ANTI-INFLAMMATORY PROPERTIES OF THE FLOWERS OF *Caesalpinia pulcherrima,* Swartz.

NANDHINI .A

(20PBC013)

A Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore – 641 043

In Partial Fulfilment of the Requirement for the Degree of

Master of Science in Biochemistry

MAY 2022

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Signature of the Head of the department

30/5/2022

Signature of the Supervisor

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NANDHINI. A

CONTENTS

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

1.0 INTRODUCTION

In the last decade, tremendous progress to understand the physiopathology of inflammation and the involvement of free radicals in its pathogenesis. The reaction is caused by oxygen species (ROS). During inflammation, an imbalance between the oxidant molecules and antioxidant system was induced by free radicals. Which increases the oxygen concentration of molecular level. This oxidative stress causes inflammatory cascades that damage cellular tissue components (Amri *et al.*, 2018).

Inflammation is initiated by the innate immune system in response to infection or tissue damage. Activated macrophages and neutrophils release active oxygen (ROS) such as superoxide, hydrogen peroxide, nitric oxide, and proinflammatory cytokines including TNFα (Abbas *et al.*, 2012). Both ROS and TNFα are activate NFkB, a proinflammatory transcription factor and a key regulator of the inflammatory response (Liu *et al.*, 2017; Kunnumakkara *et al*., 2020).

Despite an immune response to infection or injury is essential for life, uncontrolled production of ROS can lead to chronic inflammation. Generation ROS may cause damage beyond cellular antioxidant capacity DNA, proteins, lipids and cell membranes and lead to the emergence of various chronic diseases (Mueller *et al.*, 2010). Balancing ROS production and removal is achieved by activating the cellular antioxidant defenses systems.

The determination of inflammation is affected by several anti-inflammatory mediators and the recruitment of monocytes for cell elimination or tissue debris. Resolution may not occur in the acute phase, thus, it turns into a chronic stage. Chronic inflammation has been play role in the development of obesity-associated diabetes secondary to insulin resistance (Matshetshe *et al.*, 2018).

Inflammation has been described as a response activated by harmful stimuli and other injurious conditions. Moreover, the relationship between oxidative stress induced by free radicals and the inflammatory response has been reported by numerous studies (Allegra, 2019).

Research on medicinal plants is being carried out in different parts of the world. Related with the advancement of research in this area, an increase in interest in advanced knowledge

about Natural chemical compounds derived from them medicinal plant used by communities for maintain health and quality of life is observed (Bubalo *et al.,* 2018).

There are many factors that cause inflammatory process, such as infectious agents, ischemia, antigen-antibody interactions, heat or body shock. Steroids, non steroidal anti-inflammatory drug (NSAIDs) and immunosuppressants are commonly used to relieve inflammatory diseases (Oguntibeju, 2018). They require long term treatment and their use is often associated with serious side effects such as gastrointestinal bleeding and peptic ulcer. This lead to the search for an alternative treatments. In this regard, secondary metabolism of various medicinal plants have been shown to effective in treating inflammation and pain. Several studies reported that the different parts of the plant as fruits, galls and oil, possess biological effect including anti-inflammatory activities (Amri *et al.,* 2018*).*

Many plants and their active compounds have been tested for their anti-inflammatory property. One such plant is *Caesalpinia pulcherrima* (L.) Swartz. Which is used as an ornamental plant and as therapeutic agent in medicine for various diseases (Moteriya and Chanda, 2017).

Caesalpinia pulcherrima is a species of flowering plant in the pea family and is used in traditional Indian medicine. *Caesalpinia pulcherrima* has been used to treat tridosha, fever, ulcer, adortifacient, emmenaggue, asthma, tumors, vata and skin diseases (Premila *et al.*, 2018).

Kingdom : Plantae

Class : Tracheophytes

Order :Fabales

Family : Fabaceae

Subfamily :Caesalpinioideae

Genus : Caesalpinia

Species :*C. pulcherrima*

Caesalpinia pulcherrima is commonly known as peacock flower, pride of barabados. This palnt is widely used in traditional medicinal systems of India and also has been believed to possess antibacterial, anti-inflammatory, wound healing, antioxidant, anticancer, analgesic, antifertility, pharmacological activities, cytotoxic, anti-diabetic, anti-fungal, hepatoprotective, gastroprotective, analgesic, antiarthritic, anxiety, cardioprotective, anti-HIV activities, antiulcer and immunosuppressive potentiality (Vardhan *et al.*, 2019).

Caesalpinia pulcherrima is a plant known for its richness flavonoids that have different pharmacological properties, including antibacterial, antifungal, antiviral, anti-cancer and anti-inflammatory effects. *Caesalpinia pulcherrima* shows exceptionally high cytotoxic properties against malignant growth cells (Mallapur *et al*., 2021).

The methonalic extract of *Caesalpinia pulcherrima* lignum showed anti-inflammatory activity. Brazilin dye is reported to have anti-inflammatory activity (Pawar *et al.,* 2009). The trunk has antibacterial, demulcent and Hemostatic properties. It is used on bruises, wounds, Dysmenorrhea, excitement, leucorrhoea and anemia (Aksoy, *et al.,* 2016). In addition, manipulation of new vessel formation by plant derived compounds in anti inflammatory conditions such as wound healing, inflammatory diseases have also been studied to develop new therapeutic options (Zihlif *et al.,* 2013).

The flowers of this plant has been extensively studied in our laboratory and found that these flowers are rich in both enzymic and non-enzymic antioxidants. In accordance with this, the present study is aimed to investigate the anti-inflammatory activity of three different flowers (yellow, pink and orange) extracts obtained from flowers of *Caesalpinia pulcherrima*.

The study was formulated with various assays and tests with the following objective;

- to determine the anti-inflammatory activity of the flowers (yellow, pink and orange) extracts of *Caesalpinia pulcherrima* using protein denaturation and membrane stabilization assays.

REVIEW OF LITERATURE

2.0 REVIEW OF LITERATURE

2.1 INFLAMMATION:

Inflammation is a normal, essential, and protective response to any noxious stimulus that may threaten the host and may vary from a localized reaction to a complex response involving the whole organism. Many subtances called mediators are formed or realized either concurrently or in successive time sequences at the site of injury. Various cell sources are responsible to an etiological factor. Various cells containing potent mediators and in some instances, inhibitors of the inflammatory response. These cell sources may include neutrophils, basophils, mast cells, platelets, macrophages and lymphocytes (Davis and Granner, 1996; Parag, 2017).

The process of inflammation involves;

- Initial injury to tissues causing release of mediators.
- An acute transient phase characterized by local vasodilatation and increased capillary permeability.
- A delayed sub acute phase, most prominently characterized by infilteration of leukocytes and phagocytic cells.
- A chronic proliferative phase, in which tissue degeneration and fibrosis occurs.

2.1.1 INFLAMMATION A PART OF INNATE IMMUNITY:

Human innate immunity is naturally occur in the body from birth, and is not an adaptive immunity after an infection or vaccination. Innate immunity is generally non-specific, while adaptive immunity is very specific to particular pathogen / bacteria.The important causative agents responsible for inflammation include, burns, chemical irritants, frostbite, toxins, infection caused by pathogens, physical injury, ionizing radiation, foreign bodies (splinters, dirt and debris etc.) entry, stress, trauma, alcohol etc. Inflammation helps to heal the wounds and injury. Inflammation is the body's attempt to self-protection and to remove harmful stimuli and to begin the healing process. Human's immediate reaction to causative agent is swelling by inflammation and brings it down the swelling. The first stage is irritation, which later becomes inflammation - the immediate healing process, followed by suppuration (discharging of pus). There is a granulation stage, the formation in wounds of tiny, rounded masses of tissue

the process of healing. Thus without inflammation, the infections and wounds will never heal (Rao *et al.,* 2008; Rajeshwari *et al.*, 2015).

2.1.2 SYMPTOMS OF INFLAMMATION:

The ancients represent inflammation by five cardinal signs based on their visual observation such as heat (calor), pain (dolor), redness (rubor), swelling (tumour), and loss of function (functiolaesa). The final cardinal sign was named by Galen (Jenson *et al.,* 2019).

- Redness (Rubor) may be the presence of excess erythrocytes passes in to the area. It may also happen due to the persistent dilation of capillaries, venules, arterioles in injured part.
- Swelling (edema) is due to extra flow of fluid from permeable blood vessels to nearest tissues during prolonged inflammatory responses. Swelling (Tumour) may happen due to the permeability of small blood vessels which permits the exudates to release into tissues of injured area.
- Heat (Calor) is due to increased blood flow.
- Pain (Dolar) may be due to the effect of mediators of inflammatory response or from the initial damage or by the release of chemical substances such as bradykinin, serotonin and prostaglandins.
- The loss of function mention loss of motion in joint with pain, and edema, or due to functional cell removal with scar tissue. Inflammation is one of the defense mechanism of the body and is considered as drug target and better choice to treat disorders.

2.2 DISTINCT PHASES OF INFLAMMATORY RESPONSES:

- The first phase of inflammation is caused by an increase in vascular permeability resulting in exudation of fluids from the blood into the interstitial space.
- The second phase involves the infiltration of leukocytes from the blood into the tissue, followed by,
- Granuloma formation and tissue repair.

2.2.1 VASODILATATION AND INCREASED PERMEABILITY OF BLOOD VESSELS:

Within minutes after an injury, dilatation of arterioles and increased permeability of capillaries produce heat, redness and swelling in the affected area. The large amount of warm

blood flowing through the area produces both heat and redness. Edema results from increased permeability of blood vessels, which permits more fluid to move from blood into tissue spaces, pain whether immediate or delayed is cardinal symptom of inflammation; it can result from injury of nerve fibres or from irritation by toxic chemicals from micro-organisms. Kinins affect some nerve endings causing much of the pain associated with inflammation (Modak *et al.*, 2017).

2.2.2 EXUDATION OF LEUKOCYTES CHANGES IN THE FORMED ELEMENTS OF BLOOD:

In the early stage of inflammation, the rate of flow of blood is increased due to vasodilatation. But subsequently, there is slowing or stasis of blood stream. With stasis, changes in the normal axial flow of blood in the microcirculation take place. Due to slowing and stasis, the central stream of cells widens and peripheral plasma zone becomes narrower because of loss of plasma by exudation. This phenomenon is known as margination. As a result of this redistribution, the neutrophils of the central column come close to the vessel wall, this is known as pavementing (Modak *et al.*, 2017).

2.2.3 ADHESION:

In this, peripherally marginated and pavemented neutrophils stick briefly to the endothelial cells lining the vessel wall or roll over it (Agarwal *et al.*, 2003).

2.2.4 EMIGRATION:

After sticking of neutrophils to endothelium, they begin to squeeze through the wall of the blood vessel to reach the damaged area. This process is known as emigration. Simultaneous to emigration of leukocytes, escape of red cells through gaps between the endothelial cells, diapedesis takes place (Gopal *et al.*, 2014).

2.2.5 CHEMOTAXIS:

The chemotactic factor mediated transmigration of leukocytes after crossing several barriers to reach the interstitial tissues is called chemotaxis (Khajuria *et al.*, 2018).

2.2.6 PHAGOCYTOSIS:

Phagocytosis is defined as the process of engulfment of solid particulate material by the cells. There are 2 main types of phagocytosis cells (Krishnaraj *et al.*, 2010).

- Polymorphonuclear neutrophils
- Macrophages

Phagocytosis involves the following 4 steps:

- Attachment stage
- Engulfment stage
- Secretion stage
- Killing stage (Krishnaraj *et al.,* 2010)

2.3 TYPES OF INFLAMMATION:

2.3.1 ACUTE INFLAMMATION:

Acute inflammation is a response to any type of tissue damage and the process focusses mainly at removing the damaging agent as it is a short period process, it occurs in minutes or hours after tissue damage, and shows the inflammatory symptoms such as redness, heat and edema etc. (Toth *et al.,* 2004). It is due to passage of neutrophils into the damaged area, capillary infiltration, increased vascular permeability and exudation of fluids and plasma proteins from blood vessels to the area of tissue damage. Acute inflammation is a rapid self-limiting process, mainly mediated by prostaglandins, leukotrienes and vasoactive amines which elevate the movement of leukocytes and plasma into injured area. The gradual development from acute to chronic stage of inflammation in many inflammatory diseases was due to excess formation of pro-inflammatory mediators (Jenson *et al.,* 2019).

2.3.2 CHRONIC INFLAMMATION:

Chronic inflammation may begin slowly, or even in an unnoticed manner, but it can exist for several weeks, months, or years. This prolonged inflammatory condition may be the causative factor in the formation of degenerative diseases like cancer, multiple sclerosis, Rheumatoid arthritis, Alzheimer, atherosclerosis, congestive heart failure etc. The factors which may lead to chronic inflammation include improper functioning of mitochondria, uric acid crystals, oxidised products of lipoproteins, and advanced glycation end products. They are characterised by the infiltration of macrophage cells, mononuclear immune cells, neutrophils, monocytes, activation of fibroblasts, angiogenesis and results in fibrosis and tissue necrosis. Based on irritant behaviour, various inflammatory mediators known as cytokines are generated, which cause various morphological levels of inflammation. The general effects of

chronic inflammation are mediated largely by these cytokines. Chronic inflammation is always accompanied by weight loss, fatigue, wasting, sleepiness etc (Jenson *et al.*, 2019).

2.4 MEDIATORS OF INFLAMMATION:

The inflammatory response is a complex and highly regulated sequence of events that start with an initial production of pro-inflammatory mediators that recruit professional inflammatory cells to the site of injury to clear the offending trigger. Macrophages play major roles in the immune and inflammatory responses involved in host defence (Kaplanski *et al.*, 2003). Activated macrophages secrete a number of different inflammatory mediators, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL6), reactive oxygen species (ROS), prostaglandin E2 (PGE2), nitric oxide (NO), etc (Bosca *et al.*, 2005; Dhara *et al.*, 2013).

2.4.1 CYCLOOXYGENASE (COX):

COX is the key enzyme that catalyses the first two steps in the biosynthesis of the prostaglandins (PGs). The COX pathway leads to the generation of prostaglandins and thromboxanes, which mediate the pain and edema associated with inflammation. There are two isoforms of COX: COX-1 and COX-2. COX-1 is detectable, but COX2 is not detectable in most normal tissues, however, COX-2 can be induced by many factors such as pro-inflammatory cytokines, phlogistic factors, etc. Studies indicated that COX-2 plays an important role in inflammation (Shu *et al.*, 2006). Thus, those agents that could suppress the activity or protein expression of COX-2 are likely to be valuable medicine for anti-inflammation and pain. Thus, decreasing of synthesis and activity of COX-2 can result in anti-inflammatory action both in localized and systemic conditions (Gupta *el al.*, 2006).

2.4.2 PROSTAGLANDINS:

Prostaglandins (PGs) are generated by a variety of cell types, including activated macrophages (Harris *et al.*, 2002). The rate-limiting enzyme in PG synthesis is cyclooxygenase (COX). Prostaglandins are the end products of the metabolism of arachidonic acid by cyclooxygenases (COX) and prostaglandin synthases (PGS), and comprise a series of classical pro-inflammatory mediators like PGD2, PGE2, PGF2α, and PGI2 (Rajeshwari *et al.*, 2015).

2.4.3 ARACHIDONIC ACID:

The lipoxygenase pathway utilizes arachidonic acid by 5-lipoxygenase to produce the lipoxygenase products e.g. leucotrienes (LTs) which are also involved in inflammatory reactions as pro-inflammatory mediators. Leukotrienes, i.e. LTC4 and LTD4 cause edema together with increased microvascular permeability (Waghole *et al.,* 2022).

2.4.4 THROMBOXANE:

Thromboxane A2 (TXA2) is an arachidonic acid metabolite produced during the catalysis of arachidonic acid by the sequential action of COX and thromboxane synthase (TXS), and is well established as a potent vasoconstrictor. This metabolite participates in various physiological and pathological processes ranging from synaptic transmission to inflammation (Baravalia *et al.,* 2010). Platelets represent the best known cell type to produce TXA2 in response to various stimuli. However, many other cells and tissues are also able to synthesize TXA2 (Nakahata, 2008).

2.4.5 LEUKOTRIENES:

Leukotrienes (LT) are end products of the metabolism of arachidonic acid by 5-lipoxygenase. Leukotrienes have physiological roles in innate immune responses and pathological roles in a variety of inflammatory and allergic diseases, such as rheumatoid arthritis, inflammatory bowel disease, psoriasis, allergic rhinitis, but most prominently in bronchial asthma (Werz and Steinhilber, 2005).

2.4.6 POLYUNSATURATED FATTY ACIDS (PUFA):

Linoleic acid (LA) and α-linolenic acid (ALA) belong to the n−6 (ω−6) and n−3 (ω−3) series of polyunsaturated fatty acids (PUFA). LA and ALA are precursors for the synthesis of higher unsaturated species: arachidonic acid deriving from LA, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) deriving from ALA. One possible mechanistic explanation for these anti-inflammatory and antitumorigenic effects may be that an increased consumption of EPA and DHA results in an increased incorporation of these fatty acids into phospholipids at the expense of arachidonic acid. Consequently, they also replace arachidonic acid as a substrate for COX and LO that results in a reduced formation of PGE2, TXA2, LTB4 and LTE4 (Huwiler and Pfeilschifter, 2009).

2.4.7 HISTAMINE:

Histamine (HA) is a biogenic amine that affects a variety of functions in the human body. It has been known to play a role in inflammation, gastric acid secretion, and neurotransmission (Passani *et al.,* 2007; Huang and Thurmond, 2008). Multiple receptors exist for histamine in mammalian tissues and these have been classified into 4 distinct receptor types (H1R, H2R, H3R, and H4R), all of which are G-protein coupled receptors (GPCR). Histamine appears to play a complex role in pain modulation. Histamine released from mast cells is an established mediator of acute allergic reactions and chronic inflammation. Histamine and other mediators of inflammation increase vascular permeability at various times after injury. Chemical-induced vascular permeability (such as seen with acetic acid) causes an immediate sustained reaction that is prolonged over 24 h (Okoli *et al*., 2007).

2.4.8 NITRIC OXIDE (NO):

It is widely known that nitric oxide (NO), synthesized from L-arginine by nitric oxide synthase (NOS), is involved in diverse physiological processes. An excess in NO production is largely thought of as causing a variety of inflammatory diseases, such as sepsis, psoriasis, arthritis, multiple sclerosis, and systemic lupus erythromatosus (Clancy *et al.,* 1998).

2.5 OXIDATIVE STRESS AND INFLAMMATION:

During normal inflammatory process, the activated phagocytes (neutrophils and macrophages) generate enormous amount of ROS, reactive nitrogen and chlorine species for phagocytosis process. But under pathological inflammatory conditions, the generation of reactive species is enhanced, some of which may diffuse out of the phagocytes and induce a localized oxidative stress (Fialkow *et al.,* 2007; Jagathambal *et al., 2017)*). In addition to professional phagocytes, the non-phagocytic cells are also involved in the production of reactive species in response to proinflammatory cytokines. ROS induces inflammation by activating the transcription factor and inflammasome, which, in turn, induces maturation and secretion of several proinflammatory cytokines, resulting in localized inflammation. The base excision repair of oxidatively damaged/modified DNA base (7,8-dihydro-8- oxoguanine) by 8-oxoguanine-DNA glyoxalase-1 triggers a signaling cascade that promotes proinflammatory gene expression and inflammatory cell accumulation (Shimada *et al.,* 2012; Jenson *et al.,* 2019).

2.6 ANTI-INFLAMMATORY AGENTS:

Various therapeutic approaches are available for reducing long term inflammatory responses and thus the complications associated with them.

2.6.1 CORTICOSTEROIDS:

The corticosteroids, which are derivatives of cholesterol prednisone, prednisolone and methylprednisolone. These potent anti-inflammatory agents exert a variety of effects that result in a reduction in the number and activity of immune system cells. They are regularly used in anti-inflammatory therapy (Debnath *et al.,* 2013).

Corticosteroid treatment causes a reduction in the number of circulating lymphocytes due to steroid-induced lymphocyte lysis (lympholysis) or changes in lymphocyte circulation patterns. some species like hamsters, mice, rats and rabbits are particularly sensitive to corticosteroid-induced lympholysis. Corticosteroids also reduce both phagocytic and killing ability of macrophages and neutrophils, and this effect may contribute to their anti-inflammatory action. In addition, they reduce Chemotaxis so that fewer inflammatory cells attach to the site of TH cell activation. In the presence of corticosteroids, expression of class II MHC molecules and production of IL-1 by macrophages decreases rapidly; such reduction would be expected to lead to corresponding reductions in TH-cell activation. Corticosteroids also stabilize lysosomal membranes, so Where are the decreased levels of lysosomal enzymes released at the site of inflammation (Vangalapati *et al.,* 2011).

2.6.2 NON STEROIDAL ANTI INFLAMMATORY DUGS (NSAIDs):

Non-steroidal anti-inflammatory drugs (NSAIDs) are the centrepiece of pharmacotherapy for most rheumatological disorders, and are used in large numbers as analgesics and antipyretics, both as prescription drugs and over the counter purchases. Non-steroidal anti-inflammatory drugs (NSAIDs), which are often used for the relief of non-specific fever (Lesjak *et al.,* 2018), continue to be important for the palliation of pain. They are the most frequently used medications for the treatment of a variety of common chronic and acute inflammatory conditions (Manoukian and Carson, 1996), and continue to be important for the palliation of pain and in decreasing inflammation and fever (Baravalia *et al.*, 2011; Truong *et al.,* 2019).

Nearly all of the NSAIDs have been implicated in causing liver injury (Rabinovitz and Van Thiel, 1992). Diclofenac, and particularly sulindac, are reported to be more commonly associated with hepatotoxicity (Bjorkman, 1998). Several NSAIDs have been withdrawn from clinical use because of associated hepatotoxicity (Nunes *et al.,* 2020). The new high selective COX-2 inhibitors are celecoxib, rofecoxib and nimesulide are also associated with hepatotoxicity (Abate *et al.,* 2021). Hepatotoxicity from NSAIDs can occur at any time after drug administration, but like most adverse drug reactions, most commonly occurs within 6–12 weeks of initiation of therapy (Jenson *et al.,* 2019).

There are two main clinical patterns of hepatotoxicity due to NSAIDs. The first is an acute hepatitis with jaundice, fever, nausea, greatly elevated transaminases and sometimes eosinophilia. Some of the NSAIDs which causes the liver damage (Rawat *et al.,* 2012).

2.7 FACTORS RESPONSIBLE FOR INFLAMMATION:

The environmental pollutants damage the biomolecules like DNA which in turn leads to the formation of pyrimidine dimers, depurination, single-strand breaks, cross-linking of bases, alkylation or methylation. Radiations or mutagens also cause production of free radicals and reactive oxygen species (ROS) and result into lipid peroxidation of the cell membranes and cell organelles.

The ROS are unpaired electrons which are often short lived (10^{-10} s) and are highly reactive. ROS are generated in the living systems because of the effect of many external and internal factors and are also regularly produced during various life processes. Under normal conditions, ROS are generated through various mechanisms and are scavenged by a number of endogenous antioxidants like glutathione reductase, superoxide dismutase and catalase in the living system. When the magnitude of the ROS production exceeds the capacity of endogenously available antioxidants the ROS attach to various biomolecules like DNA, proteins, lipids, membranes etc. leads to the alteration in their permeability, structure and functionality. Presence of excessive ROS inside the body may affect the functioning of various vital systems which leads to various disorders. One of the most vital system in our body is gastrointestinal system which is exposed to microbes, non-sterile particles and leads to the enormous generation of oxidative species and have wide implication in the induction of pathogenesis, intestinal dysbiosis and changes in the permeability of gut mucosa (Kim *et al.,* 2012). Such oxidative stress in the gut may lead to the generation of diverse immune reactions

and inflammatory responses of the host tissue. In some cases it may lead to the emergence of various autoimmune disorders (Resnick *et al.,* 2010) like intestinal bowel syndrome (IBS) and diseases like cancer (Namazi *et al.,* 2009).

2.8 PLANT DESCRIPTION:

C. pulcherrima is a fast-growing shrub or small tree in the legume family. It is listed as 'naturalised', 'cultivation escape', and 'weed' in the Global Compendium of Weeds (Pournaghi *et al.,* 2020). The species is considered native to Asia and introduced to the West Indies and tropical regions around the world, as it has been cultivated for its striking and colorful flowers (Sharma *et al.,* 2011). It reproduces by seeds, which are produced profusely and are self-propelled by its dehiscent pods upon maturity. Although the species currently has a low risk of invasiveness score of 5 [score of 6 and above = reject the plant for import (Australia) or species likely to be of high risk (Pacific and Florida (U.S.)], it is known to be invasive in parts of Australia, Ecuador, the Philippines and Cuba (Chiang *et al.,* 2003).

C. pulcherrima is considered a weed in central Africa and southern Florida (Pournaghi *et al.,* 2020) and known to be a cultivation escape in Puerto Rico, Madagascar, and parts of the Caribbean. In the Netherlands Antilles, *C. pulcherrima* is listed as a species known to be invasive elsewhere but without sufficient information available to determine its potential threat to the Dutch Caribbean (Petal *et al.,* 2010). It is widely cultivated and has naturalized in many parts of the tropics including Micronesia, Mexico, Galapagos, and parts of Ecuador (Pournaghi *et al.,* 2020). In South America, it is possibly naturalized in parts of the Guiana Shield (Guyana, Suriname and French Guianas).

Caesalpinia pucherrima **(Yellow flower)**

Picture source;

https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.plantslive.in%2Fproduct%2Fbuy-caesalpinia-pulcherima-yellow-plant -online

***Caesalpinia pulcherrima* (Pink flower)**

Picture source

https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.amazon.in%2FM-Tech-Gardens-Peacock-Caesalpinia-pulcherrima%2Fdp%2FB094CG1VMP&psig=AOvVaw2qjTgRD17Dt

***Caesalpinia pulcherrima* (Orange flower)**

Picture source

https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.westhawaiitoday.com%2F2018%2F11%2F04%2Ffeatures%2Fplant-of-the-month-dwarf

TAXONOMIC TREE

Domain : Eukaryota

Kingdom : Plantae

Phylum : Spermatophyta

Subphylum : Angiospermae

Class : Dicotyledonae

Order : Fabales

Family : Fabaceae

Subfamily : Caesalpinioideae

Genus : Caesalpinia

Species : Caesalpinia pulcherrima

Shrub or small tree, unarmed or armed with spines or recurved prickles on branches, leaf rachises, and sometimes on nerves. Leaves are bipinnately compound, leaflets opposite or occasionally alternate, stipules various, large and leafy or minute. Flowers are caesalpinaceous, perfect or unisexual, in terminal and/or axillary racemes that are often aggregated into branched paniculate inflorescences, bracts caducous, bracteoles absent; calyx tube short, 5 lobed, lower lobe often covering the others, hood like; petals 5, imbricate, subequal or the upper one smaller and with a more developed claw; stamens 10, distinct, alternately longer and shorter; ovules usually 2-10. Fruit pods are flattened, rarely cylindrical, dehiscent or indehiscent, winged along the upper suture or unwinged. Seeds are 1-9, transversely arranged, ellipsoid or subglobose (Pankaj *et al.,* 2011). In its natural form in North America the species grows as a low branched, full, widespreading shrub about 10 feet tall and wide and requires plenty of room (Osuntokun *et al.,* 2017).

Caesalpinia pulcherrima is a striking ornamental plant and is widely grown in domestic and public gardens in warm climates with mild winters. It is a small sized shrub and tolerates pruning very well. This allows it to be planted in groups to form a hedgerow. It can be trained into a small accent tree in warmer locations. *C. pulcherrima* is an exceptionally good choice for xeriscaping with its brilliant, long-lasting summer blooms. It can be used in a variety of ways including as an ornamental accent plant or hedge. Its showy flower colours attract birds, hummingbirds, and butterflies.

2.8.1 THERAPEUTIC USES:

The availability of *Caesalpinia pulcherrima* as a coastal sand dune Legumes in the Mukka, Someshwara and Padubidri areas along the south the west coast of India was reported by (Ayaz *et al.,* 2015). According to the flowers of *C. pulcherrima* contain numerous compounds, such as lupeol, lupeol acetate, myricetin, quercetin and rutin. Infusion of flowers is used in the treatment bronchitis, asthma, intestinal worms and malarial fever. According to (Torre *et al.,* 2017) the bark showed potent antibacterial and cytotoxic activities. According to ethanobotanical research, seeds of *C. pulcherrima* was used to improve digestion and also used in killing the intestinal worms. *C. pulcherrima* dried seeds were eaten after frying. Le seeds were used as mouthwash by people of South and Central Tamil Nadu. The seeds are used for stomach pain (Aguiar *et al*., 2019).

The flower and leaves have been used for anticancer, antidiabetic and antimicrobial activities. The seeds are useful in the treatment of skin rash, rheumatism and as herbicides. Pulcherrima is rich in many pharmaceutical and biological products. active ingredients such as flavonoids, carotenoids, glycosides, phenols and steroids (Nowak *et al*., 2018). Next Dhaked *et al.,* (2011) reported that the A phytochemical analysis of C.bonduc revealed the existence of alkaloids, glycosides, sterols, saponins and essential oils. Ability analysis of current research of phytochemicals includes carbohydrates, tannins, saponins, flavonoids, alkaloids, betacyanins, quinones, terpenoids, phenols, glycosides, cardiac glycosides. A quantitative analysis of the main phytochemicals was also performed (Sakle *et al.,* 2019).

2.9 ASSAYS:

2.9.1 ANTI-INFLAMMATORY ACTIVITY:

1. Protein denaturation assay;

Denaturation of protein as one of the causes of inflammation. A number of anti-inflammatory drugs are known to inhibit protein denaturation. Denaturation was induced by incubating drugs with bovine serum albumin under some experimental conditions and compared to control. The method was used with slight modifications (Ngoua *et al.,* 2018).

2. Membrane stabilization test (Hemolysis assay);

The membrane stabilization test was evaluated by erythrocyte hemolysis. This hemolysis is caused on the one hand by heat, and on the other hand by distilled water with some modifications. In vitro hemolysis assay evaluating hemoglobin release in plasma. Immobilization of human red blood cells by hypotonicity-induced membrane lysis. HRBC membrane stabilization techniques to investigate the in vitro anti-inflammatory action of the extract have been incorporated by the following (Wang *et al.*, 2020). In the hemolysis test The human red blood cells and test material were co-incubated in a buffer at a predetermined pH that mimics extracellular. which is the endometrium in the early stages After a centrifugation step to pellet intact red blood cells, the amount of hemoglobin released into the medium is measured spectrophotometrically. In this model system the red blood cell membrane acts as a substitute for the lipid double membrane that surrounds the lipid bilayer membrane (Henneh *et al.*, 2018).

2.9.2 CONTROLS:

1. Sodium diclofenac:

Diclofenac sodium has antipyretic, analgesic and anti-inflammatory effects, is an inhibitor of cycloxygenase enzyme. Like other nonsteroidal anti-inflammatory drugs, clinical use of diclofenac has been associated with a small but significant incidence of hepatotoxicity, ranging from mild, asymptomatic, reversible increases in liver function tests to jaundice and hepatitis, including several reports of fatal hepatitis (VanderJagt *et al.*, 2022).

In many cases, the clinical and biochemical features of diclofenac hepatotoxicity suggest the involvement of reactive or toxic metabolites. These products presumably were formed via the hepatic cytochrome P450 (CYP)-catalyzed oxidation of diclofenac to reactive benzoquinone imines that are trapped by (GSH) glutathione conjugation. It is, therefore, possible that reactive benzoquinone imines may be formed and contribute to diclofenac mediated hepatic injury (Tang *et al.*, 1999; de Paiva *et al., 2019*).

Picture source;

https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.indiamart.com%2Fproddetail%2Fdiclofe nac-sodium-tablet-20620678862.html&psig=AOvVaw1yvFEeS8ftwFXmYD3GWAiU&ust=1619946926592000&source=images&cd=vfe&ved=0CAkQjhxqFwoTCJCsqMeSqPACFQAAAAAdAAAAABAD

EXPERIMENTAL PROCEDURE

3.0 EXPERIMENTAL PROCEDURE

This study has been designed to evaluate the anti-inflammatory activity of the flowers (yellow, pink and orange) extract of *Caesalpinia pulcherrima*.

The anti-inflammatory potential of *Caesalpinia pulcherrima* extracts was determined using protein denaturation and membrane stabilization stabilization assay.

3.1 PREPARATION OF *C. pulcherrima* FLOWERS (Yellow, Pink and Orange) EXTRACTS:

The plant sample was collected from nearby garden in Coimbatore. 5g of fresh flowers (yellow, pink and orange) petals were collected, cleaned, grinded and extracted using methanol (50ml). The extract was evaporated to dryness at 60°C and concentrations ranging from 20mg were dissolved in 5µg of Dimethyl sulfoxide.

Figure 1

Caesalpinia pulcherrima

(Yellow flower)

Figure 2

Caesalpinia pulcherrima

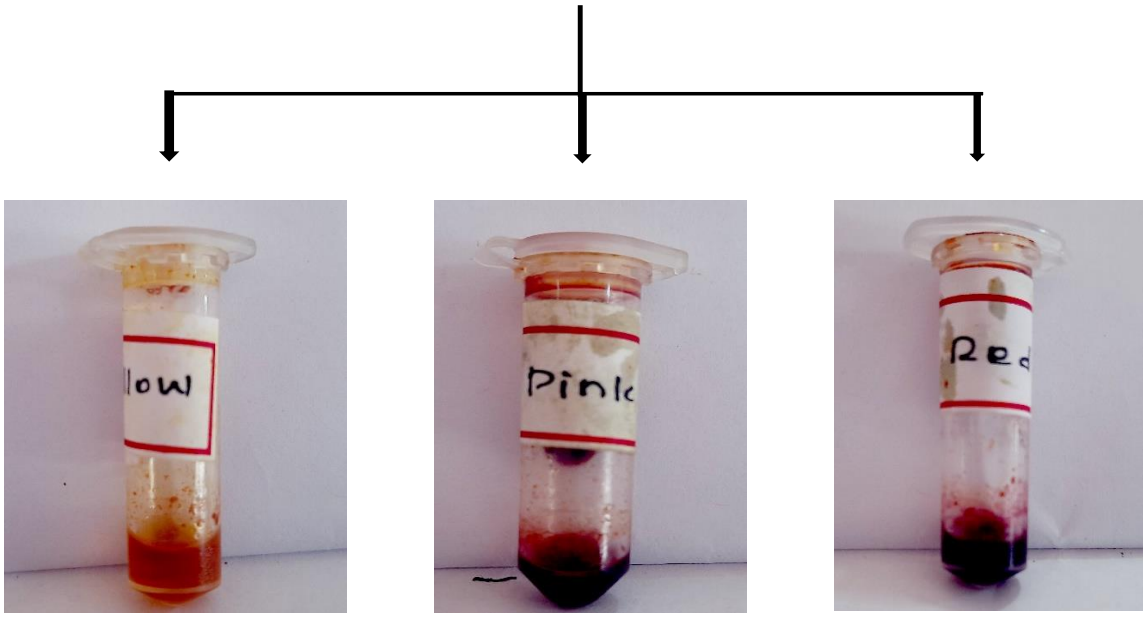
(Yellow flower)

Figure 3

***Caesalpinia pulcherrima* (Orange flower)**

PLANT SAMPLE;

The flowers (yellow, pink and orange) petals extracted, filtered and after evaporation the samples are



Yellow flower **Pink flower** **Orange flower**

FIGURE 4

***Caesalpinia pulcherrima* FLOWERS SAMPLE**

3.2 DETERMINATION OF ANTI-INFLAMMATORY ACTIVITY OF *Caesalpinia pulcherrima* FLOWERS (Yellow, Pink and Orange) EXTRACTS:

3.2.1 PROTEIN ANTI-DENATURATION ASSAY:

The protein anti-denaturation assay was carried out based on the method proposed by Williams *et al*.

Principle

Protein denaturation is considered as one of the reason for inflammation. This experiment is mainly done to study *in vitro* anti-inflammatory activity of test sample by protein denaturation (egg albumin) method.

Reagents

1. Bovine egg albumin
2. Phosphate buffered saline (pH 6.4)
3. Standard drug (sodium diclofenac)
4. Plant extracts

Procedure

About 0.1ml of fresh chick egg albumin was mixed with 1.9ml of phosphate buffered saline (p^H 6.4) and 1ml of extract with varying concentration. A similar volume of distilled water was used as negative control. Then the mixtures were incubated at 37°C in an incubator for 20 min and then heated at 70°C for 5 min. After cooling the absorbances were measured at 660 nm on the spectrophotometer. Sodium diclofenac in the final concentration, was used as a reference drug and similarly treated for the determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula,

$$\text{Inhibition (\%)} = [\text{Abs(sample)} - \text{Abs(control)} / \text{Abs(control)}] \times 100$$

Abs = absorbance. The concentration of the extract for 50% inhibition (IC50) was determined by the dose response curve.

3.2.2 MEMBRANE STABILIZATION ASSAY:

The membrane stabilization assay was evaluated by using hemolysis of red blood cells based on the method proposed by (Shinde *et al*. ; Mounnisaamy *et al*.).

Preparation of the suspension of erythrocytes;

Fresh whole blood (3 mL) collected from healthy volunteers in EDTA tubes was centrifuged at 2500 rpm for 10 min at 4 °C. A volume of normal saline equivalent to that of supernatant was used to dissolve the red blood cells. The volume of dissolved red blood cells obtained was measured and reconstituted in the proportion of a 40% v/ v suspension with isotonic buffer solution (10 mM sodium phosphate buffer, pH 7.4). The buffer solution contained 0.2 g of NaH2PO4, 1.15 g of Na2HPO4 and 9 g of NaCl in 1 L of distilled water. Reconstituted red blood cells (supernatant resuspended) were used.

i. Hemolysis induced by heat;

Principle

The membrane stabilization test was evaluated by the method of hemolysis of red blood cells. This hemolysis was induced on one hand by heat on the other hand by distilled water with some modifications.

Reagents

1. Blood cell suspension
2. Isotonic phosphate buffer (pH 7.4)
3. Methanol
4. Standard drug (sodium diclofenac)
5. Plant extract

Procedure

Samples of used extracts were dissolved in isotonic phosphate buffer solution. A set of 5 centrifugation tubes containing 2ml of extracts at increasing concentrations. Sodium diclofenac, with same concentration range were used as reference. The negative control contained 2 ml of distilled water. A suspension of 0.1ml of red blood cells are added to each of the tubes and mixed gently. The tubes were incubated at 54 ^{0}C for 20 min in a water bath. After incubation, tubes were centrifuged at 2500 rpm for 10min at 4^{0}C and the hemoglobin

content of the supernatant was estimated using the spectrophotometer at 540nm. The percentage of inhibition of haemolysis by the extract was calculated by using the following formula,

$$\% \text{ Inhibition of hemolysis} = [1 - \text{Abs(sample)} / \text{Abs(control)}] \times 100$$

Where Abs(sample) = absorbance of the sample; Abs(control)= absorbance of the control. The concentration of the extract for 50% inhibition (IC50) was determined by the dose-response curve.

ii. Hemolysis induced by hypotonicity;

Principle

This assay is based on the membrane stability of human RBCs subjected to lysis induced by hypotonicity.

Reagents

1. Blood cell suspension
2. Suspension of erythrocytes
3. Standard drug (sodium diclofenac)
4. Plant extract

Procedure

The extracts were dissolved in distilled water with different concentrations obtained from double dilution. Sodium diclofenac with the same concentrations was used as a reference drug. Distilled water was used as a negative control. 2ml of sample were mixed with 0.1ml of a suspension of erythrocytes and then the mixtures were incubated for 1 hour at 37^0 C. The tubes were then centrifuged at 2500 rpm for 10 min at 4^0C. The hemoglobin content of the supernatant was estimated using the spectrophotometer at 540 nm. The percentage of hemolysis was calculated assuming hemolysis produced in the presence of distilled water as 100%. The percentage inhibition of hemolysis by the extract was calculated by using the following formula,

$$\% \text{ Inhibition of hemolysis} = [1 - \text{Abs(sample)} - \text{Abs(control)}] \times 100$$

WhereAbs(sample) = absorbance of the sample; Abs(control) = absorbance of the control. The concentration of the extract for 50% inhibition (IC50) was determined by the dose-response curve.

RESULT AND DISCUSSION

4.0 RESULT AND DISCUSSION

Redness, soreness, swelling and a feeling of heat are all symptoms of inflammation, which is one of the body's self defense processes. Inflammatory reactions are crucial for the survival of the host, but they can also contribute to chronic inflammatory disorders as asthma (Fahy, 2015), cancer (Elinav *et al.,* 2013), rheumatoid arthritis (Innala *et al.,* 2016), Crohn's disease, and ulcerative colitis (UC) (Gobbi *et al.,* 2016). Inflammtion can be initiated by a microbial pathogen such as lipopolysaccharide (LPS). LPS is a prototypical endotoxin, which an directly activate macrophages (Rossol *et al.,* 2011). Inflammatory disorders are growing more common as people get older all across the world. Anti-inflammatory medications in clinical use have the disadvantages due to side effects and expensive treatment. Hence traditional medicines and natural goods especially from plant are an excellent alternate for allopathic remedies.

Medicinal herbs have been used as a source of a wide range of biologically active substances, either as crude plant extracts or as pure components, to cure a variety of diseases. Plants produce a variety of chemicals, the majority of which are secondary metabolites that operate as predator defences and are responsible for the usual odours and coloured characteristics of plants. Leaves, roots, rhizomes, stems, barks, flowers, fruits, grains, or seeds of such a plant will be used in the control or treatment of a disease state, and so include chemical components that are medically active. As a result, identifying therapeutically effective natural phytoconstituents from many types of plants has become a necessity (Stagos, 2019).

Caesalpinia is a genus with over 100 species found in tropical and temperate climates around the world. *Caesalpinia pulcherrima*, a fast growing shrub or small tree in the legume family, is one such promising plant. Because it has been grown for its beautiful and colourful blossoms, it is described as a "naturalised cultivation escape" and a "weed" in the Global Compendium of weeds (Pulipati *et al.,* 2012).

The quantitative screening of phytochemical compounds in the methanolic extract of *Caesalpinia pulcherrima* flowers revealed the presence of alkaloids, tannin, saponin, carbohydrates, terpenoids, flavonoids and amino acids whereas steroids, protein and phenol were absent (Premila *et al.,* 2019). It is vital to uncover bioactive compounds that will support *Caesalpinia pulcherrima*'s therapeutic qualities, given its diverse uses. Various research have

been conducted to assess the effects of *Caesalpinia pulcherrima* in the treatment of various diseases, but there is a scarcity of scientific material available online to demonstrate its anti-inflammatory properties (Shelar, 2019).

The three different flowers of *Caesalpinia pulcherrima* has been extensively studied in our laboratory for their anti-oxidant and anti-cancer properties using *in vitro* models. The present study was formulated to evaluate the anti-inflammatory activity of the methanolic extract of *Caesalpinia pulcherrima* flowers (yellow, pink and orange) and the results obtained are presented in this chapter.

4.1 DETERMINATION OF ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACTS OF *Caesalpinia pulcherrima* FLOWERS (Yellow, Pink and Orange):

The anti-inflammatory activity was assessed by protein denaturation method (Chatterjee *et al.,* 2012), and membrane stabilization method (Nguoa *et al.,* 2018).

4.1.1. PROTEIN ANTI-DENATURATION ASSAY:

Protein denaturation is the primary cause of inflammation. The potential of the extract to reduce protein denaturation was investigated as a part of the inquiry into the mechanism of anti-inflammatory effect. In comparison to sodium diclofenac, which was used as a standard anti-inflammation drug, this result indicates that *Caesalpinia pulcherrima* flowers (Yellow, Pink and Orange) extracts possess anti-denaturation activities.

Table 1

Protein anti-denaturation test

SAMPLE	% INHIBITION OF PROTEIN DENATURATION
Water	0.00 ± 0.00
Plant extract (Yellow flower)	26.84 ± 1.59
Plant extract (Pink flower)	47.22 ± 2.44
Plant extract (Orange flower)	64.81 ± 1.60
Sodium diclofenac	82.40 ± 4.24

The values are Mean ± S.D. of triplicates

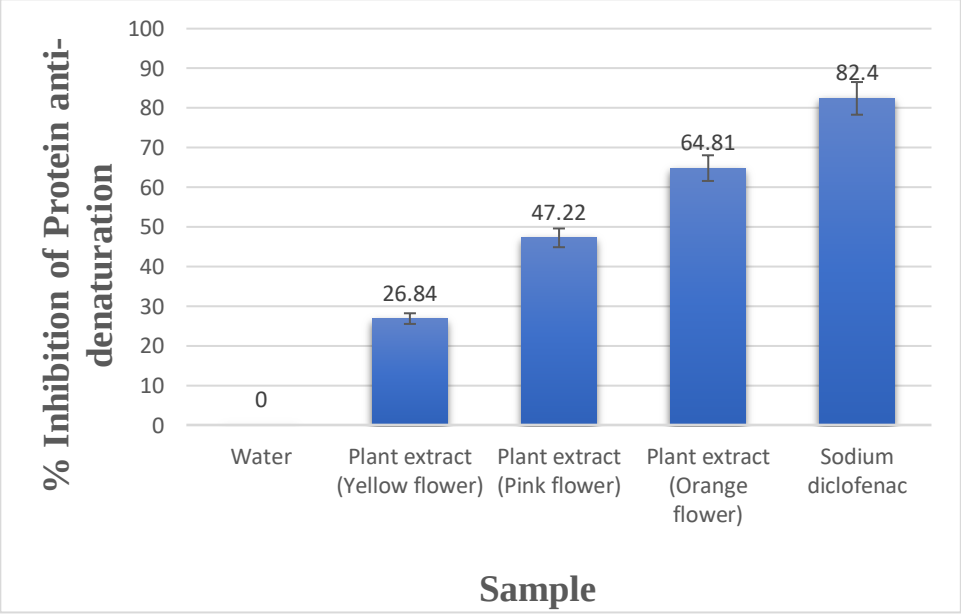


Figure 5

Effect of *Caesalpinia pulcherrima* flowers (yellow, pink and orange) extracts and sodium diclofenac against protein anti-denaturation

The results of protein anti-denaturation test are shown in Table 1 and figure 5. Protein anti-denaturation test results indicate that *Caesalpinia pulcherrima* flowers (Yellow, Pink and Orange) extracts have good anti-denaturation activities compared to the reference drug, sodium diclofenac. Inhibition percentage of methanol extracts are 26.84 ± 1.59 µg/ml (yellow flower), 47.22 ± 2.44 µg/ml (pink flower), 64.81 ± 1.60 µg/ml (orange flower) at the concentration of 100 µg/ml. The anti-denaturation activity is similar to that of the reference drug, sodium diclofenac is 82.40 ± 4.24 µg/ml. The maximum potential was exhibited by the orange flower extracts followed by pink and yellow flower.

Kousalya *et al.* (2020) studied at a concentration of 10 mg/ml of *Erythrina indica* has shown 33.98% of proteinase inhibitory ctivity and *Cardiospermum halicacabum* has shown 57.88% activity. And *mukia maderaspatana* has shown 73% of protease inhibition whereas *Delonix elata* has shown only 30% activity.

Yesmin *et al.* (2020), reported that the active compounds obtained from the Choi (*piper chaba*) part also had more or less anti-inflammatory activity. Acetyl salicylic acid a standard drug, showed the maximum inhibition of 78% at the concentration of 500 µg/ml, whereas CEE (crude ethanolic extract) of *P. chaba* showed 52.6% at that concentration.

Banerjee *et al.* (2014), reported that the leaf extracts of *M. scandens* at the dose of 16 mg/ml exhibited an anti-inflammatory activity that become significant ($p \leq 0.01$) with a 65% more inhibitory effect than the stem extract. The results were statistically significant ($p \leq 0.01$), whereas the 1000 mg/kg dose of the leaf extracts shows 37.62% greater activity than the recommended dose of the standard drug (Diclofenac sodium), which also exhibited concentration dependent inhibition of protein denaturation.

Similarly Gunathilake *et al.* (2018), reported that the methanolic extracts of leafy vegetables were able to inhibit protein denaturation in a concentration-dependent manner. Percent inhibition of protein denaturation of these leafy vegetables was within the range from 36.0% to 75.0% at the concentration range tof 25-100 µg/ml. Leaves of *C. auriculate* exhibited a significantly higher ($p \leq 0.05$) level of inhibition compared to other leafy types, whereas leaves if *C. asiatica* showed the lowest inhibition levels.

Ruiz-Ruiz *et al.* (2017), observed the effect of methanolic extract of *M. beecheii* honey and its fractions on protein denaturation was. Flavonoid fraction can effectively inhibit heat-induced albumin denaturation. About 50% inhibition was observed at 0.11 mg/ml.

acetylsalicylic acid, whereas standard anti-inflammatory drug showed inhibition of 50% at a concentration of 0.05 mg/ml. This indicate that the stronger anti-inflammatory activity was found in extracts and their fractions, compared to standard drug.

Similarly Dharmadeva *et al.* (2018), reported the inhibition rate of protein denaturation on treatment with cold water extract, hot water extract and prednisolone gradually increases with the increase in the concentration. In addition, a decreasing inhibition rate was observed with ibuprofen. It was found that 1000 μg/ml of the *F. racemose* bark extract showed the highest anti-inflammatory activity against 1;32ZEX denaturation of protein. Similarly, Mandal et al. (2000) reported that *F. racemosa* Linn. leaf extract (400 mg/kg) showed 32% of anti-inflammatory activity.

4.1.2. ERYTHROCYTE MEMBRANE STABILIZATION TEST:

Another important anti-inflammatory parameter is membrane stabilization which leads to inhibition of release of some proteases and bacterial components form the cells commonly seen during acute inflammation.

To study the effect of the plant extract on the stability of the cell membrane, human erythrocytes were used. The effect of plant extract on the stabilization of human RBC's were assayed in two separate conditions each as heat induced and hypotonicity induced haemolytic condition (Misra and Nisha, 2013).

The methanolic extract of *Caesalpinia pulcherrima* flowers (yellow, pink and orange) were tested for their ability to protect the erythrocytes from heat and hypotonicity induced haemolysis (Ngoua *et al.*, 2018).

4.1.2.A) HEAMOLYSIS INDUCED BY HEAT:

The anti-inflammatory activity of extracts of *Caesalpinia pulcherrima* flowers (yellow, pink and orange) was assessed by *in vitro* HRBS membrane stabilization method. Heat-induced haemolysis test results are showed in Table 2 and Figure 6.

Table 2

Hemolysis induced by heat

SAMPLE	% inhibition of hemolysis induced by heat
Water	0.00 ± 0.00
Plant extract (Yellow flower)	23.01 ± 1.81
Plant extract (Pink flower)	38.22 ± 2.25
Plant extract (Orange flower)	64.15 ± 2.97
Sodium diclofenac	90.07 ± 4.18

The values are Mean ± S.D. of triplicates

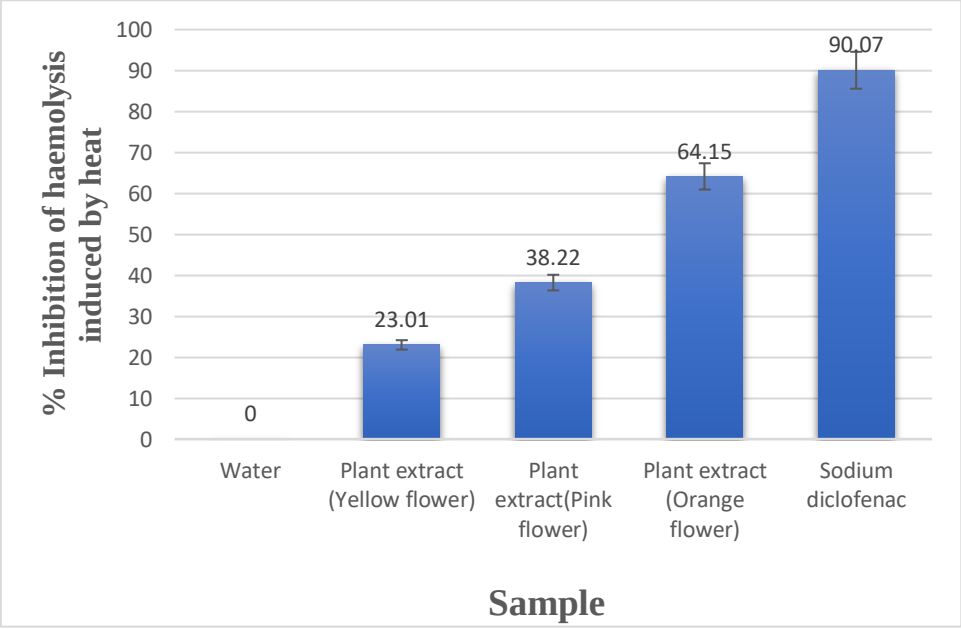


Figure 6

Effect of *Caesalpiniapulcherrima* flowers (yellow, pink and orange) extracts and sodium diclofenac on hemolysis induced by heat

For heat induced hemolysis, all the three flowers (yellow, pink and orange) extracts showed the good anti-hemolytic activity, compared to the reference drug, sodium diclofenac. The inhibition percentages of methanolic extracts are 23.01 ± 1.81 (yellow flower), 38.22 ± 2.25 (pink flower) and 64.15 ± 2.97 (orange flower) at respective concentration of 100 µg/ml. The heat-induced hemolysis activity is similar to that of the reference drug, sodium diclofenac 90.07 ± 4.18 µg/ml.

Misra, (2013), reported that in heat-induced hemolysis, the *Crinum asiaticum* extract was more effective at higher dose of 400 µg/ml (52.10%) in comparison to lower dose of 200 µg/ml (33.60%).

Several studies have been demonstrated the anti-inflammatory potential of medicinal plants through their ability to inhibit protein denaturation. Kargutkar, (2016) reported that all the three *Ananas comosus* fruit peel extracts were effective in significantly inhibiting heat induced albumin denaturation.

Similarly Gunathilake *et al.* (2018), reported that heat-induced hemolysis of red blood cells at different concentrations of each leafy vegetable, in the range of 25-100 µg/ml. Inhibition % of hemolysis from these leaf extrats (*Passiflora edulis, Olax zeylanica, Gymnema lactiferum, Sesbania grandiflora, Centella asiatica, and Cassia auriculata L.)* were within the range from 3.8% to 23.1% at the concentrations of 25-100 µg/ml. Leaves of *Passiflora edulis and O. zeylanica* showed higher ($p \leq 0.05$) levels of hemolysis inhibition compared to other leafy types.

Chippada *et al.* (2011), reported the percentage of membrane stabilization for methanolic extract of *Centella asiatica* and sodium diclofenac were done at 50, 100, 250, 500, 100, 2000 µg/ml. The methanolic extracts of *C. asiatica* are effective in inhibiting the heat induced hemolysis of HRBC at different concentrations (50-2000 µg/ml) and it showed the maximum inhibition of 94.97% at 2000 µg/ml.

Anosike *et al.* (2012), reported that the garden egg extract preserved the human erythrocyte membrane against lysis caused by heat at all dosages (100-800 µg/ml).

Ruiz-Ruiz *et al.* 2017, results showed the effect of methanolic extract of *M. beecheii* honey and its flavonoid fraction at a concentration of 0.42 mg/ml protect the erythrocyte membrane against the lysis induced by heat. Acetylsalicylic acid 0.07 mg/ml offered a 50% of

protection against the damaging effect of heat. It has been reported that certain flavonoids exerted profround stabilizing effect on lysosomal membrane both *in vivo and in vitro.*

Kardile *et al.* 2016, reported haemolysis in negative control samples as 100% and relative percentage of haemolysis in samples treated with different concentrations (100µg/ml, 10µg/ml, 1µg/ml, 0.1µg/ml, 0.01µg/ml, 0.001µg/ml) of ethanol and methanol. The samples are treated with diclofenac 50 µg/ml. Even at alcohol level in test media as low as 0.01 µl/ml, ethanol and methanol were protected against haemolysis, and this protective effect was statistically significant.

4.1.2.B. HEMOLYSIS INDUCED BY HYPOTONICITY:

The lysosomal enzyme released during inflammation produces a variety of disorders. The nonsteroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. The HRBC membrane is similar to lysosomal membrane, the study was undertaken to check the stability of HRBC membrane by the extracts to observe the anti-inflammatory activity *in vitro.* Hypotonicity-induced haemolysis results are shown in table 3 and figure 7.

Table 3

Hemolysis induced by hypotonicity

SAMPLE	% INHIBITION OF HEMOLYSIS INDUCED BY HYPOTONICITY
Water	0.00 ± 0.00
Plant extract (Yellow flower)	41.69 ± 0.85
Plant extract (Pink flower)	51.58 ± 1.03
Plant extract (Orange flower)	65.19 ± 1.27
Sodium diclofenac	70.68 ± 1.33

The values are Mean ± S.D. of triplicates

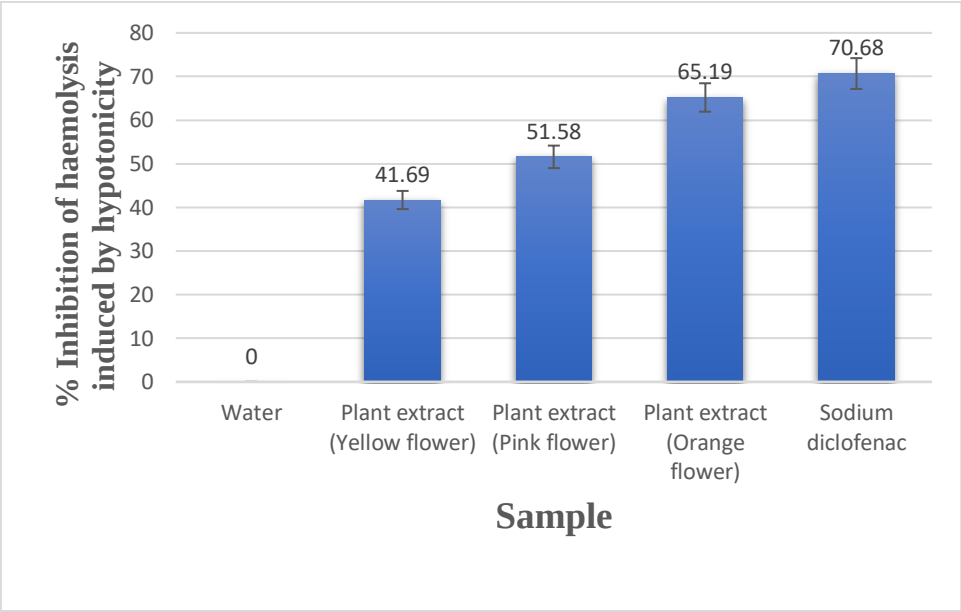


Figure 7

Effect of *Caesalpinia pulcherrima* flowers (yellow, pink and orange) extracts and sodium diclofenac on hemolysis induced by hypotonicity

For hemolysis induced by a hypotonic solution, all the extracts have good anti-hemolytic activity compared to the reference drug, sodium diclofenac. The inhibition percentages of methanolic extracts are 41.69 ± 0.85 (yellow flower), 51.58 ± 1.03 (pink flower), and 65.19 ± 1.27 (orange flower) at the concentration of 100 µg/ml. The anti-hemolytic activity is similar to that of the reference drug, sodium diclofenac is 70.68 ± 1.33 µg/ml.

Parvin *et al.* (2015), studied the CEE (crude ethanol extract) and CHF (chloroform fractions) of *Crescentia cujete* leaves and bark showed a concentration dependent anti-inflammatory activity, and the protection percent increased with increase in the concentration of the samples. At concentration of 1.0 mg/ml, the CEE of leaves and bark produced 53.86 ± 6.37 and 61.85 ± 5.56% inhibition of RBC haemolysis, respectively as compared with 75.80 ± 5.04% produced by standard drug aspirin. Likewise, CHF of leaves and bark produced 48.74 ± 0.56 and 43.55 ± 6.20% inhibition of RBC haemolysis. It is clear from the data that CEE of leaves and bark shoed grater response than the CHF.

Mirsa, (2013), examined the membrane stabilization activity of *Crinum asiaticum* plant extract the chicken erythrocyte. It was observed that the extract exhibited membrane stabilizing effect against haemolysis induced by heat and hypotonicity. But the effect was more prominence in the case of hypo-tonicity induced haemolysis of cell at a higher dose of 400 µg/ml.

Yesmin *et al.* (2020), reported that the ethanolic root extract of *P. chaba* extract may help to stabilize the by preventing the discharge of lytic enzymes and other active inflammatory mediators form the RBC membrane at lower dosage of 100 µg/ml when compared to a reference standard.

Obidoa *et al.* (2012), examined the garden egg extracts significantly ($p \leq 0.05$) inhibited lysis induced by water. The high percentage of haemolysis (40.8, 53.3 and 50.8) obtained at dosages of 400, 600 and 800 µg/ml, respectively. The hemolysis inhibition was discovered to be dosage dependent, increasing when the dose is raised the extracts concentration in the medium and similar to that obtained with indomethacin.

In this article the methanolic extract of the leaves of *Gardenia coronaria* to resist the cell lysis in small concentrations as compared to the standard drug aspirin at 100 µg/ml, even in the highest concentration of the extract at 300 µg/ml was able to prevent lysis of 33% which was 5.84% less than that of Aspirin, when used in 100 µg/ml. So the methanolic extract of plant has good membrane stability (Chowdhury, *et al.*, 2014).

Saleem *et al.* (2011), reported that the ethanolic and aqueous extract of *G. vulgaris nees* at different concentrations (200, 300, 400 µg/ml) showed considerable stabilization of HRBC membranes.

SUMMARY AND CONCLUSION

5.0 SUMMARY AND CONCLUSION

Inflammation is a biological response of tissue to stimuli including pathogens, damaged cells, chemical substances and begins the healing process for the tissue. Nowadays, both steroidal and non-steroidal anti-inflammatory drug (NSAIDs) are used in the prevention of inflammation. This led an interest among the people for the use of phytochemicals and herbal medicines to reduce pain and inflammation.

One such plant is *Caesalpinia pulcherrima* plant chosen for the present study. The present work was to evaluate the anti-inflammatory properties of *Caesalpinia pulcherrima* flowers (yellow, pink and orange) extract. The three flowers (yellow, pink and orange) extracts showed significant *in vitro* anti-inflammatory activity. As evident from protein anti-denaturation and RBC haemolysis assays. The results are summarized below;

- Methanolic extract of all the three flowers (yellow, pink and orange) of *Caesalpinia pulcherrima* were prepared and was used to assess all the assays.
- *Caesalpinia pulcherrima* flowers (yellow, pink and orange) extracts exhibit anti-inflammatory property against protein anti-denaturation and RBC haemolysis than reference drug (sodium diclofenac).
- The maximum protective effect against inflammatory process was observed in orange flower extract followed by pink and yellow flower.

Hence, it may be concluded form the present findings that the methanolic extracts of flowers (yellow, pink and orange) of *Caesalpinia pulcherrima* have significant anti-inflammatory activity in *in vitro* systems. Further research on isolating the components may be undertaken and they may be incorporated into existing anti-inflammatory compositions to improve their efficacy.

FUTURE SCOPE;

- To develop therapeutic methods with which infectious and inflammatory diseases are treated.
- The biologically active compounds present in the flowers of *Caesalpinia pulcherrima* can be isolated, purified and their structures can be elucidated.

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