"EVALUATION OF INVITRO ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *SYZYGIUM CUMINI* BARK IN WISTAR RATS"

A Dissertation submitted to
THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,
CHENNAI- 600 032

In partial fulfilment of the award of the degree of

MASTER OF PHARMACY IN

Branch-IV -- PHARMACOLOGY

Submitted by

Name: L.KARTHIKEYAN

REG.No.261925204

Under the Guidance of

Mrs. R.ELAVARASI, M.Pharm.,

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J.K.K.NATTARAJA COLLEGE OF PHARMACY
KUMARAPALAYAM – 638183
TAMILNADU.

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CERTIFICATES

EVALUATION CERTIFICATE

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Internal Examiner

External Examiner

CERTIFICATE

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Professor &Principal,

CERTIFICATE

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DECLARATON

I do hereby declared that the dissertation **"EVALUATION OF INVITRO ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *SYZYGIUM CUMINI* BARK IN WISTAR RATS",** submitted to "**The Tamil Nadu Dr. M.G.R. Medical University - Chennai",** for the partial fulfilment of the degree of **Master of Pharmacy** in **Pharmacology,** is a bonafide research work has been carried out by me during the academic year 2020-2021, under the guidance and supervision of **Mrs. R.Elavarasi, M.Pharm.,** AssociateProfessor, Department of Pharmacology,J.K.K. Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

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Date: **Reg. No: 261925204**

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L.KARTHIKEYAN

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LIST OF SYMBOLS AND ABBREVATIONS

SYMBOLS AND ABBREVATION	FULL FORM
%	Percentage
⁰C	Degree Celsius
AESC	Aqueous bark extract of *Syzygium cumini*
AMP	Adenosine monophosphate
ANOVA	Analysis of Variance
CAT	Catalase
COX	Cyclooxygenase
DPPH	1,1 diphenyl picryl hydrazine
KCl	Potassium Chloride
LPS	Lipopolysaccharide
MIF	Macrophage Migration Inhibitory Factor
NO	Nitric oxide
OTR	Oxytocin Receptor
PPARγ	Peroxisome Proliferator –activated Receptor Gamma
PPROM	Preterm premature rupture of the membrane
PROM	Premature Rupture Of The Membranes
e.g.	Example
FDA	Food and Drug Administration
FeCL3	Ferric Chloride
Fig. No.	Figure Number
PTD	Preterm Delivery
PTL	Preterm Labor
ROS	Reactive Oxygen Species
GSH	Growth stimulating hormone
S.C	*Syzygium cumini*
SEM	Standard Error Mean
H20	Water

H2O2	Hydrogen Peroxide
H2SO4	Sulphuric Acid
v/v	volume by volume
HCl	Hydrochloric Acid
Mm	Micro Molar
WHO	World Health Organization
SOD	Superoxide dismutase
Mgso4	Magnesium sulphate
Hg	Mercury
i.p.	Intra-peritoneal
I.P.	Indian Pharmacopiea
KOH	Potassium hydroxide
IU/L	International units/litre
k.g	Kilogram
K2HPO4	Potassium bi phosphate
KCl	Potassium chloride
Kg	Kilogram
$Cacl_2$	Calcium chloride
AESC	Aqueous extract of *Syzygium cumini*
Nacl	Sodium chloride

TABLE OF CONTENT

INTRODUCTION

1. INTRODUCTION

The term of medicinal plants includes a different type of plants used in herbalism and some of the plants have a medicinal activities. Medicinal plants are considered as the "backbone" of traditional medicine, i.e, more than 3.3 billion peoples are utilizing medicinal plants in the less developed countries on a regular basis. [1] .

The Indian sub-continent has a very rich diversity of plant species in a wide range of ecosystems. There are nearly 17.000 species of the plants, in which 8.000 species of the plants, are considered as medicinal activity in traditional medicinal systems, such as the Ayurveda. These medicinal plants consider as a rich resources of ingredients which can be used in synthesis and drug development. Plants play a important role in the development of human cultures around the whole world.

The use of therapeutic plants and modern medicine in most developing countries, as a basis for the maintenance of good health, has been widely observed by UNESCO, 1996[2]. The use of medicinal plants has increased in the industries has been traced to the development of several drugs and extraction, chemotherapeutics from these plants as well as from traditionally used rural herbal remedies [3] .

During the previous decade, traditional medicines have become a topic of global importance. Current estimates shows that, in many developing countries, a large proportion of the population relies heavily on medicinal plants and traditional practitioners to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines have often maintained popularity for cultural and historical reasons.

Medicines in most developing countries, using beliefs and local traditions, is still considered as health care. According to WHO, Medicinal plants can make an important contribution to ensure, that all peoples, will lead a sustainable socio-economic productive life, worldwide by the year 2000 [4].

The Indigenous system of medicine comprises of Ayurveda, Unani and Siddha. These system comprised of many crude materials and3 herbal formulations. Traditional Medicines are derived from plants mainly and used by about 60% of the world's population [5]

The World Health Organization (WHO) has registered 21,000 plants, which are used for medicinal determinations around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a honestly large scale. In the last few years there has been an exponential development in the field of herbal medicine and these drugs are in advance popularity both in established and unindustrialized countries because of their natural origin and a lesser amount of side effects. [6]

In India, many steps have been taken to improve the quality of ayurvedic medicines. Bacopa Monniera, Tribu/lus Terrestris,Asparagus Racemosus, Asiatica have shown promising results and have been the target of research for drug development. [7]

Only a fraction of the earth's natural pharmacopoeia has been analyzed with modern techniques. The threat of imminent extinction of many plant species, especially in tropical areas, make it urgent that scientists has to learn as much as possible before the plants get extinct or their raw material are destroyed or old remedies are forgotten. This process requires the recording and observation of medical techniques, identification of plant materials and experimental investigation of the ingredients and their Effects.

Ethno pharmacology can also be an important element of a developing nation's economic and medical system. Chemists have so far been not able to reproduce the complex structure of many plant compounds. Further coordinated research into old traditions, plant species growing conditions and local medical needs is urged. Care must be taken however to preserve the main advantages of traditional medical care, less expensive and easy access. [8]

In India, plants have been used in traditional medicines from the time immemorial. Rigveda which is one of the historic storage of human knowledge mentions the use of plants in medicine.

Recently around 20,000 medicinal plant species have been recorded in India, further more than 500 traditional communities utilize about 800 plant species for curing new diseases. [9]

1.1 Inflammation:

Inflammation is the word obtained from the Latin word *inflammo*. It is a part of the complex biological response of body tissues to various harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective response involving immune cells, blood vessels, and molecular mediators. The important function of inflammation is to eliminate the initial cause of cell injury, clear out the necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair. [10]

CLASSICAL SIGNS OF INFLAMMATION

Inflammation has been studied for thousands of years and Celsus in 30 A.D. described the four classical signs of inflammation [11]

- Redness (rubor)

An acutely inflamed tissue appears red, due to dilatation of small blood vessels within the damaged area (hyperemia).

- Swelling (tumor)

Swelling which occurs from edema is due to the accumulation of fluid in the extravascular space as part of the inflammatory fluid exudate, and to a much lesser extent, from the physical mass of the inflammatory cells migrating into the affected area.

- Heat (calor)

Increase in temperature is due to increased blood flow (hyperemia) through the region, resulting in vascular dilation and the delivery of warm blood to the area.

- Pain (dolor)

Pain results from the stretching partly and distortion of tissues due to inflammatory edema and, in part from some of the chemical mediators of acute inflammation, especially bradykinin and some prostaglandins.

- Loss of function (functiolaesa)

Loss of function, a well-known consequence, which was added by Virchow (1821-1902) to the list of features described in Celsus written work. Loss of function may occur from severe swelling that prevents movement in the area or from the pain that inhibits mobility. [12]

CAUSES OF INFLAMMATION

- Microbial infections**:** Microbes including viruses, bacteria, protozoa, fungi and various parasites.
- Hypersensitivity reactions
- Physical agents, irritant and corrosive chemicals: Physical trauma, ultraviolet or other ionizing radiation, burns or excessive cooling ('frostbite') may cause tissue damage leading to inflammation. Corrosive chemicals such as acids, alkalis, oxidizing agents are also inflammatory stimulus that can cause direct tissue damage.
- Tissue necrosis: Lack of oxygen or nutrients results into inadequate blood flow and it is a potent inflammatory stimulus that can cause the death of tissues. [13][10]

TYPES OF INFLAMMATION

By considering the defence capacity of the host and duration of response, inflammation can classified as;

- Acute inflammation
- Chronic inflammation

Acute inflammation is of short duration, enduring less than 2 weeks and represents the early body reaction, resolves quickly and is usually followed by healing.

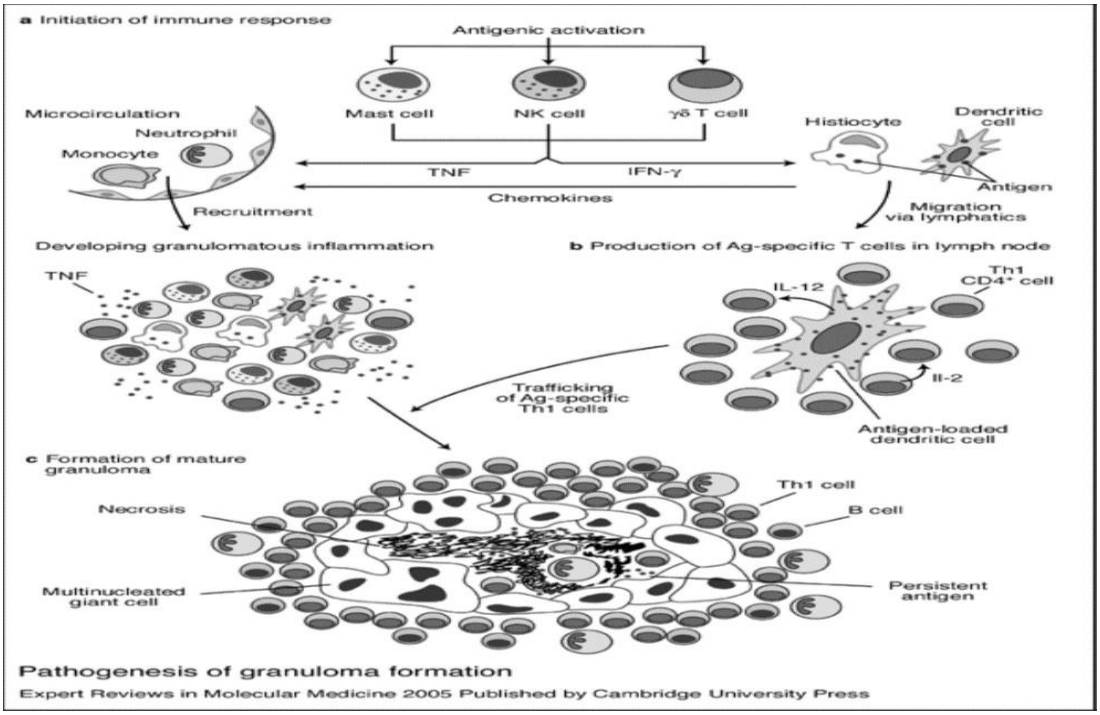
The main features of acute inflammation are:

- Accumulation of fluid and plasma at the affected site
- Intravascular activation of platelets
- Polymorphonuclear neutrophils as inflammatory cells

Chronic inflammation is of longer duration. The important feature of chronic inflammation is the presence of chronic inflammatory cells such as lymphocytes, plasma cells, macrophages, granulation tissue formation, and in specific situations as granulomatous inflammation. Chronic inflammation occurs either after the causative agent of acute inflammation persists for a long period of time, or the stimulus is such that it induces chronic inflammation from the beginning.

PATHOGENESIS OF GRANULOMA FORMATION

Figure 1: Pathogenesis of granuloma formation



Phagocytosis

Phagocytosis is defined as the process of engulfment of solid particulate material by the cells (cell-eating). The cells performing this function are called phagocytes. There are 2 main types of phagocytic cells:

i. Polymorphonuclear neutrophils (PMNs), sometimes called microphages which appears early in acute inflammatory response
ii. Circulating monocytes and fixed tissue mononuclear phagocytes, commonly called as macrophages.

Neutrophils and macrophages on reaching the tissue spaces produce several proteolytic enzymes—lysozyme, protease, collagenase, elastase, lipase, proteinase, gelatinase, and acid hydrolases. These enzymes degrade collagen and extracellular matrix. The microbe undergoes the process of phagocytosis by polymorphs and macrophages and involves the following 3 steps:

a) Recognition and attachment

b) Engulfment.

CHEMICAL MEDIATORS OF INFLAMMATION [14]

1. Plasma derived
2. Cell derived

Table 1: Plasma-derived mediators

Name	Produced by	Description
Membrane attack complex	*Complement system*	A complex of the complement proteins C5b, C6, C7, C8, and multiple units of C9. The combination and activation of this range of complement proteins forms the membrane attack complex, which is able to introduce into bacterial cell walls and causes cell lysis resulting bacterial death.

Plasmin	*Fibrinolysis system*	Able to break down fibrin clots. Also plasmin cleaves the complement protein C3, and activate Factor XII.
Thrombin	*Coagulation system*	Thrombin cleaves the soluble plasma protein fibrinogen to produce insoluble fibrin. Fibrin then aggregates to form a blood clot. Through PAR1 receptor thrombin can bind to cells to trigger several other inflammatory responses, such as production of chemokines and nitric oxide.
Factor XII (*Hageman Factor*)	Liver	It is a protein that circulates inactively. But get activated by collagen, platelets, or exposed basement membranes via conformational change. On activation it is able to activate three plasma systems involved in inflammation: the kinin system, fibrinolysis system, and coagulation system.
Bradykinin	Kinin system	Bradykinin cause vasodilation, increase vascular permeability, smooth muscle contraction, and also pain. Bradykinin is a vasoactive protein.
C3	*Complement system*	C3 Cleaves to produce C3a and C3b. C3a can produce the histamine release by mast cells and thereby produce vasodilation. C3b can bind to bacterial cell walls and act as an opsonin as a target for phagocytosis.
C5a	*Complement system*	Stimulates histamine release by mast cells. Histamine can produce vasodilation. Through chemotaxis it is also able to act as a chemoattractant to direct cells to the site of inflammation.

Table 2: Cell-derived mediators

Name	Type	Source	Description
Lysosome granules	*Enzymes*	Granulocytes	Contains a large variety of enzymes that perform a number of functions and are able to break down a number of substances, some of which may be plasma-derived proteins that allow these enzymes to act as inflammatory mediators.
Histamine	*Monoamine*	Mast cells and basophils	Stored in preformed granules, histamine is released in response to a number of stimuli. It causes arteriole dilation, increased venous permeability, and a wide variety of organ-specific effects.
IFN-γ	*Cytokine*	T-cells, NK cells	This interferon called macrophage-activating factor, and is especially important in the maintenance of chronic inflammation. Also have antiviral, immunoregulatory, and anti-tumour properties.
IL-8	*Chemokine*	Primarily macrophages	Activation and chemoattraction of neutrophils. Have weak effect on monocytes and eosinophils.

Leukotriene B_4	*Eicosanoid*	Leukocytes, cancer cells	Have the ability to mediate leukocyte adhesion and activation. And allow them to bind to the endothelium and migrate across it. In neutrophils, it is also a chemoattractant. And it is able to induce the formation of reactive oxygen species, followed by the release of lysosomal enzymes by these cells.
IL-1 and TNF-α	*Cytokines*	Primarily macrophages	IL-1 and TNF-α induce many similar inflammatory reactions such as fever, production of cytokines, endothelial gene regulation, chemotaxis, leukocyte adherence, activation of fibroblasts. Also responsible for the systemic effects of inflammation, such as loss of appetite and increased heart rate. In additionally TNF-α inhibits osteoblast differentiation.
LTC_4, LTD_4	*Eicosanoid*	Eosinophils, mast cells, macrophages	These three Cysteine-containing leukotrienes contract lung airways, increase micro-vascular permeability, stimulate mucus secretion, and promote eosinophil-based inflammation in the lung, skin, nose, eye, and other tissues.

5-oxo-eicosatetraenoic acid	*Eicosanoid*	leukocytes, cancer cells	Potent stimulator of neutrophil chemotaxis, lysosome enzyme release, and ROS formation; monocyte chemotaxis; and with even greater potency eosinophil.
5-HETE	*Eicosanoid*	Leukocytes	Metabolic precursor to 5-oxo-eicosatetraenoic acid, it is a less potent stimulator of neutrophil chemotaxis, lysosome enzyme release, and reactive oxygen species formation; monocyte chemotaxis; and eosinophil chemotaxis, lysosome enzyme release, and reactive oxygen species formation.
Prostaglandins	*Eicosanoid*	Mast cells	A group of lipids that can cause vasodilation, fever, and pain.
Nitric oxide	*Soluble gas*	Macrophage, endothelial cells, some neurons	Potent vasodilator, relaxes smooth muscle, reduces platelet aggregation, aids in leukocyte recruitment, direct antimicrobial activity in high concentrations.

THE INFLAMMATORY CELLS

The cells involved in acute and chronic inflammation are circulating leukocytes, plasma cells and tissue macrophages.

- Polymorphonuclear neutrophils are acute inflammatory cells, which are involved in initial phagocytosis of bacteria and foreign bodies, engulfment of antigen-antibody complexes.
- Monocytes are chronic inflammatory cells which are involved in bacterial phagocytosis and regulates lymphocyte response.
- Lymphocytes are chronic inflammatory cells which are involved in humoral and cell mediated immune responses and regulate macrophage responses.
- Eosinophils are chronic inflammatory cells which are involved during allergic states and parasitic infestations.
- Basophils containing electron dense molecules and functions as receptor for Ig E molecules
- Plasma cells are derived from B cells and it is larger than lymphocytes with more abundant cytoplasm and eccentric nucleus. Their number increased during prolonged infection with immunological responses, hypersensitivity states and multiple myeloma.
- Giant cells exist in normal tissues. In chronic inflammation when macrophages fail to deal with particles to be removed, they fused together and form multinucleated giant cells. Besides, morphologically distinct giant cells appear in some tumours also. [15]

CHRONIC INFLAMMATION

Chronic inflammation, is a host response to an inciting stimulus. Chronic inflammation is characterized by inflammation, tissue destruction, and attempts at repair all happening at once. Inflammation does not have as much rubor (redness) or calor (heat) as in the acute reaction. Also, exudates aren't so grossly apparent as in acute inflammation. Because of the fibroplasia and neovascularization, areas affected by chronic inflammation tend to be slightly swollen and firm. If fibrosis is extensive the lesions can be large and disfiguring. Fibrosis (granulation tissue) is the best indicator that the inflammatory response is chronic. [15]

Chronic inflammation have prolonged duration. Chronic inflammation in contrast to acute inflammation is characterised by the vascular changes, edema and a predominantly neutrophilic infiltrate. Chronic inflammation is distinguished by

- Infiltration with mononuclear cells which includes macrophages, lymphocytes and plasma cells
- Tissue destruction, largely induced by the products of the inflammatory cells.
- Repairing which involves new vessel proliferation (angiogenesis) and fibrosis.

Acute inflammation may progress to chronic inflammation. This change occurs when the acute response cannot be resolved, either because of the persistence of the inflammatory agent or because of the intervention with the normal process of healing.

GRANULOMATOUS INFLAMMATION

Chronic inflammation characterized by the aggregates of activated macrophages that assume an epithelioid appearance.

The causes of or conditions in which granuloma develops are

- Bacterial infections
- Fungal infections
- Parasites
- Foreign bodies
- Immune conditions

2. LITERATURE REVIEW

History of medicine goes practically to the existence of human being. The current accepted modern medicine or allopathy has gradually been developed over the years by the scientific observational efforts of scientists. However, the basis of its development remains in the roots of tradional medicine and therapies. The history of medicine includes many indecorous therapies. Nevertheless,the ancient wisdom has been the basis of modern medicine and will remain as one of the important source of future medicine and therapeutics. Thefuture will be more holistic, personal and involves wise use of ancient and modern therapeutic skills in a community. Medical system has a broad meaning .it incorporates diagnosis, prognosis and prevention. Treatment has a definite rational and philosophy, Therapies have a limited meaning referring to mainly treatment Within this framework of definition, two prominent system of medicine with history of existence for thousands of years include Indian system of medicine such as Siddha, Ayurveda, etc. and Chinese Medicine. Ayurveda incorporates Yoga while Chinese medicine includes Acupuncture.

1. **Sharma A, Patel VK, Chaturvedi AN et al.,(2009)[16]** has reported that Acetone, aqueous and ethanolic bark extracts were evaluated for their antibacterial effect against twelve strains of vibrio cholera, of which the ethanolic extract was found to be most effective.

2. **Jabeen K, Javaid A et al.,(2010)[17]** has reported that different concentrations of n-hexane, alcohol and aqueous extracts of different plant parts of SC (barks of stem and roots, fruits and leaves) to study their antifungal potential against Ascochyta rabiei.
   The causative agent for blight disease of Cicer arietinum. All the aqueous extracts, n-hexane extract of stem-bark and alcoholic extracts of both the barks exhibited significant antifungal activity.

3. **Haroon R, Jelani S, Arshad FK et al.,(2015)[18].**have reported that the methanolic extract of leaves, bark and seeds of SC were fractionated in different solvents: nhexane, chloroform, ethyl acetate, butanol and water. These fractions were studied for their antioxidant and free radical scavenging activities. Of all the fractions, the polar ones i.e., ethyl acetate and water fractions showed excellent results.

4. **Sood R et al., (2012)[19]** has reported that Petroleum ether, chloroform, methanolic and aqueous extracts of SC bark were evaluated for their diuretic activity in Wistar albino rats at the dose of 500mg/kg body weight. The methanolic and aqueous extracts were found to be active as diuretic as depicted by an elevation in total urine output and in the excretion of electrolyte concentration of sodium and potassium ions.

5. **Haroon R et al., (2015)[20]** has reported that the cold and hot aqueous extracts of leaves and barks of SC were evaluated for their antiviral potential against H5N1 (avian influenza virus which causes a highly contagious disease of poultry) using CPE reduction assay to establish virucidal, pre-exposure and post-exposure potential of these extracts. With hot and cold aqueous bark extracts and hot aqueous leaf extracts, 100% inhibition of the virus was observed in virus yield reduction assay and in egg based in ovo assay.
   CC50/EC50 (selective index) for cold aqueous extract (43.5) and hot aqueous extract (248) of bark exhibited their potency against H5N1 virus.
6. **Gulshum Ali, Vikram Singh et al., (2016)[21]** has reported the ethanol and aqueous extracts using bark of Syzygium cumini on Albino Westar rats whereas Glibenclamide were used as a standard.
   Biochemical and histopathological evidences indicated that using treatment with the methanol and aqueous extracts of bark of Syzygium cumini effectively protected rats against alloxan induced diabetes.

7. **Fokhrul Abedin et al., (2008)[22]** has evaluated the locomotor activity of the animals in the open field and forced swim methods at a dose of 200 and 400 mg/kg body weight and the anti-diarrheal activity was evaluated through castor oil induced method and charcoal induced GI motility tests. The ethanolic bark extract of S. cumini justify its traditional uses through good thrombolytic, CNS depressant and anti- diarrheal activity

8. **N.ARCHANA et al., (2012) [23]** has reported the ethanolic extract of Syzygium cumini seed relaxes the rat uterus smooth muscle against Potassium chloride (KCl) induced smooth muscle contraction. Considering smooth muscles, Syzygium cumini can be claimed to possibly act via calcium or potassium channels whereas in case of cardiac muscles the mode of action of Syzygium cumini still remains a question mark.

3. PLANT PROFILE

3.1 PLANT INTRODUCTION:

Syzygium cumini Linn. (synonym Eugenia jambolan Linn.) is a very largeevergreen tropical tree belonging to the family Myrtaceae [24] the plant is also mentioned in literature as Jamun, synonym as black plum or jambolan, botanical name the plant are very well known for their pharmacological properties since ancient age . The native home of the *Syzygium* is India and East Indies. This plant is also found in other countries like Thailand, Philippines, Madagascar [25], extensive work were carried out on plant of *Syzygium cumini* for their pharmacological properties. The medicinal value is due to presence of malic acid, oxalic acid, gallic acid, tannins . Various works on tannin, flavonoids essential oil and betulinic acid was reported to have diverse pharmacological activities like gastroprotective, antiulcerogenic, antibacterial [26], anti-infective [27-28], antimalarial [29]. *Syzygium cumini* belongs to family Myrtaceae, which records the occurrence of taxonomically informative molecules, namely malic acid, oxalic acid, gallic acid, betulinic acid, tannins, flavonoids and essential oil. Review detailing the chemical constituents of the *Syzygium cumini* have been reported several researcher. The widespread uses of *Syzygium cumin*i in traditional medicines have resulted in considerable chemical analysis of the plant, and active principles which attribute the plant its medicinal properties have been identified and isolated. The entire plant is used in the traditional medicine; however the leaves and stem bark is mentioned to be most powerful part.

PLANT DESCRIPTION: Fig:2 *Syzygium cumini*

3.2 *Syzygium cumini*

Table 3: Botanical illustration of *Syzygium cumini*

Binomial Name	*Syzygium cumini*
Family	Myrtaceae
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Myrtales
Genus	*Syzygium*
Species	*Syzygium cumini*

Vernacular names of *Syzygium cumini:*

Table 4: Vernacular names of *Syzygium cumini:*

English	Jamun
Hindi	Jamun
Tamil	Naval
Telugu	Nerudu
Kannada	Nerale
Malayalam	Njara

3.3 Plant characteristics:

Surface:-Lacate (shinny)

Shape:-Ovate, corky, dimidiate, rarely suborbicular.

Margin:- Thin or truncate, often slightly incurved.

Colour:- Brown with yellowish margin

Round base with truncate to narrowly rounded apex.

3.4 Morphology Characteristics:

Syzygium cumini may reach 30 m tall in India and Oceania or up to 12-15 m in Florida, USA, with a wide crown up to 11 m in diameter and a trunk diameter of 0.6-0.9 m though it usually has a multi-stemmed form branching close to the ground.

Bark is rough, fractured, flaking and discoloured on the lower part of the trunk, becoming smooth and light-grey higher up.

Evergreen leaves have a turpentine smell, and are opposite; pinkish when young, becoming leathery, glossy, dark-green above, lighter beneath, with a conspicuous, yellowish midrib when grown-up. Flowers are fragrant and appear in clusters, white at first, becoming rose-pink, detaching rapidly to leave only the numerous stamens. Fruit appear in bunches of just a few or 10-40, are round or oblong. The skin is thin, smooth, glossy, and adherent.

3.5 Microscopic Features:

Bark is rough, clacked, flaking and discoloured on the lower part of trunk, becoming smooth and light grey higher up. Leaves are 5-25cm long and 2.5-10cm wide oblong, oval or elliptic. Flowers are fragrant and appears in clusters with a funnel shaped calyx and united petals.

3.6 History:

For long in the period of recorded history, the tree is known to have grown in the Indian sub-continent, and many others adjoin regions of South Asia such as India, Bangladesh, Burma, Nepal, Pakistan, Sri Lanka and Indonesia. In southern Asia, the tree is venerated by Buddhists, and it is commonly planted near Hindu temples because it is considered sacred to Lord Krishna [30]. The plant has also been introduced to many different places where it has been utilized as a fruit producer, as an ornamental and also for its timber. In India, the plant is available throughout the plains from the Himalayas to southern India.

3.7 Active chemical constituents:

Jambolan is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin. The seeds are claimed to contain alkaloid, jambosine, and glycoside jambolin or antimellin, which halts the diastatic conversion of starch into sugar and seed extract has lowered blood pressure and this action is recognised to the ellagic acid content. The seeds have been reported to be rich in flavonoids, a well-known antioxidant, which accounts for the scavenging of free radicals and protective effect on antioxidant enzymes [31, 32] and also found to have high total phenolics with significant antioxidant activity [33] and are fairly rich in protein and calcium.

Java plums are rich in sugar, mineral salts, vitamin C, PP which fortifies the beneficial effects of vitamin C, anthocyanins and flavonoids. [34]

3.8 Leaves:

The leaves are rich in acylated flavonol glycosides [35], myricetin, quercetin, myricitin, myricetin, 3-O-4-acetyl-L-rhamnopyranoside[36], triterpenoids[37], esterase, galloyl carboxylase and tannin [38].

3.9 Stem bark

The stem bark is rich in friedelin, eugenin, betulinic acid, , epi-friedelanol, β-sitosterol, and fatty acid ester of epi-friedelanol [39], β-sitosterol, quercetin kaempferol, myricetin, gallic acid and ellagic acid [40] , bergenins [41] , flavonoids and tannins [42] . The presence of gallo- and ellagi-tannins may be responsible for the astringent property of stem bark.

3.10 Flowers

The flowers are rich in kaempferol, quercetin, myricetin, isoquercetin (quercetin-3-glucoside), myricetin-3-L-arabinoside, quercetin-3-D-galactoside, dihydromyricetin, oleanolic acid, acetyl oleanolic acid, eugenol-triterpenoid A and eugenol-triterpenoid B [43]

3.11 Roots

The roots are rich in flavonoid glycosides [44] and isorhamnetin 3-O-rutinoside [45]

3.12 Fruits

The fruits are rich in glucose, raffinose, fructose [46] , gallic acid acid, mallic acid [47] , anthocyanins [48]; malvidin-3-laminaribioside delphinidin-3-gentiobioside, petunidin-3-gentiobioside[49] , cyanidin diglycoside, petunidin and malvidin[50]. The sourness of fruits might be due to presence of gallic acid. The color of the fruits might be due to the presence of anthocyanins[51] . The fruit contains 83.70–85.80 g moisture, 0.70–0.13 g protein, 0.15–0.30 g fat, 0.30–0.90 g crude fiber, 14.00 g carbohydrate, 0.32–0.40 g ash, 8.30–15.00 mg calcium, 35.00 mg magnesium, 15.00–16.20 mg phosphorus, 1.20–1.62 mg iron, 26.20 mg sodium, 55.00 mg potassium, 0.23 mg copper, 13.00 mg

sulphur, 8.00 mg chlorine, 80 I.U. vitamin A, 0.01–0.03 mg thiamine, 0.009–0.01 mg riboflavin, 0.20–0.29 mg niacin, 5.70–18.00 mg ascorbic acid, 7.00 mg choline and 3.00 mcg folic acid per 100 g of edible portion [52] .

One of the variety of jambolan found in the Brazil possesses malvidin-3-glucoside and petunidin-3-glucoside [53]. The peel powder of jambolan also can be employed as a colorant for pharmaceuticals and foods anthocyanin pigments from fruit peels were studied for their antioxidant efficacy strength as extract and in formulations [54].

3.13 Essential oils

The essential oils isolated from the freshly collected leaf (accounting for 82% of the oil) [55] , stem, seed, fruits contain α-Pinene, camphene, β-Pinene, myrcene, limonene, cis-Ocimene, trans-Ocimene, γ-Terpinene, terpinolene, bornyl acetate, α-Copaene, β-Caryophyllene, α-Humulene, γ-Cadinene and δ-Cadinene [56] , trans-ocimene, cis-ocimene, β-myrcene, α-terpineol, dihydrocarvyl acetate, geranyl butyrate, terpinyl valerate [54], α-terpineol, β-caryophyllene, α-humulene, β-selinene, calacorene, α-muurolol, α-santalol, cis-farnesol: lauric, myristic, palmitic, stearic, oleic, linoleic, malvalic, sterculic and vernolic acids [55]. Unsaponifiable matter of the seed fat was also chemically investigated [56]

3.14 Medicinal properties: The bark is used in the treatment of sore throat, bronchitis, asthma, thirst, biliousness, dysentery and ulcers. It is also a good blood purifier. The fruit is sweet, acrid, cooling and astringent to the bowels and removes bad smell form mouth, stomachic, astringent, biliousness, diuretic and antidiabetic [57]. The fruit has a very long history of use for various medicinal purposes and currently has a large market for the treatment of chronic diarrhea and other enteric disorders [58]. The ash of the leaves is used for strengthening the teeth and gums. Vinegar prepared from the juice of the ripe fruit is an agreeable stomachic and carminative and used as diuretic
[59] and it is also useful in spleen enlargement and in chronic diarrhea.

3.15 Parts used: All parts of the plants are used medicinally especially leaves, seed, stem bark.

3.16 Traditional uses:

All parts of the jambolan can be used as medicine. From all over the world, the fruits have been used for a wide variety of ailments, including cough, diabetes, dysentery, inflammation and ringworm [60].

It is widely distributed throughout India and ayurvedic medicine (Indian folk medicine) mentions its use for the treatment of diabetes mellitus. Various traditional practitioners in India use the different parts of the plant in the treatment of diabetes, blisters in mouth, cancer, colic, diarrhea, digestive complaints, dysentery, piles, pimples and stomachache [61]. During last four decades, numerous folk medicinal reports on the antidiabetic effects of this plant have been cited in the literature. In Unani medicine various parts of jambolan act as liver tonic, enrich blood, strengthen teeth and gums and form good lotion for removing ringworm infection of the head [62].

4. AIM AND OBJECTIVE

The aim of the study is to evaluate the invitro antioxidant and anti-inflammatory effect of ethanolic extract of *Syzygium cumini* bark in Wistar rats.

The objectives of the present study include:

- Evaluation of *in vitro* antioxidant activity of EESC
- Acute toxicity study of Ethanolic extract of *Syzygium cumini* (EESC)
- Evaluation of anti-inflammatory activity of EESC

4.1 Specific Objectives:

1. Phytochemical investigation of ethanolic extract of bark of *Syzygium cumini.*

4.2 Phase-I

1. Identification and authentication of bark of Syzygium cumini renowned taxonomist.
2. Collection barks of *Syzygium cumini* and shade drying.
3. Powdering of dried barks for extraction.

4.3 Phase-II

Ethanolic Extraction:

The bark was air dried under shade, powdered mechanically and stored in airtight containers. The powdered bark was extracted with ethanol (95%) by Soxhlation process. The extract was filtered and filtrate was concentrated at reduced pressure by Rotary vaccum Evaporator.

4.4 Phase-III

Preliminary phytochemical screening

The preliminary phytochemical investigations of extracts of bark of *Syzygium cumini* were carried out for qualitative identification of the present phytochemical constituents like Alkaloids, Steroids, Carbohydrates, Triterpenes and Saponins etc. Tests were carried out using standard methods. All the chemicals and reagents to be used in the tests were of analytical grade.

4.5 Parameters

1. Quantification of total phenol and flavonoid content.

2. *In-vitro* antioxidant study.

- DPPH radical scavenging assay
- ABTS radical cation scavenging assay

3. Acute toxicity study of EESC.

4. In-vivo anti-inflammatory study.

5. METHODOLOGY

5.1Selection of animal

5.2 Housing of animals

5.3 List of chemicals

5.4 List of instruments used during experiments

5.5 Phytochemical investigation

5.6 In-vitro antioxidant studies.

5.7 Acute toxicity studies

5.8 Pharmacological Study

- Screening of anti-inflammatory activity
- Histamine induced inflammation

5.9 Statistical analysis

5.1 Selection of animal: Female Albino rats (wistar) weighing 180-200 grams were selected for study.

5.2 Housing of the animals: The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2 °C under clearance for use of the animals was obtained from the institutional animal ‘‘ethical committee’’ prior to the beginning of the project work.

5.3 List of chemicals:

Table 5: List of chemicals used during experiment

Sl. No.	Chemicals
1	Acetic anhydride
2	Acetone
3	Alkaline reagent
4	Ammonia
5	Bardford’s reagent
6	Benedict’s reagent
7	Chloroform

8	CMC
9	Copper sulphate
10	Dragendroff's reagent
11	Ethanol
12	Fehling's A & B
13	Ferric chloride
14	Formalin
15	Bark extract of Syzygium cumini
16	Glacial acetic acid
17	Hydrochloric acid
18	Lead acetate
19	Mayer's reagent
20	Millon's reagent
21	Molish's reagent
22	Ninhydrine reagent
23	Sodium chloride
24	Sodium picrate
25	Sulfuric acid
26	Wagner's reagent
27	Potassium chloride
28	Sodium bicarbonate
29	Calcium chloride
30	Glucose

5.4 List of materials/instruments used in experiment:

Table 6: List of instruments used during experiment

Sl. No	Instruments	Manufacturer
1.	Analytical weighing balance	Shimadzu
2.	Cooling centrifuge	Goorej
3.	UV spectrometer	Shimadzu
4.	Kymograph	INCO instruments and chemicals PVT.LTD
5.	Rotary evaporator	SUPERFIT ROTAVAP PBU-G
6.	Microscope	BESTO- Model 9

Table 7: Requirements of the study

DRUGS	CHEMICALS	OTHERS
Diclofenac	1. ABTS 2. DPPH 3.Phosphate Buffer 4.Quercetin 5. Potassium Persulfate 6.Potassium Ferricyanide 7.Ferric Chloride 8.Gallic Acid 9.Sodium Carbonate 10.Folin Ciocalteau Reagent	1. Distilled water 2. Sterile water for injection 3.Normal saline 4.Syringe 5.Needle 6.Gloves 7.Droppers 8.Microscopical slides

5.5 Phytochemical investigation:

5.5.1 Collection: Syzygium cumini barks were collected from Ramasagara Village, Chandapura, Bangalore.

5.5.2 Authentication: Syzygium cumini barks was authenticated by Dr. N. Karmegam, Assistant professor, Department of Botany, Govt Arts college, Salem. The barks were shade dried. It was then coarsely powdered and preserved in air tight glass container for further processing.

5.5.3 Extraction: The coarsely powdered *Syzygium cumini* barks were extracted with water in a soxhlet extractor.

The extracts were concentrated in rotary flash evaporator and stored in desicator until further use and the percentage yield of corresponding extracts were calculated. The colour and consistency of the extract were noted and summarized in table no 6.

Table 8: Percentage extractives and physical characteristics of extract of bark of *Syzygium cumini*

Solvent	**Colour and Consistency**	**Percentage yield**
Aqueous	Black and powder	22.5 %

5.5.4 Preliminary Phytochemical Analysis of the Extract :

The information about the constituents present in the plant clarifies the medicinal uses of the plant. Identification and evaluation of herbal extracts is a fundamental procedure and parts of quality control protocol. The aqueous extract of *Syzygium cumini* bark was subjected to phytochemical evaluation and identified the various plant constituents present in the test samples by qualitatively and quantitatively. The following studies were carried out in phytochemical analysis.

5.5.4.1 Qualitative chemical test

5.5.4.2 Estimation of total phenol

5.5.4.3 Estimation of total flavonoid

5.5.1 QUALITATIVE CHEMICAL TEST:

The qualitative chemical tests were carried out for the extract and identified the various secondary metabolites present in the aqueous extract of *Syzygium cumini* bark.

Preparation of test sample

500 mg of the extract was dissolved in 5ml of distilled water and then filtered. The filtrate was tested to detect the presence of various phytochemical constituents in the sample.

A. Test for Alkaloids:

The small portions of solvent free extract/fraction was stirred separately with a few drops of dil.HCl, filtered and used to test the presence of alkaloids.

- **Dragendroff's Test:** The extract was treated with Dragendroff's reagent (Potassium Bismuth iodide) to produce orange-brown precipitate.
- **Mayer's Test:** The extract on treatment with Mayer's reagent (Potassium mercuric iodide) gives creamy precipitate.
- **Hager's Test:** The extract treated with Hager's reagent (saturated solution of picric acid produce yellow precipitate.

B. Tests for Carbohydrates:-

Extract was dissolved individually in 5ml distilled water and filtered. The filtrates were used to identify the presence of carbohydrates.

- **Molisch's Test:** Extract treated with Molisch reagent (α-napphthol in 95% ethanol) and few drops of concentrated H_2SO_4 were added at the sides of the test tube, violet ring appears at the junction indicates the presence of carbohydrates.
- **Fehling's Test:** Small portion of the extract treated with Fehling's reagent (Fehling's reagent A-Copper sulphate in water and Fehling's reagent B-Sodium Potassium Tartarate), the mixture was heated; brick red colour precipitate indicates the presence of reducing sugars.

- **Barfoed's Test:** Extracts treated with Barfoed's reagent (Copper sulphate in water), the mixture was heated on a water bath, red colour precipitate indicates the presence of sugars.

- **Benedict's test:** Extracts treated with Benedict reagent (Copper sulphate, sodium citrate and sodium carbonate in water), the mixture was heated on water bath for 10 minutes, red colour precipitate indicates the presences of sugars.

C. Test for Steroids, Triterpenoids and Cardiac glycosides:-

- **Liebermann-Buchard test:** Small portion of the extracts were dissolved in 1ml of chloroform, to this 1ml of acetic anhydride was added followed by addition of 2ml of concentrated sulphuric acid from the sides of the test tube. Formation of reddish violet colour indicates the presences of steroids, cardiac glycosides and triterpenoids.
- **Salkowski test:** When few drops of concentrated sulphuric acid was added to the test solution in chloroform shaken and allowed to stand for few minutes formation of red colour in the chloroform layer suggests the presence of steroids.
- **Keller Killani test:** 5mg of extract was treated with 1ml of glacial acetic acid and few drops of ferric chloride solution was added in to the test tube. To this mixture adding 2 ml of concentrated sulphuric acid carefully along the sides of the test tubes. The formation of a reddish brown colour at the seam of two layers and formation of bluish green upper layer indicates the presence of deoxy-sugar in the carbohydrates.
- **Baljet test:** 1 ml of extract solution was treated with few drops of sodium picrate reagent. Yellowish orange colour indicates the presence of cardiac glycosides.

D. Test for Saponins:-

- **Froth test:** Diluted 1ml of the extract with distilled water to 20ml and shaken in a graduated cylinder for 15min. Formation of foam indicates the presence of saponins.

E. Test for Tannins:-

- **Ferric chloride test:** Extracts treated with ferric chloride solution, blue colour indicates the presence of tannins.
- **Lead acetate test:** Extracts treated with lead acetate solution, yellow precipitate indicates the presence of tannins.

F. Tests for Amino acids:

- **Millons's test:** Extract treated with Millon's reagent (Mercuric nitrate in nitric acid), red colour indicates the presence of proteins.
- **Biuret test:** Extract treated with sodium hydroxide and copper sulphate solution added drop wise and mixed, violet colour indicates the presence of proteins.
- **Ninhydrin test:** Extract treated with Ninhydrin reagent and ammonia, heated, violet colour indicates the presence of amino acids.

5.5.2 ESTIMATION OF TOTAL PHENOLIC CONTENT [63]

Total phenolic content determined by standard Folin-Ciocalteu reagent method, using tannic acid as a standard phenolic compound in the range 100 μg/ml to 1000 μg/ml. 250 μl of extract was mixed with 1 ml of distilled water followed by the addition of 250 μl of Folin-Ciocalteu reagent. Mixed well and left to stand for 5 min at room temperature. 2.5 ml of 7 % sodium carbonate aqueous solution were added and the final volume was made up to 6 ml with distilled water. The absorbance was measured at 760 nm. The Phenolic content is expressed in terms.

5.5.3 ESTIMATION OF TOTAL FLAVANOIDS CONTENT: [64]

Aluminium chloride method was used for flavonoids determination. Mix 0.5 ml plant part extracts separately with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M sodium potassium tartrate and 2.8 ml of distilled water. Allow it to stand for 30 min room temperature. Absorbance measured at 415 nm. The flavonoid content is expressed in terms.

5.6 *IN-VITRO* ANTIOXIDANT STUDIES:

Various methods are used to investigate the antioxidant property of samples. In the present study the antioxidant properties of the extract was evaluated by *in vitro* methods. The antioxidant properties could not be concluded based on the single antioxidant test method.

It is in practice that generally several *in vitro* test procedure are carried out to conclude the antioxidant properties of the sample. Among various free radical scavenging methods DPPH and ABTS assays was carried out in the present study.

5.6.1 DPPH

Principle:

The free radical scavenging activity of sample and the standard, L-Ascorbic Acid (Vitamin C) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH

Chemicals & Reagents:

1. DPPH (Prepare 0.1mM in methanol, protect from light)
2. Sample: Different concentrations of given sample diluted in warm distilled water (50-250 µg/ml)
3. Ascorbic acid (50–250 µg/ml)

Method: Various concentrations of sample (50-250 µg/ml) were added to a 0.1mM DPPH solution in methanol and the reaction mixture was shaken vigorously. After incubation for 30min at 27°C in dark the absorbance at 517 nm was recorded spectrophotometrically. Ascorbic acid is used as a references in the same concentration range as the test extract. A control solution, without a tested compound, was prepared in the same manner as the assay mixture. The degree of decolourization indicates the radical-scavenging efficiency of the sample. Ascorbic acid used as reference compound.

Calculations: The percentage inhibition activity was calculated from $[(A_0-A_1)/A_0]$ x 100, where A_0 is the absorbance of the control and A_1 is the absorbance of extract/standard taken as Ascorbic acid. All the values are mean ± SEM of three independent experiments and expressed as % DPPH radical scavenging activity.

Note: Since these are coloured samples, OD of coloured sample alone added to control OD values.

5.6.2 ABTS Radical Scavenging assay

Principle:

The ABTS radical is produced by the reaction of ammonium persulphate with ABTS under dark conditions. The persulphate ions are involved in a nucleophilic attack on ABTS and thereby, greenish blue ABTS radicals ($ABTS^{+}$) are produced. Whichare spectrophotometrically measured at 734 nm. The presence of antioxidants, which includes polyphenolics and flavonoids in the sample will scavenge the formed ABTS radical and thereby, a decreased colour intensity will be observed.

Reagents:

1. ABTS: Dissolve 5ml of ABTS and 5ml of 4.9 mM ammonium persulphate.
2. Ammonium per sulphate (4.9mM): Dissolve 22mg of APS in 20ml of distilled water.

Procedure:

Freshly prepare the ABTS radical solution by adding 5ml of a 4.9mM APS solution to 5ml of a 14mM ABTS solution and keep for 10hrs under dark conditions. The solution is diluted with distilled water to yield an absorbance of 0.70±0.02 at 734 nm and the same is used for the assay. To 900µl of ABTS radical solution, add 100 µl the extract (25-125 µg/ml) and the reaction mixture is vortexed for 10 s. Six minutes after record the absorbance at 734nm against distilled water by using spectrophotometer. Ascorbic acid used as reference compound.

Calculations: The percentage inhibition activity was calculated from $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of extract/standard taken as Ascorbic acid. All the values are mean ± SEM of three independent experiments and expressed as % ABTS radical scavenging activity.

Note: Since these are coloured samples, OD of coloured sample alone added to control OD values.

5.7 PHARMACOLOGICAL STUDY

ANIMALS

Male Wistar rats of 6-8 weeks old and 180-200 g body weight were selected for study. All rats were housed and maintained under standard conditions of temperature (25 ^{0}C ± 5 ^{0}C), relative humidity (55 ± 10%), and 12/12 h light/dark cycle. Animals were fed with commercial pellet diet and water *ad libitum* freely throughout the study. Protocols for the study were approved by the Institutional Animal Ethical Committee (IAEC) for Animal Care and were in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India.

5.7.1 ACUTE TOXICITY TEST

Acute oral toxicity study was performed as per OECD-423 guidelines. The mice were fasted overnight with free excess of water and were grouped into four group consisting of 3 animals each, to which the extract was administered orally at the dose level of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg body weight. They were observed for mortality; toxic symptoms such as behavioral changes, locomotor activity, convulsions; direct observation parameters such as tremor, convulsion, salivation, diarrhoea, sleep, coma, changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and CNS, somatomotor activity etc. periodically for 30 min during first 24 h. And specific attention given during first 4 hours daily for a total period of 14 days.

5.7.2. SCREENING OF ANTI-INFLAMMATORY ACTIVITY

Experimental method

Anti-inflammatory activity was assessed by histamine induced rat paw edema method.

- **HISTAMINE INDUCED RAT PAW EDEMA [65]**

Experimental design

Wistar albino rats were divided into four groups of 6 animals each.

Table :9 Experimental design for histamine induced paw edema

GROUPS	TREATMENT
Group I	0.1 ml of freshly prepared histamine (0.1%)
Group II	Diclofenac (20 mg/kg), p.o
Group III	EESC (200 mg/kg), p.o
Group IV	EESC (400 mg/kg), p.o

Procedure

One hour after the drug treatment, inflammation was induced by injection of 0.1 ml of freshly prepared histamine (1%) in normal saline underneath the plantar tissue of the right hind paw of rats. Paw volume, measured using a plythesmometer before histamine administration and at 1, 2, 3 h after histamine injection. The percentage inhibition (PI) of paw edema was calculated by using the following formula;

$$\textbf{Percentage of edema inhibition} = (V_c - V_t/V_c) \times 100$$

V_c = Mean edema volume in control

V_t = Mean edema volume in group treated with standard or extract

6. RESULTS AND DISCUSSION

6.1 EXTRACTIVE YIELD

Percentage Yield of EESC

Coarsely powdered bark of *Syzygium cumini* were extracted with ethanol (95%) and the percentage yield was found to be 22% w/w.

6.2 PRELIMINARY PHYTOCHEMICAL ANALYSIS

Table 10: Qualitative Chemical Tests

Sl. No	Phytochemical Constituents	EESC.
1	Carbohydrates	_
2	Alkaloids	+
3	Glycosides	+
4	Triterpenoids	_
5	Flavonoids	++
6	Phenols	++
7.	Tannins	+
8	Steroids and sterols	+
9	Saponins	_
10	Proteins and amino acids	++

+= Present, ++ = More present, - = Absent

6.3.QUANTIFICATION OF TOTAL PHENOL AND FLAVONOIDS CONTENT

6.3.1. Estimation of total phenolic content of EESC

Table 11 : Preparation of calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Absorbance (Mean±SD) λmax 760 nm
1	0	0
2	25	0.251±0.011
3	50	0.542±0.015
4	75	0.797±0.021
5	100	0.999±0.023
6	125	1.168±0.014

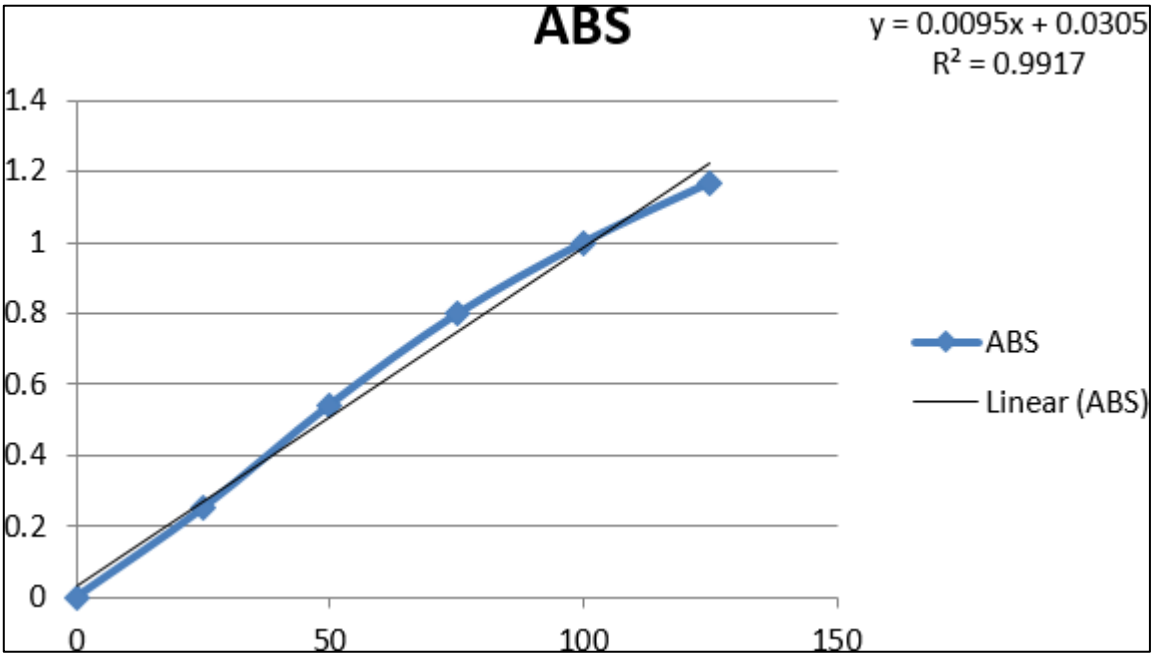


Fig: 3 Calibration curve of Gallic acid

The presence of phenolic compounds (TPC) is expressed as mg/g of gallic acid equivalent of dry concentrate test utilizing the mathematical expression obtained from the calibration curve: Y = 0.0095X + 0.0305, R2= 0.991, where Y is the absorbance and X is the Gallic acid equivalent (GAE).

In the present study, the ethanolic bark concentrate of *Syzygium Cumini* displays 71.94 mg/g of gallic acid equivalent phenols.

6.3.2 Estimation of total flavonoid content of EESC

Table: 12 Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Absorbance (Mean±SD) λmax 420 nm
1.	0	0
2.	25	0.108±0.012
3.	50	0.195±0.018
4.	75	0.297±0.023
5.	100	0.397±0.012
6.	125	0.517±0.018

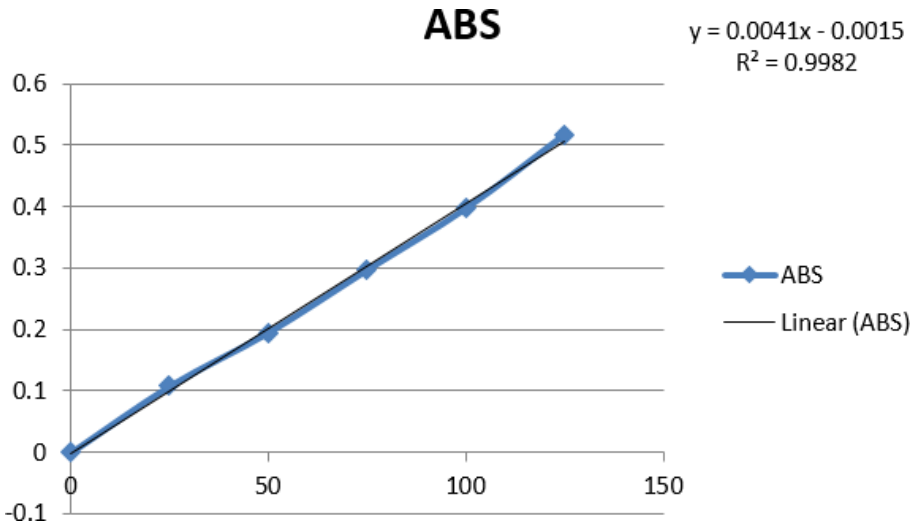


Fig: 4 Calibration curve of Quercetin

Absolute concentration of flavonoids was computed as quercetin proportionate (mg/g) utilizing the mathematical statement in light of the calibration curve: Y=0.0041 X-0.0015, R2=0.998, where Y is the absorbance and X is the quercetin comparable (QE).

The result reveals that the ethanolic bark concentrate of *Syzygium Cumini* exhibits 1.81 mg/g of quercetin equivalent of flavonoids.

6.4 IN-VITRO ANTIOXIDANT STUDY:

Table: 13 DPPH RADICAL SCAVENGING ACTIVITY

	% inhibition			**DPPH**
Conc	STD	SCBE	**Extract**	**IC 50 in µg/ml**
50	12.905	1.869	STD	134.4747
100	30.96	12.81	SCBE	240
150	56.111	28.26		
200	68.298	43.65		
250	86.66	49.77		

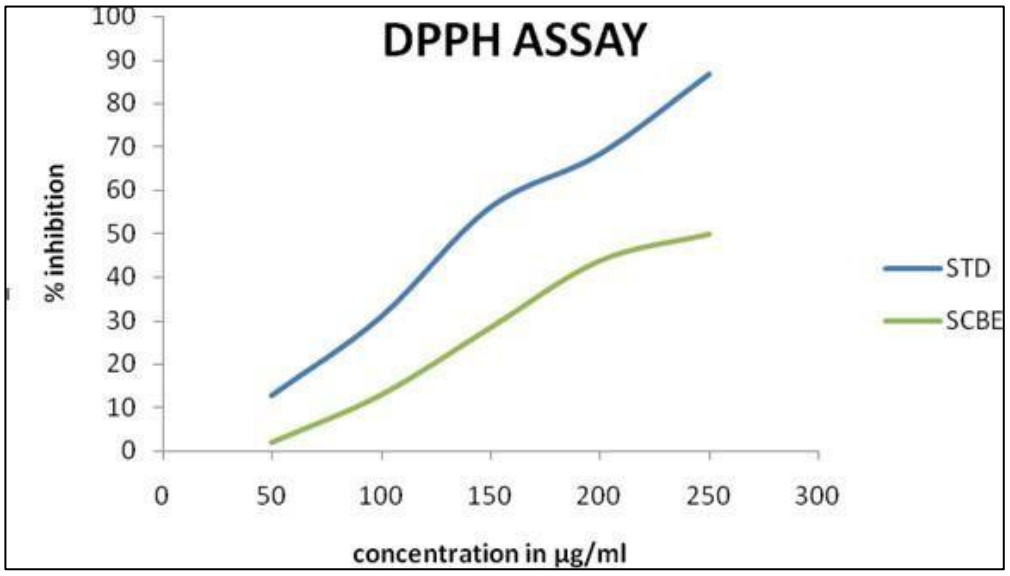


Fig :5 DPPH assay

6.4.2 ABTS RADICAL SCAVENGING ACTIVITY

Table: 14 ABTS RADICAL SCAVENGING ACTIVITY

ABTS	% inhibition			**ABTS**
Conc	**STD**	**SCBE**	**Extract**	**IC 50 in µg/ml**
25	20.9736	17.3761	STD	44.495
50	35.0822	22.89	SCBE	97.928
75	47.2373	38.0606		
100	66.0193	51.5586		
125	86.3962	64.2219		

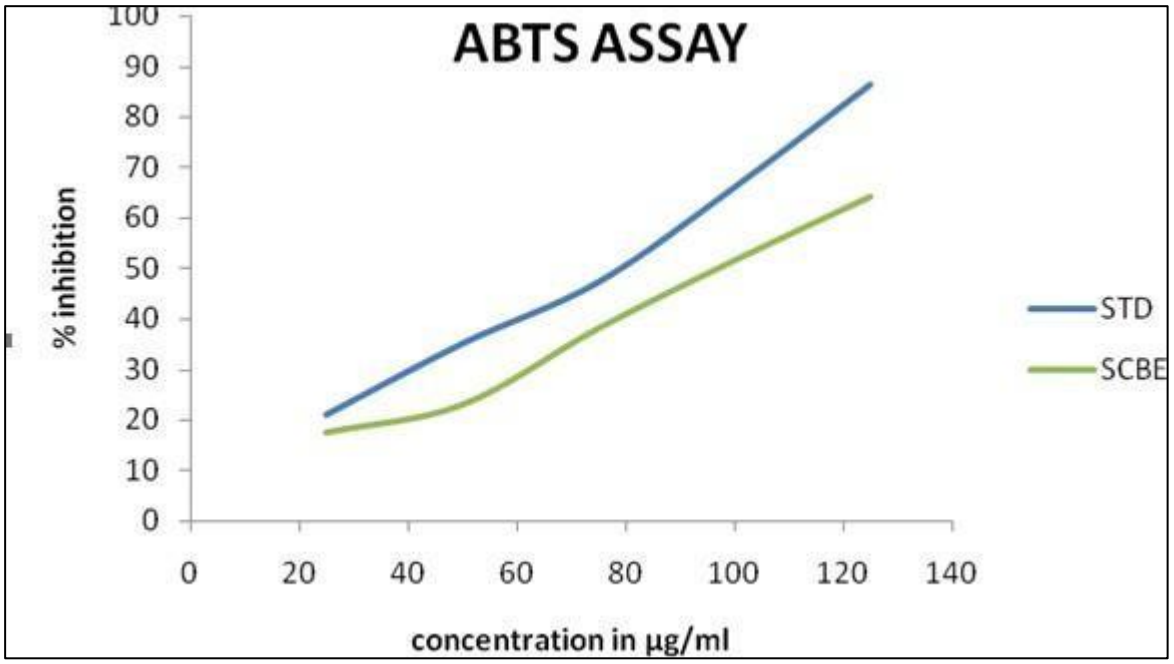


Fig.6 ABTS assay

6.5 ACUTE TOXICITY TEST

The acute toxicity test was performed by using the EESC at concentrations of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg. Toxicity study was performed as per OECD guidelines 423. It was observed that the EESC was not lethal to the rats at 2000 mg/kg dose. Hence the dose fixed for study as 200 mg/kg as low dose and 400 mg/kg as high dose. The observations are summarized in table:

Table 15: Acute toxicity study

Response	Group 1	Group 2	Group 3	Group 4
Alertness	Normal	Normal	Normal	Normal
Grooming	Normal	Normal	Normal	Normal
Touch response	Normal	Normal	Normal	Normal
Torch response	Normal	Normal	Normal	Normal
Pain response	Normal	Normal	Normal	Normal
Tremor	Normal	Normal	Normal	Normal
Convulsion	Normal	Normal	Normal	Normal
Lighting reflex	Normal	Normal	Normal	Normal
Gripping strength	Normal	Normal	Normal	Normal
Pinna reflex	Normal	Normal	Normal	Normal
Corneal reflex	Normal	Normal	Normal	Normal
Urination	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Skin color	Normal	Normal	Normal	Normal
Lacrimation	Normal	Normal	Normal	Normal
Diarrhoea	Normal	Normal	Normal	Normal

- **HISTAMINE INDUCED RAT PAW EDEMA METHOD**

Table 16: Effect of EESC on Histamine induced rat paw edema in rats

Group	Treatment and Dose	Mean edema volume(ml) and % inhibition			
		0 h	**1 h**	**2 h**	**3 h**
I	Histamine 0.1% (0.1 ml)	0.066±0.003	0.120±0.002	0.217±0.003	0.3133±0.002
II	Diclofenac (20 mg/kg)	0.067±0.004	0.061±0.0015*** (49.166%)	0.082±0.001*** (62.24%)	0.092±0.001*** (70.60%)
III	EESC (200 mg/kg)	0.063±0.004	0.101±0.005** (15.97%)	.112±0.001*** (48.112%)	0.130±0.002*** (58.46%)
IV	EESC (400 mg/kg)	0.064±0.002	0.076±0.002*** (36.83%)	0.095±0.003*** (55.89%)	0.103±0.003*** (67.09%)

Values in brackets denote percentage inhibition of the edema paw volume

Statistical comparison: Values represent mean ± SEM, n=6 compared with control, statistical analysis was done by one way analysis of variation (ANOVA) followed by Dunnett's test. ***P<0.001, **P<0.01, *P<0.05 and ns- non significant.

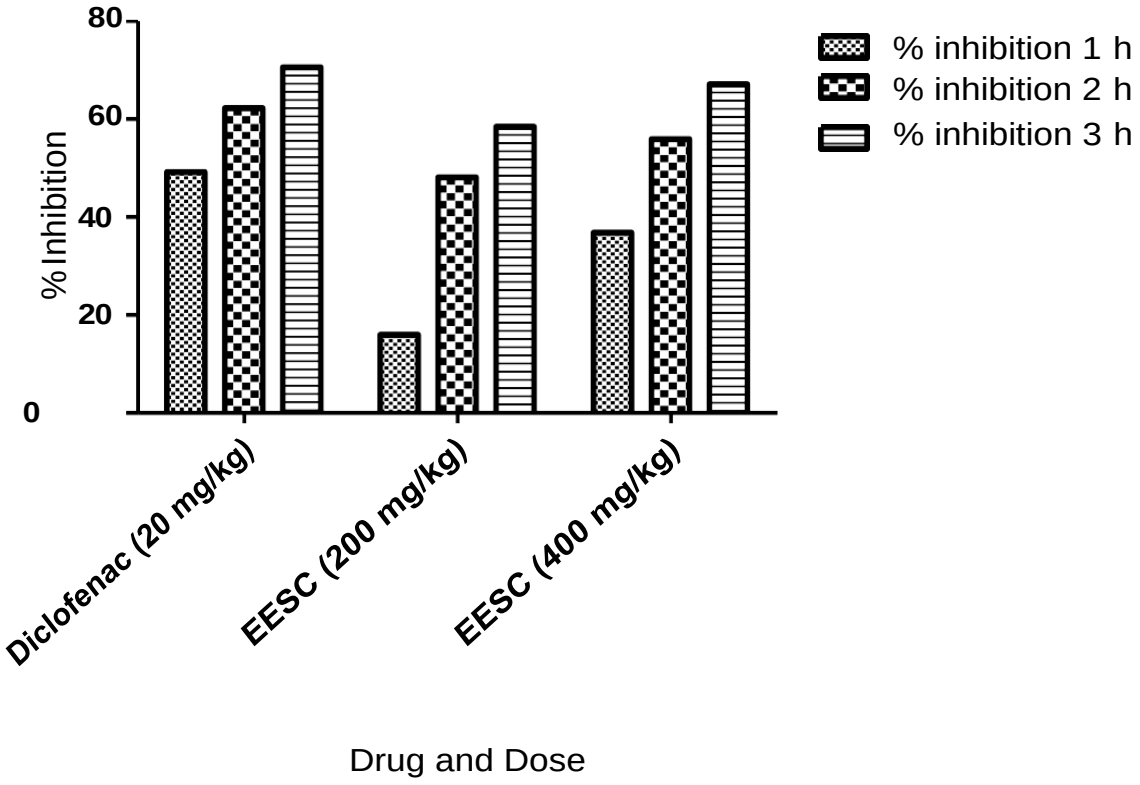


Figure 7: Anti-inflammatory effect of Diclofenac and EESC in histamine induced paw edema in rats.

6.8 STATISTICAL ANALYSIS

Data were analyzed by one way ANOVA followed by Tukey's multiple comparison test using Graph pad 5.0 software. The values were expressed as Mean ± SEM.

6.9 DISCUSSION

The study aims at the evaluation of the anti-oxidant and anti-inflammatory activity of ethanolic bark extract of *Syzygium cumini*.

The preliminary phytochemical screening of EESC were performed and the results revealed the presence of carbohydrates, flavonoids, triterpenoids, phenols, glycosides, tannins, steroids, alkaloids, saponins, proteins and amino acids. The various phytoconstituents present in the extract is shown in the Table 7.

Phenolics and flavanoid normally scavenge the free radicals and play an essential role in prevention and therapy of PTL, and many more diseases by inducing antioxidant defense system, drug metabolizing enzymes, modulating diverse events incellular level and inhibiting inflammation, hyperplasia, proliferation and oxidative DNA damage. Poly phenolic compounds (quercetin, gallic acid, rutin) are natural antioxidants which decreases oxidation of bio molecules essential for life [95]. The total

Phenol content present in *Syzygium cumini* bark extract was found to be 0.714 mg/g equivalent to gallic acid. The total flavanoid content in EESC was found to be 0.0059 mg/g of extract calculated as Quercetin equivalent.

Herbal drugs containing radical scavengers are gaining importance in treating oxidative stress related diseases.[130] DPPH radical scavenging activity of the extract at different concentration was compared with the standard quercetin. Smaller IC50 value indicates a higher antioxidant potential.

The IC_{50} value of the standard and test extract was found to be 134.47 µg/ml and 240 µg/ml respectively. The higher IC_{50} values of the sample indicated lesser scavenging activity of the sample when compared with the standard.

The study showed the promising radical scavenging activity of the extract due to hydrogen-donating ability of the extract. The ability of the extract to donate hydrogen atoms or electrons to scavenge the radical cation was reflected by the decolourization of ABTS radical cation. The radical scavenging activity of the extract was compared with that of the standard and the IC_{50} values obtained were 44.495 µg/ml and 97.928 µg/ml for standard and extract respectively.

Both doses of extract (200 mg/kg and 400 mg/kg) exerted a significant inhibition of 15.97% and 36.83% at 1 h, 48.11% and 55.89% at 2 h, 58.46% and 69.09% at 3 h respectively in the histamine induced rat paw edema model. It was observed that the extract was capable of inhibiting edema induced by histamine and the effectiveness for suppression of edema might be due to the ability of extract to inhibit the synthesis, release or action of histamine involved in the inflammation.

To improve the safety of this traditional herbal remedy, additional research is needed to define the stability and bioactivity of this product. Therefore, further studies are needed for the isolation and characterization of the active constituents.

7.CONCLUSION

Syzygium cumini Linn (family Myrtaceae) is a well-known bioactive plant in Ayurvedic system of medicine. The present investigation was aimed at determining the anti-inflammatory and anti-oxidant activity of ethanolic bark extract of *Syzygium cumini*.

EESC showed significant *in-vitro* antioxidant activity by terminating the actions of free radicals. The extract was studied for its anti-inflammatory activity using Histamine induced inflammation in which Diclofenac (20mg/kg) was used as the standard.

Thus the study proved that the EESC possesses significant ($P<0.05$) anti-inflammatory activity which was evident with reduction in mean paw edema volume in histamine induced inflammatory models.

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