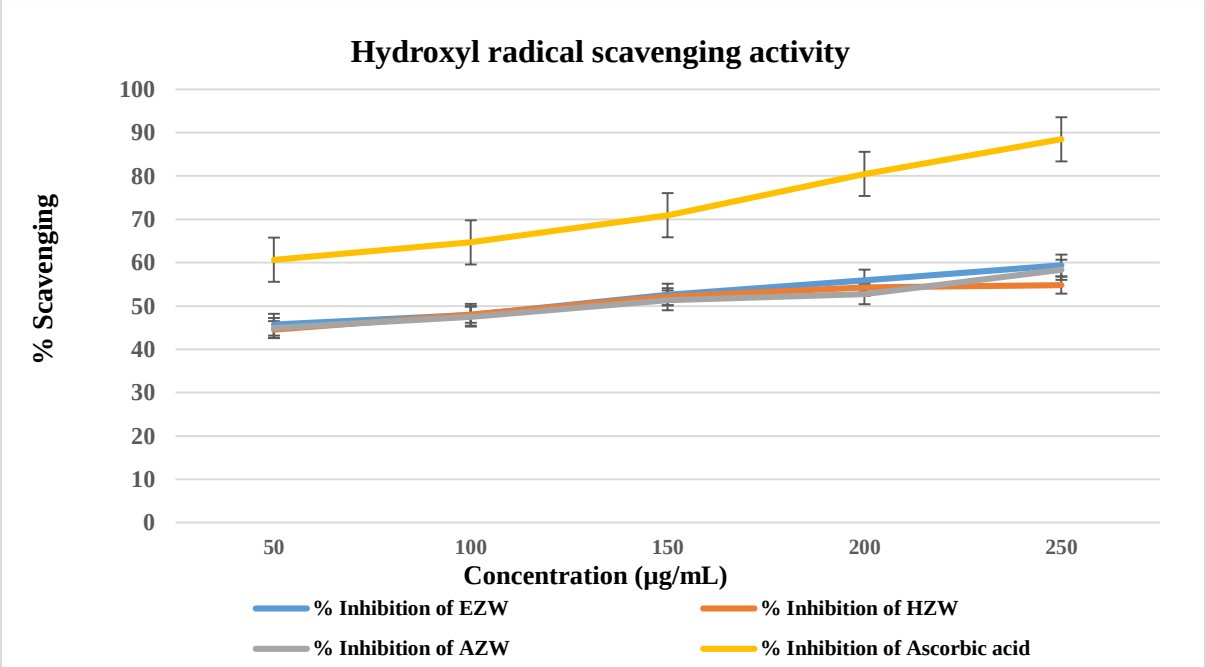

Figure 5. Hydroxyl radical scavenging activity of ethanolic, hydro ethanolic and aqueous extract of *Z. wightianum*

Values are expressed as mean ± SD, for n=3.

4.3.2 Nitric oxide radical scavenging assay

The nitric oxide radical scavenging of extracts of *Z. wightianum* is shown in table and the findings were compared with that of standard L-Ascorbic acid. IC_{50} of ethanolic extract is 86.27 µg/mL whereas hydro ethanolic and aqueous extract showed IC_{50} value of 69.85 µg/mL and 135.82 µg/mL. Standard, ascorbic acid showed a highest scavenging activity (58.13 %) with IC_{50} of 20.84 µg/mL.

Table 7. Effect of extracts of Z. wightianum on nitric oxide radical scavenging activity

Sl No	Conc. (µg/mL)	% scavenging of EZW (546 nm)	% scavenging of HZW (546 nm)	% scavenging of AZW (546 nm)	% scavenging of Ascorbic acid (546 nm)
1	50	48.15 ± 0.98	49.15 ± 1.10	43.15 ± 1.46	60.25 ± 0.44
2	100	50.70 ± 0.85	51.29 ± 1.09	45.80 ± 1.02	64.13 ± 1.05
3	150	53.43 ± 0.93	54.22 ± 0.77	51.66 ± 0.83	71.16 ± 0.89
4	200	55.97 ± 1.91	58.05 ± 1.78	55.42 ± 1.07	84.08 ± 1.09
5	250	61.41 ± 1.41	61.21 ± 1.72	58.88 ± 2.62	90.74 ± 1.12
6	IC_{50}	86.27 µg/mL	69.85 µg/mL	135.82 µg/mL	20.84 µg/mL

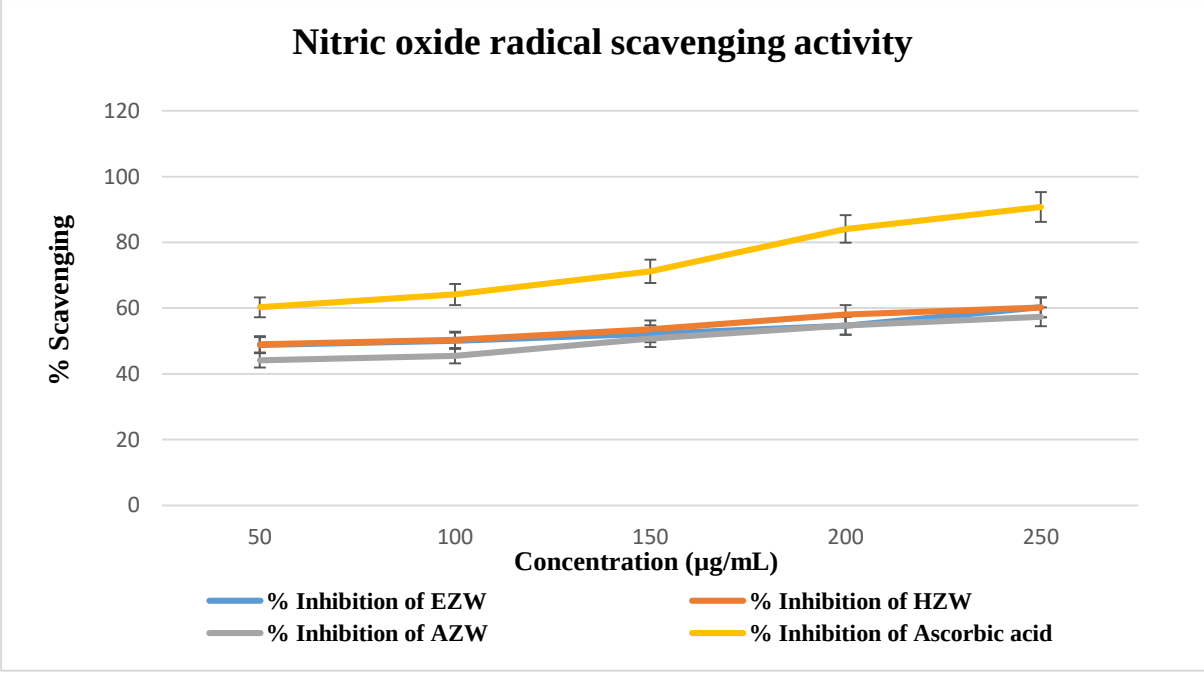

Figure 6. Nitric oxide radical scavenging activity of ethanolic, hydro ethanolic and aqueous extract of *Z. wightianum*,

values are expressed as mean ± SD, for n=3.

4.3.3 Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP value of EZW, HZW and AZW was found to be 822.89 µM, 724 µM and 771 µM Trolox/g dry weight of sample.

From the mixture of rhizome extract and FRAP reagent, the absorbance at 593 nm of EZW, HZW and AZW was found to be 0.1656, 0.1641 and 0.1541 respectively. Ascorbic acid was used as the standard and the standard curve was plotted. The absorbance of ascorbic acid at concentrations (µM) 50, 100, 150 and 200 are 0.1664, 0.1772, 0.2091 and 0.2240 respectively. Using the trendline obtained from plotting the graph using these values, an equation for deriving the values for EZW, HZW and AZW was obtained (Figure 5).

$$y = 0.0004x + 0.1437$$

From the equation, the FRAP value of EZW, HZW and AZW was found to be 414 mg, 410 mg and 385.25 mg /g dry weight respectively.

Table 8. Effect of extracts of Z. wightianum on Ferric Reducing Antioxidant Power (FRAP) assay.

Sl No	Concentration of ascorbic acid (mg/mL)	Absorbance (593 nm)
1	10	0.1664 ± 1.02
2	20	0.1772 ± 0.83
3	30	0.2091 ± 1.32
4	40	0.2248 ± 0.91
5	50	0.2437 ± 1.26
6	EZW (1 mg/ ml)	0.1808 ± 1.52
7	HZW (1 mg/ ml)	0.1595 ± 1.06
8	AZW (1 mg/ ml)	0.1637 ± 0.97

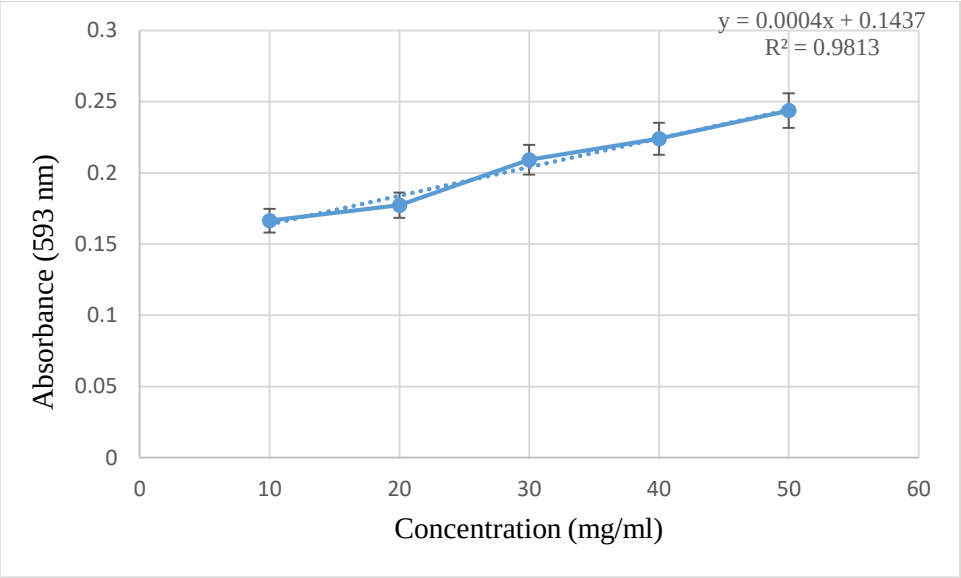

Fig. 7. Standard curve of trolox.
values are expressed as mean ± SD, for n=3.

4.3.4 DPPH radical scavenging assay

DPPH radical scavenging activity of extracts of *Zingiber wightianum* is shown in table 9 and the results were compared with that of standard L-Ascorbic acid. IC_{50} of ethanolic extract is 71.02 µg/mL whereas hydro ethanolic and aqueous extract showed IC_{50} value of 137.48 µg/mL and 137.94 µg/mL. Standard, ascorbic acid showed a highest scavenging activity (58.13 %) with IC_{50} of 18.75 µg/mL.

Table 9. Effect of extracts of Z. wightianum on DPPH radical scavenging assay.

Sl No	Conc. (µg/mL)	% scavenging of EZW (517 nm)	% scavenging of HZW (517 nm)	% scavenging of AZW (517 nm)	% scavenging of Ascorbic acid (517 nm)
1	50	47.70 ± 1.96	29.73 ± 0.99	35.03 ± 1.02	68.30 ± 0.90
2	100	53.17 ± 1.23	39.91 ± 1.96	40.30 ± 1.82	76.87 ± 1.24
3	150	56.67 ± 1.96	53.37 ± 0.98	53.08 ± 1.15	80.18 ± 1.01
4	200	61.04 ± 1.07	61.15 ± 1.37	61.79 ± 0.53	86.28 ± 0.94
5	250	69.20 ± 1.16	70.77 ± 1.00	66.02 ± 1.58	92.53 ± 0.14
6	IC_{50}	71.02 µg/mL	137.48 µg/mL	137.94 µg/mL	18.75 µg/mL

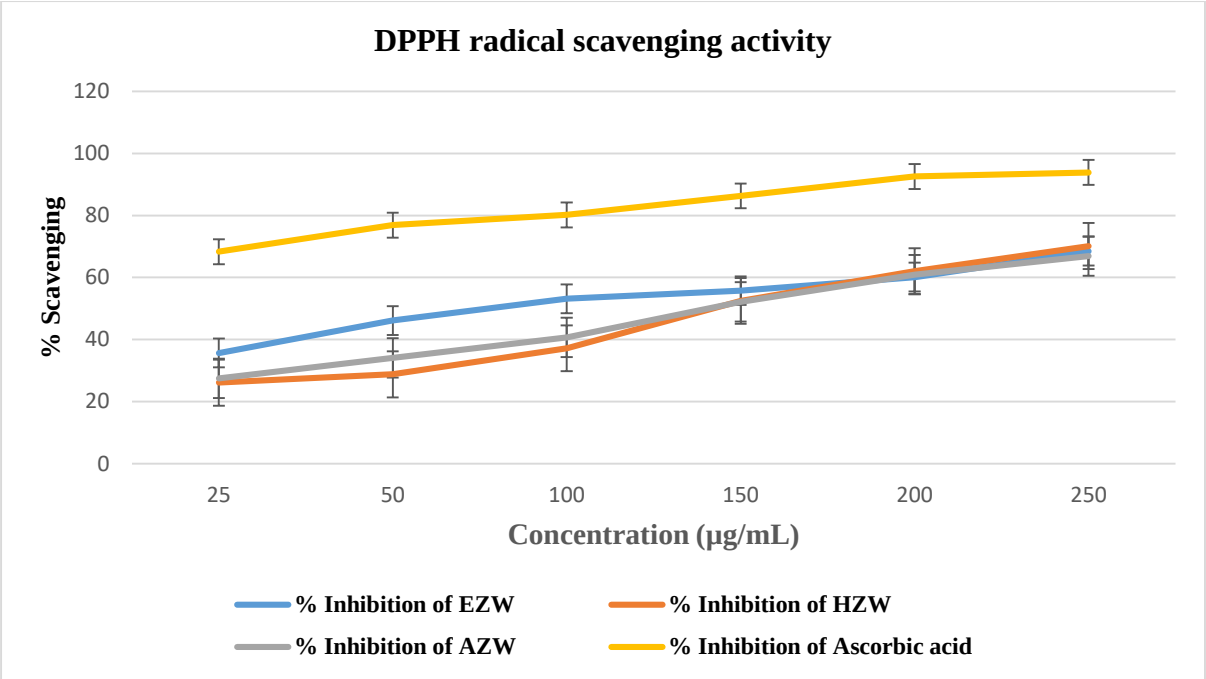

Figure 8. DPPH radical scavenging activity of ethanolic, hydro ethanolic and aqueous extract of *Z. wightianum,*

values are expressed as mean ± SD, for n=3.

4.3.5 Superoxide radical scavenging assay

Superoxide radical scavenging activity of extracts of *Zingiber wightianum* is shown in the table and the results were compared with that of standard trolox. IC_{50} of ethanolic extract is 43.75 µg/mL whereas hydro ethanolic and aqueous extract showed IC_{50} value of 100.07 µg/mL and 90.90 µg/mL. Standard, ascorbic acid showed a highest scavenging activity (58.13 %) with IC_{50} of 16.27 µg/mL.

Table 10. Effect of extracts of Z. wightianum on Superoxide scavenging assay.

Sl No	Conc. (µg/mL)	% scavenging of EZW (560 nm)	% scavenging of HZW (560 nm)	% scavenging of AZW (560 nm)	% scavenging of Trolox (560 nm)
1	25	41.55 ± 1.36	42.27 ± 1.46	37.83 ± 1.15	64.27 ± 0.63
2	50	53.07 ± 0.50	44.52 ± 0.82	41.79 ± 1.33	71.13 ± 1.49

3	100	60.79 ± 1.73	49.99 ± 0.73	52.42 ± 1.40	76.00 ± 1.91
4	150	65.36 ± 1.03	56.73 ± 1.58	56.07 ± 1.17	80.72 ± 0.91
5	200	67.36 ± 1.18	61.06 ± 1.51	60.23 ± 0.89	90.04 ± 0.57
6	IC_{50}	43.75 µg/mL	100.07 µg/mL	90.90 µg/mL	16.27 µg/mL

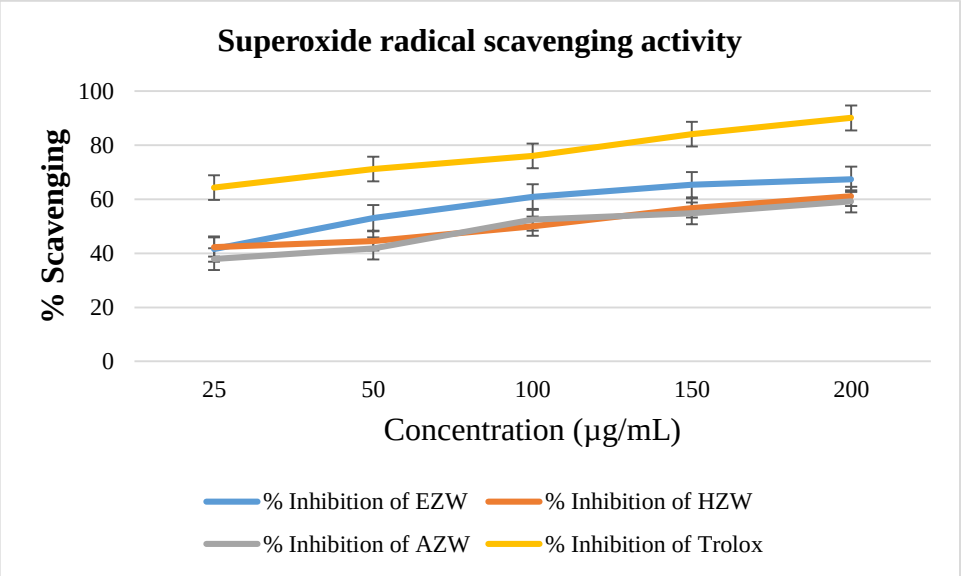

Figure 9. Superoxide radical scavenging activity of ethanolic, hydro ethanolic and aqueous extract of *Z. wightianum*,

values are expressed as mean ± SD, for n=3.

4.3.6 ABTS radical scavenging assay

ABTS radical scavenging activity of extracts of *Zingiber wightianum* is shown in the table 11 and the results were compared with that of standard L-Ascorbic acid. IC_{50} of ethanolic extract is 132.77 µg/mL whereas hydro ethanolic and aqueous extract showed IC_{50} value of 180.28 µg/mL and 190.86 µg/mL. Standard, ascorbic acid showed a highest scavenging activity (58.13 %) with IC_{50} of 18.75 µg/mL.

Table 11. Effect of extracts of Z. wightianum on ABTS radical scavenging assay.

Sl No	Conc. (µg/mL)	% Scavenging of EZW (734 nm)	% Scavenging of HZW (734 nm)	% Scavenging of AZW (734 nm)	% Scavenging of Ascorbic acid (734 nm)
1	50	34.50 ± 1.16	34.49 ± 1.26	39.53 ± 1.35	65.42 ± 0.10
2	100	40.26 ± 1.25	43.49 ± 2.56	43.20 ± 1.62	72.76 ± 0.58
3	150	55.11 ± 1.79	49.14 ± 0.59	46.96 ± 1.82	81.21 ± 0.09
4	200	58.31 ± 1.35	52.84 ± 2.19	50.68 ± 1.11	90.31 ± 0.85
5	250	64.95 ± 1.23	64.82 ± 1.11	62.41 ± 0.95	93.13 ± 1.77
6	IC_{50}	132.77 µg/mL	180.28 µg/mL	190.86 µg/mL	18.75 µg/mL

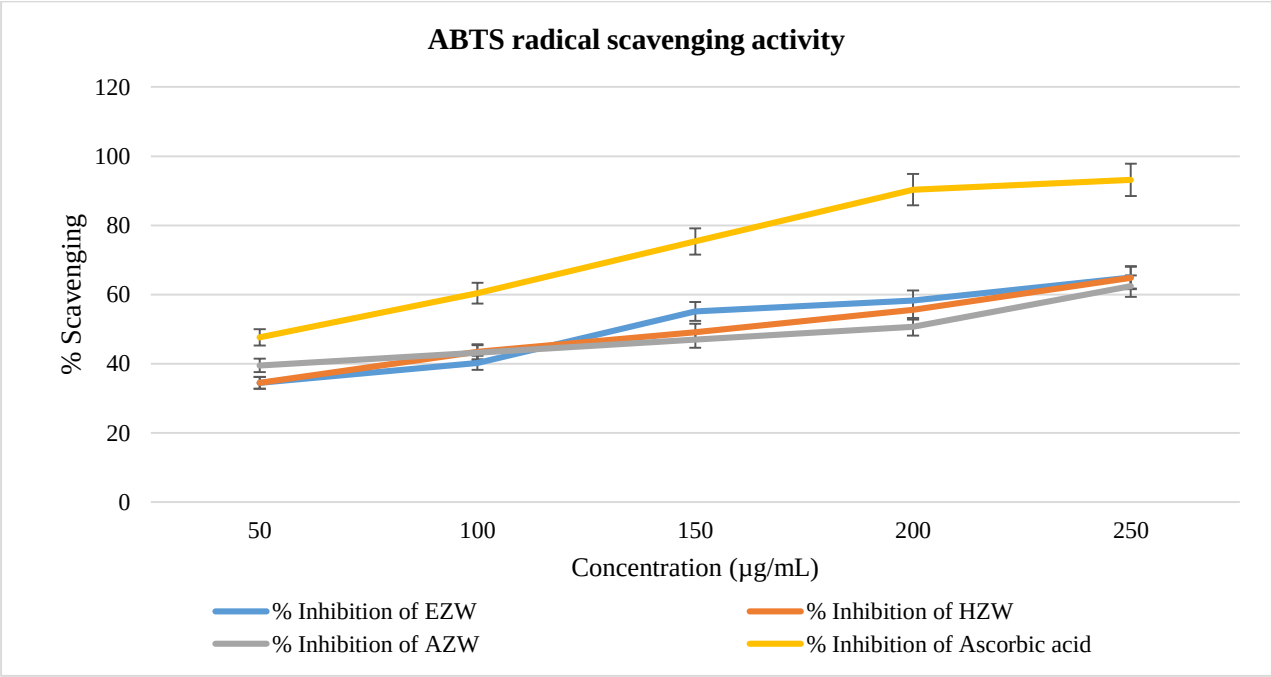

Figure 10. ABTS radical scavenging activity of ethanolic, hydro ethanolic and aqueous extract of *Z. wightianum*.

Values are expressed as mean ± SD, for n=3.

4.3.7 Total antioxidant activity

From the mixture of 200 µl of extract solution (mg / mL), 2 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4mM ammonium molybdate) in the respective solvent, the absorbance at 635 nm of EZW, HZW and AZW was found to be 0.1392, 0.1162 and 0.1350 respectively. Ascorbic acid was used as the standard and the standard curve was plotted. The absorbance of gallic acid at concentrations (mg/ml) 12.5, 25, 50, 75 and 100 are 0.3190, 0.4762, 0.8175, 1.3235 and 1.8596 respectively. Using the trendline obtained from plotting the graph using these values, an equation for deriving the values for EZW, HZW and AZW was obtained (Figure 1).

$$y = 0.0169x + 0.0615$$

From the equation, the total antioxidant activity of ethanolic, hydro ethanolic and aqueous extract of rhizomes of *Z. wightianum* was found to be 83 µg AAE/g, 70 µg AAE/g and 80 µg AAE/g of dry extract.

Table 12. Total antioxidant activity of Zingiber wightianum

Sl No	Conc. of Ascorbic acid(µg/mL)	Mean Absorbance (695 nm)
1	12.5	0.3124 ± 1.00
2	25	0.4762 ± 1.34
3	50	0.8176 ± 1.69
4	75	1.3236 ± 1.86
5	100	1.8596 ± 1.12
6	**EZW (1mg/mL)**	0.1392 ± 0.99
7	**HZW (1mg/mL)**	0.1162 ± 1.47
8	**AZW (1mg/mL)**	0.1351 ± 1.83

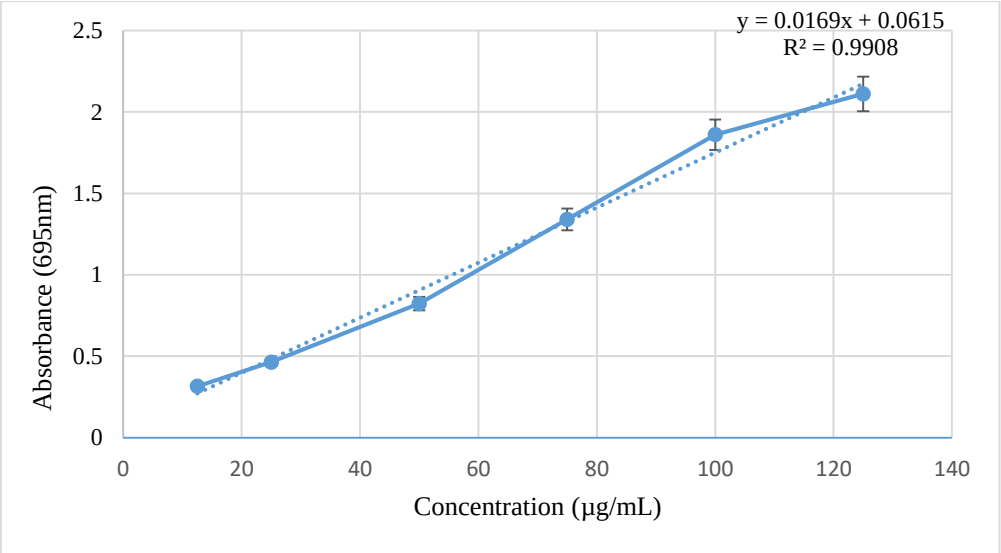

Figure 11. Standard curve of ascorbic acid
Values are expressed as mean ± SD, for n=3.

4.4 ORAL ACUTE TOXICITY STUDIES

For the acute toxicity study, the ethanolic extract of *Z. wightianum* was administered orally to mice at doses of 5, 150, 450, 1000 and 2000 (mg / kg body weight) for each group. Even at the maximum dosage (2000 mg / kg), no mortality and behavioural changes were observed. The other parameters of test animals such as respiration, skin conditions, behaviour pattern etc. have been monitored and the results are tabulated.

Table 13. Acute toxicity studies of ethanolic extract of Z. wightianum

Sl No	Parameters	Normal	EZW 50 (mg/kg)	EZW 150 (mg/kg)	EZW 450 (mg/kg)	EZW 1000 (mg/kg)	EZW 2000 (mg/kg)
a) Difference in body weight							
1	a) Difference in body weight	Nil	Nil	Nil	Nil	Nil	Nil
b) Changes in food and water intake							
2	Food and water intake	Normal	Normal	Normal	Normal	Normal	Normal

c) Cage side observations							
3	Respiration	Normal	Normal	Normal	Normal	Normal	Normal
4	Breathing abnormalities	Nil	Nil	Nil	Nil	Nil	Nil
5	Condition of the fur	Normal	Normal	Normal	Normal	Normal	Normal
6	Skin	Normal	Normal	Normal	Normal	Normal	Normal
7	Subcutaneous swelling	Nil	Nil	Nil	Nil	Nil	Nil
8	Abdominal distension	Nil	Nil	Nil	Nil	Nil	Nil
9	Pupil diameter	Normal	Normal	Normal	Normal	Normal	Normal
10	Wetness or soiling of the perineum	Nil	Nil	Nil	Nil	Nil	Nil
11	Condition of teeth	Normal	Normal	Normal	Normal	Normal	Normal
12	Gait	Normal	Normal	Normal	Normal	Normal	Normal
13	Colour and consistency of the faeces	Normal	Normal	Normal	Normal	Normal	Normal

4.5 *IN VIVO* ANTI-INFLAMMATORY STUDY

4.5.1 Carrageenan induced paw oedema in Wistar rats

In adult wistar rats the effect of ethanolic extract of *Z. wightianum* on paw oedema induced by carrageenan has been examined. The percentage of paw oedema volume inhibition is shown in Table 14 and the difference in volume of paw oedema is shown graphically in Figure 10. The maximum percentage of inhibition of paw oedema (38.91%) was exhibited by a dose of EZW (100 mg/kg). The standard drug Indomethacin exercised a good inhibition level (40.59%) at a dosage of 10 (mg / kg) body weight.

Table 14. Effect of ethanolic extract of Z. wightianum on Carrageenan induced paw oedema.

Sl No	Concentration (mg/kg)	Percentage of inhibition
1	Control (Distilled water)	……
2	Indomethacin (10 mg/kg)	40.59
3	EZW (50mg /kg)	32.64
4	EZW (100mg /kg)	38.91

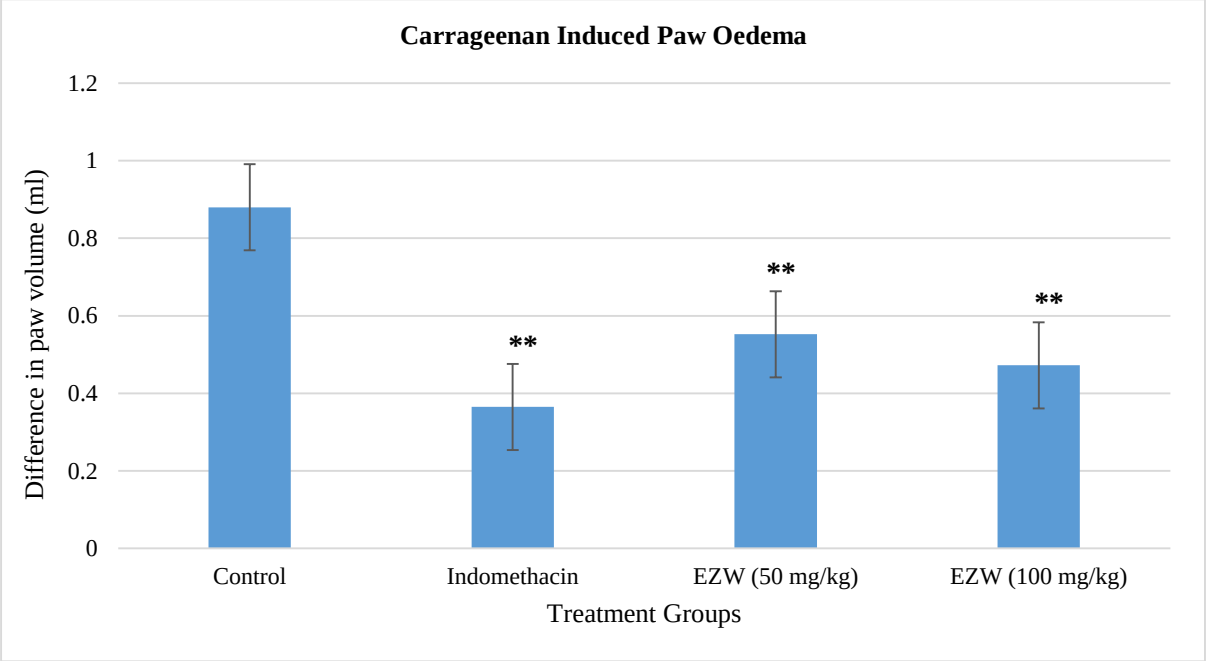

Figure 12. Effect of ethanolic extract of *Z. wightianum* on Carrageenan induced paw oedema in rats.

Values are expressed as mean ± SD, for n=3, one way ANOVA followed by Duncan's multiple comparison test, ***P≤0.05 and ns- no significant difference compared with the control group.

4.5.2 Formalin induced paw oedema in Wistar rats

The effect of ethanolic extract of *Z. wightianum* on formalin induced paw oedema in wistar rats has been studied. The percentage inhibition of paw oedema volume is shown in Table and difference in paw oedema volume is shown graphically in Figure 11. The standard drug Aspirin exhibited a percentage inhibition of 51.07% at a dose of 10 mg/kg

whereas the maximum percentage of inhibition of paw oedema (35.08%) was exhibited by a dose of EZW (100 mg/kg).

Table 15. Effect of ethanolic extract of Z. wightianum on Formalin induced paw oedema.

Sl No	Concentration (mg/kg)	Percentage of inhibition
1	Control (Distilled water)	…..
2	Aspirin (10 mg/kg)	51.07
3	EZW (50mg /kg)	34.03
4	EZW (100mg /kg)	35.08

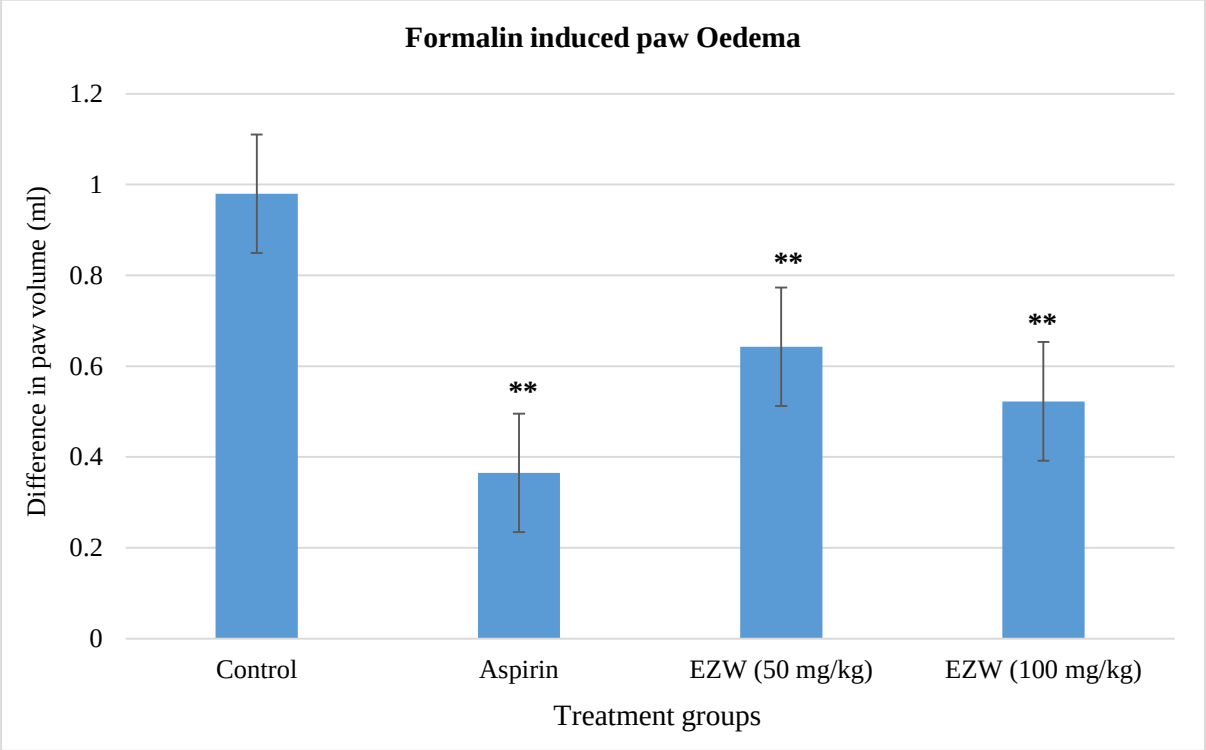

Figure 13. Effect of ethanolic extract of *Z. wightianum* on Formalin induced paw oedema in rats.

Values are expressed as mean ± SD, for n=3, one way ANOVA followed by Duncan's multiple comparison test, ***P≤0.05 and ns- no significant difference compared with the control group.

4.6 *IN VITRO* ANTI-INFLAMMATORY STUDY

4.6.1 HRBC membrane stabilization assay

Ethanolic extracts demonstrated substantial stabilisation of the HRBC membrane at different concentrations. Ethanolic extract at 100 µg / mL concentration offered maximum protection of the HRBC membranes. It exhibited a maximum inhibition at 100 µg / mL of 49.29 %. The EC_{50} extract value was estimated to be 103 µg / mL. Maximum inhibition shown by standard is 63.79% with EC_{50} value of 62µg/mL. The results were shown in Table 16.

Table 16. In vitro anti-inflammatory activity of Zingiber wightianum

Concentration (µg/mL)	(% protection) EZW	(% protection) Standard
Control	….	….
50	44.17	47.61
100	49.29	59.34
150	55.44	63.79
EC_{50} value (µg/mL)	103 µg/mL	62µg/mL

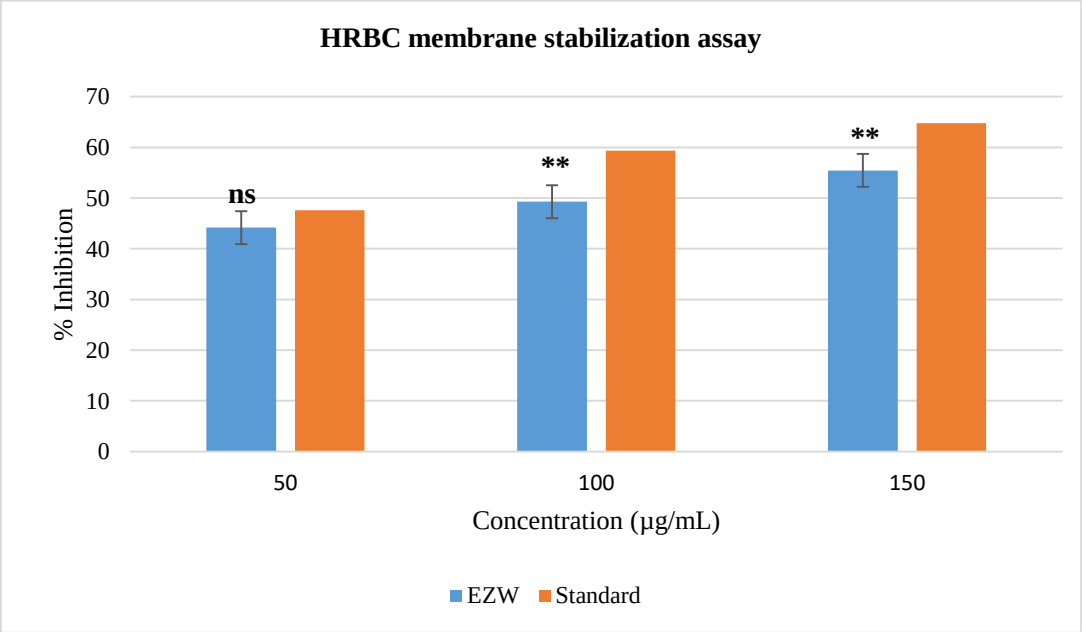

Figure 14. Percentage of inhibition of hemolysis *in vitro* due to ethanolic extract of *Z. wightianum*

.Values are expressed as mean ± SD, for n=3, one way ANOVA followed by Duncan's multiple comparison test, ***P≤0.05 and ns- no significant difference compared with standard.

4.6.2 Albumin denaturation assay

Ethanolic extracts demonstrated substantial protection of albumin from denaturation at different concentrations. Ethanolic extract at 200 µg / mL concentration offered maximum protection of 65.68% with EC_{50} value 168.50 µg/mL where the standard showed maximum protection of 79.41% with EC_{50} value 107.24 µg/mL .The results were shown in Table 17.

Table 17. Percentage of inhibition of denaturation of albumin in vitro due to EZW

Sl No	Conc. (µg/mL)	% Inhibition of standard (660nm)	% Inhibition of EZW (660 nm)
1	50	23.33	33.05
2	100	47.23	36.24
3	150	66.34	40.79
4	200	79.41	65.68
EC_{50} value (µg/mL)		107.24 µg/mL	168.50 µg/mL

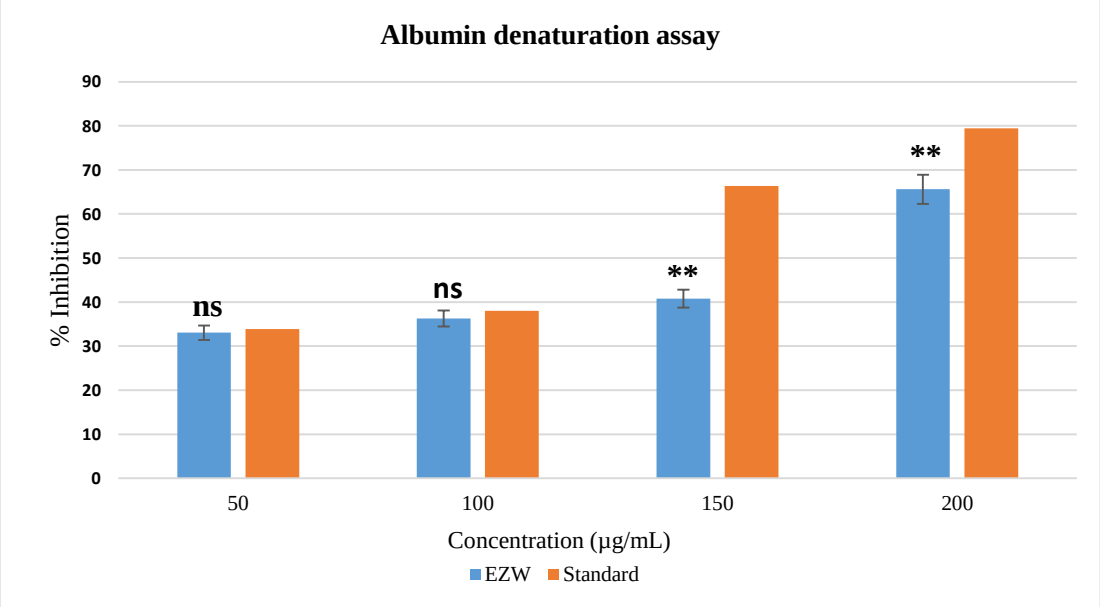

Figure 15. Percentage of inhibition of denaturation of albumin *in vitro* due to ethanolic extract of *Z. wightianum* extract.

Values are expressed as mean ± SD, for n=3, one way ANOVA followed by Duncan's multiple comparison test, ***P≤0.05 and ns- no significant difference compared with standard.

5. DISCUSSION

Plants have been explored comprehensively as potential sources of secondary metabolites or phytochemicals proven to be outstanding compounds with unique properties, making them perfect candidates for much-needed therapeutics. In addition to improving protection against both biotic and abiotic stress, these phytochemicals are an important source of anticancer, antioxidant, antidiabetic, immunosuppressive, antifungal, anti-inflammatory, antimalarial, anti-oomycetic, antibacterial, antifever, antidiabetic, insecticidal and antiviral lagents (Borges *et al*., 2016). Plants have traditionally been used as a source of medicine in India by indigenous people of different ethnic groups inhabiting various terrains for the control of various ailments afflicting human and their domestic animals. In Kerala, the traditional healers of Wayanad uses rhizomes of *Zingiber wightianum* for the treatment of swelling and pain. Based on this traditional knowledge, this particular study envisage evaluation of anti-inflammatory and antioxidant potential of *Zingiber wightianum* Thwaites.

Ethanolic (EZW), hydro ethanolic (HZW) and aqueous (AZW) rhizome extract of *Zingiber wightianum* was used in this study. It is the first study on *Zingiber wightianum* for its phytochemical analysis. Qualitative phytochemical analysis of these extracts revealed the presence of phenols, saponins, flavonoids, glycosides, anthocyanins and steroids. In earlier studies, carbohydrates, flavonoids, saponins, anthocyanins, steroids were present in rhizome of *Zingiber officinale* and *Alpinia galanga* which belong to the same family; Zingiberaceae. These phytochemicals give these plants significant antioxidant activity (Singh *et al*., 2014).

In Quantitative phtochemical analysis of *Zingiber wightianum*, the content of total phenols in the ethanolic, hydro ethanolic and aqueous extract of *Z. wightianum* was found to be 10.3 mg, 9.9 mg and 9.1 mg Gallic acid equivalents per gram of dry extract; which means, compared to hydro ethanolic and aqueous extract, ethanolic extract was found to be more effective. These values were lower than those reported by El-ghorab *et al*. (2010) (95.2 ± 6.2 and 87.5 ± 2.3 mg gallic acid/g) from the methanolic and hexane extracts of fresh ginger (*Zingiber officinale*) rhizome.

In this study, the content of total flavonoid in the ethanolic, hydro ethanolic and aqueous extract of *Z. wightianum* was expressed as Gallic acid equivalents per gram of dry extract and is 7.5 mg, 6.8 mg and 6.5 mg GAE/g of extract. But Ali *et al*., (2018) reported that the petroleum ether extract of ginger contained low concentrations (6.55 ± 0.20 mg quercetin/g). Flavonoid content in extract of *Zingiber wightianum* is comparable to the petroleum ether extract of ginger (*Zingiber officinale*).

In this study, we further evaluated the antioxidant potential of EZW, HZW and AZW. The extract with high antioxidant potential has been chosen for anti-inflammatory tests *in vitro* and *in vivo*. Antioxidants constitute the first line of defense against free radical damage. They play a critical role in maintaining plant cells in optimal safety. Many phenolic compounds (in addition to tocopherols) are potential antioxidants in plant tissues: flavonoids, tannins, and lignin precursors may act as ROS-scavenging compounds. The scavenging of active oxygen in plants includes many antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR), peptides and metabolites, and their activation is known to increase when exposed to oxidative stress (Neha *et al*., 2019).

In the present investigation, *in vitro* antioxidant activity of *Zingiber wightianum* was assessed on the basis of stable Hydroxyl radical, Nitric oxide radical scavenging activity, Superoxide radical scavenging activity, ABTS radical scavenging activity, DPPH radical scavenging activity, Ferric Reducing Antioxidant Power assay and Total antioxidant capacity.

In the study, *Z. wightianum* has been found to significantly scavenge radicals in a dose dependent manner. The hydroxyl-radical scavenging potential of extracts was measured by its ability to compete for hydroxyl radicals with deoxyribose. In Similar study done by Huiduan *et al.* (2017), with the increase in concentration of ginger (*Zingiber officinale*) extract, the scavenging ratio for hydroxyl radicals also rises, which depicted a dose dependent relationship.

In this study, with increase in *Z. wightianum* extract concentrations, nitric oxide radical scavenging activity of the extracts was found to be increased. The highest activity of EZW, HZW and AZW was found to be with 250 µg/mL. In a study done by

Bhattacharya *et al*., (2009), NO radical scavenging activity of hexane fraction of ginger (*Zingiber officinale*) improved with the increase in extract concentration in the similar manner.

In this study, the ferric reducing ability of *Z. wightianum* extracts was in the range of 300- 400 mg /g dry weight. Whereas, in the study done by Jeena *et al*., (2013), ferric reducing ability of ginger oil (*Zingiber officinale*) for 50 µg was found to be 1.8 mM.

In DPPH radical scavenging assay, Ethanolic, hydro ethanolic and aqueous extract of *Zingiber wightianum* exerted an inhibition in the range of 60- 70 %. But in a study done by Ali *et al.*, (2018), petroleum ether extract of ginger rhizome (*Zingiber officinale*) showed maximum DPPH radical inhibition of 97.47 % while in a study done by El- ghorab *et al*., (2010), the maximum DPPH radical inhibition by dried ginger (*Zingiber officinale*) essential oil was 83.87% and fresh ginger essential oil was 83.03. These data suggests that DPPH radical scavenging activity of *Zingiber wightianum* is comparable with that of *Zingiber officinale*.

In a study done by Jeena *et al*. (2013), ginger oil offered an IC_{50} of 36 µg/ml for superoxide radical scavenging whereas in this study, the ethanolic extract of *Zingiber wightianum* offered an IC_{50} of 43.75 µg/ml.

Total antioxidant capacity (TAC) is an analyte usually used to evaluate the antioxidant response against the free radicals produced and to determine the antioxidant status of biological samples (Rubio *et al*., 2016). The total antioxidant activity of EZW, HZW and AZW was found to be 83 µg AAE/g, 70 µg AAE/g and 80 µg AAE/g of dry extract. While in a study conducted by Shirin *et al*., (2010) found that the total antioxidant activity was highest in methanolic extract of *Zingiber officinale* at 98822 µmol/g followed by ethanolic extract at 91176 µmol/g.

Before conducting anti-inflammatory studies, oral toxicity studies were performed. Oral acute toxicity studies are carried out in animal experimental models such as mice. The acute toxicity study was conducted by oral administration of 5 doses (50 mg/kg, 150 mg/kg, 450 mg/kg, 1000 mg/kg, 2000 mg/kg) of plant extract in mice. Observations for mortality, behavioural pattern, and changes in physical appearance,

injury, pain and signs of illness had been monitored. Mice did not show any toxicity symptoms or changes in behavioral or physiological patterns at the highest dose (2000 mg/kg). From this it is concluded that EZW is not toxic and safe for oral administration within this range for studies. A similar study conducted in *Zingiber zerumbet* by Chang *et al*., (2012) revealed that the ethanolic extract was found to be safe even at the highest dose (3000 mg/kg) in wistar rats.

We studied *in vitro* and *in vivo* anti-inflammatory potential of EZW extracts. Anti- inflammatory response is often short-term adaptive response of tissue repair and involves the integration of numerous complex signals into separate cells and organs (Hotamisligil, 2006). An effective inflammatory response leads to the removal of infectious agents (Medzhitov, 2008).

For determining *in vitro* anti-inflammatory activity of rhizome extracts, Albumin denaturation assay and HRBC membrane stabilization assay was used. Ramesh *et al*., (2018) suggested that, HRBC or erythrocyte membrane is similar to lysosomal membrane and its stabilisation indicates that lysosomal membranes may also be stabilized by the extract and therefore stabilization of HRBC membrane by hypo-tonicity mediated membrane lysis is an *in-vitro* indicator of drug or anti-inflammatory activity of plant extract.

In a study done by Ezzat *et al*. (2018), the 80 and 90% ethanolic extracts of *Zingiber officinale* (500 µg / ml) demonstrated a maximum stability of the membrane of human red blood cells by 37%, the standard reference drug at the same dosage exhibited stabilization of 34%. In our study, the results demonstrated that EZW at the concentration of 150 µg/mL significantly protected the hypotonicity induced haemolysis of RBC. EZW showed maximum inhibition of haemolysis 55.69 % at the highest concentration (150 µg/mL) as compared with the standard Diclofenac sodium which showed 63.79 % inhibition of RBC haemolysis. Significant inhibition of the hemolysis of RBC indicates its anti- inflammatory activity.

Compounds with anti-inflammatory potential value are measured by stabilizing the heat treated albumin by NSAIDs, proposed by Williams *et al* (2008). In a study done by Anwar *et al.* (2020), *Zingiber officinale* exhibited a maximum inhibition of 39.66% at a concentration of 600 µg/mL. In our study, EZW at the concentration of

200 µg/mL significantly protected the heat induced denaturation of albumin. EZW showed maximum inhibition of 65.68 % at the highest concentration (200 µg/mL) as compared with the standard Diclofenac sodium which showed 79.41 % inhibition of albumin denaturation. Significant inhibition of albumin denaturation indicates its anti-inflammatory activity.

For determining *in vivo* anti-inflammatory activity of *Zingiber wightianum* rhizome extracts, Carrageenan induced paw oedema and Formalin induced paw oedema was used. Carrageenan, is a mucopolysaccharide derived from Chondrus, the Irish Sea moss. The lambda form is injectable to induce an inflammatory response. Carrageenan mediated inflammation is typically acute, non-immune, and highly reproducible. Peripheral inflammation includes an increase in cyclooxygenase mediated prostaglandin synthesis, which leads to allodynia and hyperalgesia. Carrageenan induced rat paw inflammation is a classic model of oedema formation and hyperalgesia, commonly used in the development of non-steroidal anti-inflammatory drugs and selective COX1 -2 inhibitors. The prostaglandin production mediated via COX -2- increase contributes to the severity of the inflammatory and pain responses in rat model (Guay *et al*., 2004). In a study done by Anwar *et al*., (2020), intraperitoneal treatment with ginger oil (*Zinigiber officinale*) in mice at 100 mg / kg was able to decrease carrageenan odema development by 27.8 %. But in this study, the ethanolic extract of *Z. wightianum* showed inhibition of carrageenan induced paw oedema in a dose dependent manner and the maximum inhibition was observed at 100 mg/kg (38.91%), and the maximum inhibition shown by the standard drug Indomethacin is (40.59%). The inhibitory effect of *Zingiber wightianum* extract may be due to the secondary metabolites in the plant extract either in an independent manner or in a synergistic way by inhibition of inflammatory mediators such as prostaglandins, histamines etc.

Next *in vivo* anti-inflammatory study is formalin induced paw oedema. Formalin induced paw oedema in rats, which causes local paw tissue damage, was used as a model for acute pain and localized inflammatory pain (Lee *et al*., 2002). In this study, EZW administration in formalin induced paw oedema showed a maximum reduction in paw volume at the dose 100 mg/kg (35.08). In a similar study conducted by Habib *et al*., (2008), the action of *Zingiber officinale* extract on paw thickness

(oedema) of rats at two separate doses (100 and 200 mg / kg body weight) revealed that the anti-inflammatory agent made a significant drop in paw thickness in rats.

The present study thus substantiates the traditional claim of medicinal properties of *Zingiber wightianum* for the treatment of swelling and pain by possessing anti-inflammatory and antioxidant potentials which were scientifically validated. The presence of secondary metabolites like phenols, saponins, flavonoids, glycosides, phlobatannins, anthocyanins and steroids contributes for its antioxidant properties. In the future, further studies are needed to explore the exact mechanism of action behind *Zingiber wightianum* Thwaites by analyzing their bioactivities.

6. SUMMARY

The thesis entitled 'Evaluation of anti-inflammatory and antioxidant potentials of *Zingiber wightianum* Thwaites (Malayinchi), an Ethnomedicinal plant of Kerala' was carried out in the Ethnomedicine and Ethnopharmacology Division of KSCSTE-Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram during the academic year 2019-2020. The objective of the study was to scientifically evaluate anti-inflammatory and antioxidant potentials of an ethnomedicinal plant *Zingiber wightianum* Thwaites (Malayinchi).

Zingiber wightianum Thwaites (Malayinchi) of the Zingiberaceae family is an important medicinal plant. The rhizome was collected from the Western Ghats hills and maintained at JNTBGRI to conduct pharmacological studies. Plant extraction procedures were performed to prepare different-dose drugs for the study. Acute oral toxicity and anti-inflammatory experiments were conducted in mice and rats.

The preliminary phytochemical screening of rhizome of *Zingiber wightianum* has shown the presence of phenols, saponins, flavonoids, glycosides, phlobotanins, anthocyanins and steroids which could be responsible for its medicinal properties. The total phenolic content of EZW, HZW and AZW was found to be 10.3 mg GAE/g, 9.9 mg GAE/g and 9.1 mg GAE/g of extract respectively. The total flavonoid content of EZW, HZW and AZW was found to be 7.5 mg, 6.8 mg and 6.5 mg GAE/g of extract.

Toxicity studies of *Zingiber wightianum* rhizome extract investigated in Swiss albino mice for 14 days with the oral administration of 5 doses (5, 150, 450, 1000 and 2000 mg/kg body weight) showed no signs of toxicity in animals.

The anti-inflammatory potential of rhizome 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 and albumin denaturation asssay. The EZW extracts were administered at doses (50 and 100 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 EZW 150 mg/kg in both the methods. In HRBC membrane stability study, EZW at a higher concentration significantly prevented the hypotonicity induced RBC haemolysis. In albumin denaturation assay, EZW at a higher concentration

significantly prevented the denaturation of the protein, albumin. These reveals the anti-inflammatory potential of *Zingiber wightianum*

The antioxidant effect of *Zingiber wightianum* was evaluated by Hydroxyl, Nitric oxide, DPPH, ABTS and Superoxide radical scavenging assay, Ferric Reducing Antioxidant Power (FRAP) assay and total antioxidant capacity. *Zingiber wightianum*'s antioxidant potential was comparable to the standard which indicated its potential activity.

The present study thus substantiates the traditional claim of medicinal properties of *Zingiber wightianum* for the treatment of swelling and pain by possessing anti-inflammatory and antioxidant potentials which were scientifically validated. The presence of secondary metabolites like phenols, saponins, flavonoids, glycosides, phlobatannins, anthocyanins and steroids may be responsible for its medicinal properties. In the future, further studies are needed to explore the exact mechanism of action behind *Zingiber wightianum* Thwaites by analyzing their bioactivities.

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8. APPENDICES

APPENDIX I

Dragendorff's reagent

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

APPENDIX II

Griess reagent

Napthylethylenediamine dihydrochloride	0.1 %
Sulfanilamide	1 %

APPENDIX III

Hager's reagent

Picric acid	1.00 g
Water	100 ml

APPENDIX IV

Mayer's reagent

Mercuric chloride	1.36 g
Potassium iodide	5.00 g
Water	100 ml

APPENDIX V

Wagner's reagent

Iodine	2.00 g
Potassium iodide	6.00 g
Water	100 ml

9. ABSTRACT

The thesis entitled 'Evaluation of anti-inflammatory and antioxidant potentials of *Zingiber wightianum* Thwaites (Malayinchi), an Ethnomedicinal plant of Kerala.' 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 2019-2020. The objective of the study was to scientifically evaluate anti-inflammatory and antioxidant properties of an ethnomedicinal plant *Zingiber wightianum* Thwaites (Malayinchi).

Zingiber wightianum Thwaites (Malayinchi), herbaceous plant of the family Zingiberaceae, is one of the important medicinal plants seen in dense tropical forests of the southern Indian peninsula. The rhizomes of *Zingiber wightianum* were collected from the hills of Western Ghats and maintained at JNTBGRI to conduct pharmacological studies. Extraction procedures were carried to prepare the drugs of different doses for the study. Acute oral toxicity studies in mice and anti-inflammatory studies in rats, were done as pharmacological analysis. In the preliminary phytochemical investigation, rhizomes of *Zingiber wightianum* have shown the presence of secondary metabolites like phenols, saponins, flavonoids, glycosides, phlobatannins, anthocyanins and steroids which may be responsible for its medicinal properties.

The total phenolic content in the ethanolic, hydro ethanolic and aqueous extract of *Zingiber wightianum,* expressed as gallic acid equivalents per gram of dry extract, was 10.3, 9.9 and 9.1 mg per gram of the extract respectively. The total flavonoid content of EZW, HZW and AZW was found to be 7.5, 6.8 and 6.5 mg GAE/g of extract. Oral administration of 50, 150, 450, 1000 and 2000 mg/kg body weight of rhizome extract to Swiss albino mice for 14 days did not produce any toxic symptoms, even at the highest dose. Anti-inflammatory potential of rhizome extract was investigated *in vivo* by carrageenan induced paw oedema and formalin induced paw oedema methods and *in vitro* by Albumin denaturation assay and HRBC membrane stabilization assay. The extracts were administered at doses of 50, and 100 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 EZW 100 mg/kg in both the methods. EZW at higher concentration protected

significantly the hypotonicity induced haemolysis of HRBC and prevented the denaturation of albumin by *in vitro* anti-inflammatory analysis.

The antioxidant effect of ethanolic, hydro ethanolic and aqueous extract of *Z. wightianum* showed IC_{50} of 121.83 µg/mL, 123.67 µg/mL and 132.66 µg/mL in hydroxyl radical scavenging assay, 86.27 µg/mL, 69.85 µg/mL, 135.82 µg/mL in nitric oxide radical scavenging assay. While in superoxide radical scavenging assay, ethanolic, hydro ethanolic and aqueous extract of *Z. wightianum* showed IC_{50} of 21.32µg/mL, 53.15 µg/mL and 44.28 µg/mL. In ABTS radical scavenging assay, of ethanolic, hydro ethanolic and aqueous extract of *Z. wightianum* showed IC_{50} of 132.77 µg/mL, 180.28 µg/mL and 190.86 µg/mL. While in DPPH radical scavenging assay, ethanolic, hydro ethanolic and aqueous extract of *Z. wightianum* showed IC_{50} of 71.02 µg/mL, 137.48 µg/mL and 137.94 µg/mL. Total antioxidant capacity of ethanolic, hydro ethanolic and aqueous extract of *Z. wightianum* was found to be 83 µg AAE/g, 81 µg AAE/g and 69 µg AAE/g of dry extract. The ferric reducing ability of ethanolic, hydro ethanolic and aqueous extracts of *Z. wightianum* were found to be 414 mg, 410 mg and 385.25 mg /g dry weight.

The antioxidant potential of *Z. wightianum* was compared with a standard and the outcome portrayed a significant impact. These results may offer assistance to set up a comprehensive investigation utilizing the drug as the reference. Thus the above findings scientifically validated the traditional claim of *Zingiber wightianum* for its medicinal use.

EVALUATION OF ANTI-INFLAMMATORY AND ANTIOXIDANT POTENTIALS OF *Zingiber wightianum* Thwaites (MALAYINCHI), AN ETHNOMEDICINAL PLANT OF KERALA

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മലയാളസംഗ്രഹം

സിഞ്ചിബർ വൈറ്റിയാനത്തിന്റെ ആന്റി ഇൻഫ്ലമ്മേട്ടറിയും ആന്റി ഓക്സിഡന്റ് സാധ്യതകളുടെയും മൂല്യനിർണയം

സിഞ്ചിബർ വൈറ്റിയാനത്തിന്റെ ആന്റി ഇൻഫ്ലമേറ്ററിയും ആന്റി ഓക്സിഡന്റ് സാധ്യതകളുടെയും മുല്യനിർണയത്തെ കുറിച്ചുള്ള പ്രബന്ധമാണ് പാലോട് ജെ എൻ ടി ബി ജി ആർ ഐയിൽ ഈ എം ആന്റ് ഈ പി ഡിവിഷനിൽ തയാറാക്കിയിട്ടുള്ളത്. പശ്ചിമഘട്ടത്തിലെ മലനിരകളിൽ കാണപ്പെടുന്ന സിഞ്ചിബറെസിയെ കുടുംബത്തിലെ സിഞ്ചിബർ വൈറ്റിയാനം എന്ന മലയിഞ്ചി ഒരു പ്രധാന ഔഷധസസ്യമാണ്. പ്രിലിമിനറി ഫൈറ്റോ കെമിക്കൽ വിശകലനത്തിൽ ഫിനോൾ, സാപോണിൻ, ഫ്ലാവനോയിട്, ഗ്ലൈകോസൈട്, ഫ്ലോബാറ്റാനിൻ, ആന്തോസയാനിൻ, സ്റ്റിറോയിട് എന്നിവ ഉൾപ്പെട്ടിട്ടുള്ളതായി കണ്ടെത്തി. ആന്റി ഓക്സിഡന്റ് പരിശോധകളിലൂടെ മലയിഞ്ചിക്ക് ശ്രദ്ധേയമായ ആന്റി ഓക്സിഡന്റ് പൊട്ടെൻഷ്യൽ ഉണ്ടെന്ന് കണ്ടെത്താൻ സാധിച്ചു. ഉയർന്ന കോൺസൻട്രേഷനിൽ മലയിഞ്ചി ഫോർമാലിനും കരാജീനാൻ ഇൻഡ്യൂസ്ഡ് പോ എഡിമ കുറയ്ക്കുന്നതായി കണ്ടെത്തി. സിഞ്ചിബർ വൈറ്റിയാനം മനുഷ്യന്റെ ചുവന്ന രക്താണുക്കളുടെ ഹീമോ ലൈസിസിനേയും ആൽബുമിന്റെ ഡിനാച്ചുറേഷനെയും ശ്രദ്ധേയമായി തടയുന്നതായി കണ്ടെത്തി. സിഞ്ചിബർ വൈറ്റിയാനത്തിന്റെ ഇത്തരത്തിലുള്ള കണ്ടെത്തലുകൾ പിൻകാലത്ത് സിഞ്ചിബർ വൈറ്റിയാനത്തിന്റെ ഉപരി പഠനങ്ങൾക്ക് ഉപയോഗിക്കാവുന്നതാണ്.