PHYTOCHEMICAL, ANTIMICROBIAL, AND ANTIINFLAMMATORY STUDY ON THE FRUIT PEELS OF *PITHOCELLOBIUM DULCE*

(MANILA TAMARIND)

PRITHIKA, S.

(20PBT011)

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 Biotechnology

May 2022

Certificate

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PRITHIKA S

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Introduction

1. INTRODUCTION

Plants have long been known to be a rich source of innovative medicinal molecules, and herbal blends have made significant contributions to human health and happiness. The World Health Organization has also urged that plants be evaluated in situations where contemporary medications are not available. As a result, the quest for plant-based medications and nutritional supplements has increased in recent years. Currently, a lot of studies are being done to look at the ethnobotanical uses of plants that are common among indigenous peoples (Bamola *et al.*, 2018).

The medicinal potential of plant products and their use in the treatment of diseases can be evidenced the than 5000 years ago. Medicinal plants are valuable not only in traditional medicine but also in trade commodities, meeting the demand for novel pharmaceuticals in far-flung markets. In India, medicinal plants are used to treat a variety of ailments. It is widely utilized by people from all walks of life, as in various indigenous systems of medicine such as Siddha, Ayurveda, and Unani medicine, as well as India's pharmaceutical industry's processed products. There are around 4.5 million plant species in the world, and among them, only 250,000-500,000 plant species have been identified. Phytochemical research whether carried out of a plant possesses any biological or chemical implications (Sekhon *et al.*,2019).

In developing countries, herbal treatments have been used as an alternative to pharmaceutical products for health concerns and expenses. At least 25% of medications in today's pharmacopeia are still derived from plants. Such health-care delivery systems exist officially in China, India, Sri Lanka, and a few other countries (Ripple *et al.*, 2017). Secondary metabolites are biosynthetically created from primary metabolites by plants, and these phytocompounds are the main ingredients in herbal, pharmaceutical, and nutraceutical compositions. The therapeutic and nutritional needs of the human system are met by a variety of phytomolecules with specific biological activities. Phytomedicine is prepared using diverse components of plants, that act as a panacea for cultures, civilizations, and society (Kumar *et al.*, 2018).

1.1 THE CANDIDATE PLANT OF THE-*PITHOCELLOBIUM DULCE*

Pithecellobium dulce is used as a medicinal plant. *Pithecellobium* species is a flowering and fruit-bearing plant and the *Fabaceae* family. It is commonly known as Manila tamarind in English and seeni puliyangai in Tamil. Antimicrobial agents play a critical role in lowering the global burden of infectious illnesses. However, the growth and spread of multidrug-resistant (MDR) strains of pathogenic bacteria have become a huge public health hazard (Manandhar *et al.,* 2019).

Various research investigations have academically or experimentally tested the antibacterial activity of traditional herbs with findings on pathogenic microorganisms resistant to antimicrobials. Medicinal plants' antimicrobial activity, or the bioactive substances derived from various functional activities, may be capable of suppressing virulence factors as well as targeting microbial cells. Some bioactive chemicals produced from traditional plants have been shown to be capable of reversing antibiotic resistance and enhancing the synergetic impact of existing antibiotics (Mickymaray *et al.,* 2019). In drug treatment, resistance to antibiotics is becoming more frequent, and yet there is a dearth of new antibiotics. Inflammation is usually generated by damage to living tissues caused by bacterial, viral, and fungal infections, physical agents, and a faulty immune response, according to reports. The primary goal of the inflammatory response is to locate and eliminate harmful chemicals; secondarily, to remove damaged tissue components, resulting in tissue, organ, or system recovery. Many plants and their active compounds are studied for their antimicrobial and anti-inflammatory potential (Oguntibeju *et al.,* 2018).

Pithecellobium dulce is the only species that has spread beyond its native range. In India, it is currently common and naturalized. It has been found to possess many medicinal properties. The bark and pulp of the Manila Tamarind are traditionally used to treat gum problems, toothaches, and bleeding. The extracts of the bark are used to treat pain, fever, cold, dysentery, diarrhea, and constipation. Leaves are used to treat gall bladder problems and to prevent miscarriage. The seed powder is used to treat ulcers. The anti-oxidant, anti-inflammatory, anti-

diabetic, and anti-cancer effects of Manila tamarind have been studied extensively (Kulkarni *et al.*,2018).

Dried Fruit Peel of *Pithocellobium dulce* (Manila Tamarind)

In folk medicine, it's used as an emollient, anodyne, and larvicidae. Different components of the plant have been used to cure ailments in the past, including the skin of the stem for dysentery, the leaves for digestive issues, and the seeds for ulcers, among others. Leaves can also be used as a plaster to treat the discomfort of venereal sores and convulsions (Verma *et al.,* 2012). Chemical studies of the plant's many components have resulted in the isolation of a few new and fascinating metabolites, some of which have medicinal properties. Long before civilization realized the existence of germs, it was widely accepted that certain plants had healing properties and that they included what we now call antimicrobial principles (Gorlenko *et al.,* 2020).

The present study was formulated to evaluate the effect of the fruit peel extract of *Pithocellobium dulce* on antimicrobial and inflammatory conditions induced *in vitro*. The literature collection relevant to this study was done and the review of the vast literature is presented in the following chapter.

Review of literature

2. REVIEW OF LITERATURE

Medicinal plants and plant-derived medicine are widely used in traditional cultures all over the world and they are becoming increasingly popular in modern society as natural alternatives or supplements to synthetic chemicals. As more and more natural remedies are being commercialized, there is a need for a user-friendly but scientifically accurate reference guide to the plants and their products. This book is a photographic guide to the most commonly used and best-known medicinal plants of the world, including their botany, main traditional uses, active ingredients, pharmacological effects, and evidence of efficacy (Van Wyk *et al.*, 2018).

The review of literature pertaining to the present study" Phytochemical and Anti-inflammatory study on the fruit peel of *Pithocelobium duce"*

2.1 Medicinal plants

2.2 Phytoconstituents

2.3 Pathogenic microorganisms

2.4 Anti-microbial activity

2.5 Anti-inflammatory activity

2.6 *P. dulce* –The medicinal herb

2.1 MEDICINAL PLANTS

Medicinal plants are currently used widely for the treatment of various ailments. The demand for them is growing, as is their acceptance. Plant, perform a significant role health system of many developing countries as folklore medicine. Therapeutic herbs have always served as an overall ecosystem indicator of health (Jamshidi *et al.*, 2018).

The use of medicinal plants to heal ailments extends back to the beginning of human life on the planet. Throughout their lives on the earth, humans have treated themselves with medicinal plants based on their own experiences, knowledge, and thought, and have employed them to treat their maladies. Plants can alter many of these processes and enhance illness outcomes due to the availability of active chemicals. Humans have discovered some of the qualities and components of these medicinal plants through trial and error, over time (Ahandani *et al*., 2018).

Until today, the biological qualities of many plant species traditionally used in conjunction with their bioactive components had remained unknown. The identification of a large number of bioactive phytochemicals was possible using both traditional bioassay-guided natural drug discovery, procedures such as high-throughput screening, and even the novel reverse pharmacognosy approach (Marrelli *et al*.,2021).

Each year, roughly two million patients in the United States experience chemical drug-related adverse events, resulting in around 100,000 deaths. For example, the US Food and Drug Administration Advisory Council recently recommended that the popular pain medications Percocet and Vicodin be banned due to severe adverse responses Hence, the need for alternative medicine using natural products has become inevitable in the past few decades (Marrelli *et al*., 2021).

2.2 PHYTOCONSTITUENTS

Herbal substances have been reported to induce null allergic reactions, adverse effects, and comedogenic properties. herbal ingredients, are more effective, rich in instability, safety, purity, and cost-effectiveness, and are easily available and are found in a wide variety of plants (Prasanth *et al.*, 2020). Phytoconstituents, found naturally in plants, are gaining popularity. More than 10,000 phytoconstituents and phytopharmaceuticals have been identified and tested for the treatment of various ailments such as liver diseases, viral infections, cancer, and metabolic disorders (Karpuz *et al*., 2020).

Herbal product markets have grown significantly over the previous few decades, with diverse end-uses including flavors, colorants, essential oils, sweeteners, antioxidants, and nutraceuticals. Phytotherapy now employs around 8000 phenolic chemicals produced from medicinal plants in the form of herbal teas, classic and novel medications, industrial/pharmaceutical auxiliary goods, functional foods, and galenic items (Fierascu *et al.*, 2020). Medicinal compounds from a plant with increased efficacy require physicochemical and preliminary phytochemical analysis. Many plant species are capable of thriving in an environment brimming with bacteria, fungus, and viruses since they produce defensive natural chemicals against these pathogens that possess bactericidal, fungicidal, or virucidal effects in humans. Many plant-derived phytoconstituents like phenols and flavonoids have been reported

to possess anti-inflammatory, antioxidant, antimutagenic, and anticarcinogenic qualities (Dasari, *et al.*, 2021).

2.3 PATHOGENIC MICROORGANISMS

In the ecosystem, nonpathogenic microorganisms play key roles in the carbon and nutrient cycle, animal (including human) and plant health, and agriculture. The global food web relies majorly on microorganisms. Microorganisms reside in all places on earth that are populated by macroscopic organisms, and in some settings, such as the deep subsurface and 'extreme' environments. On the other hand, they can cause significant infections to humans and animals, putting their health and lives in jeopardy. A pathogenic organism is one that can infect a host (human) and cause sickness. The World Health Organization (WHO) classified potentially hazardous bacteria, viruses, poisons, parasites, and chemicals as potential food dangers. According to the WHO, tainted food causes one out of every ten people to become unwell, and 420,000 people die each year. Salmonellosis, listeriosis, campylobacteriosis, and yersiniosis are examples of foodborne disorders caused by pathogenic bacteria (Pigłowski *et al*., 2019).

2.3.1 *Escherichia coli*

E. coli is an essential member of the normal intestinal microflora of humans and other mammals. On the other hand, it is more than a laboratory workhorse or an innocuous intestine dweller; it may also be a highly adaptable, and frequently deadly, pathogen. Virulence factors affect a wide range of cellular functions and several different *E. coli* strains cause a variety of intestinal and extraintestinal illnesses. Cell signaling, ion secretion, protein synthesis, mitosis, cytoskeletal function, and mitochondrial function are all eukaryotic cellular processes that *E. coli* virulence factors can influence (Denamur *et al*.,2021).

2.3.2 *Staphylococcus aureus*

S. aureus is highly adapted to its human host and the health-care setting, having been first detected in purulent pus from a leg abscess by Ogston in the 1880s and properly isolated by Rosenbach not long after. Endocarditis, bacteremia, osteomyelitis, and skin and soft tissue infections are all caused by *S. aureus*, which is a common commensal. *S. aureus* has become a prominent cause of health-care-associated infections with the advent of hospital-based medicine. Penicillin provided temporary relief, but resistance developed in the 1940s, mediated by the blaZ -lactamase gene. Around 1960, the first semi-synthetic anti-staphylococcal penicillins were

created, and within a year after their first clinical usage, methicillin-resistant *S. aureus* (MRSA) was discovered. In reality, genomic data reveal that methicillin resistance existed before the first antibiotic was developed (Turner *et al*., 2019).

2.3.3 *Bacillus* species

Bacillus species are Gram-positive or Gram-negative aerobic rod-shaped bacteria. They produce spores that are resistant to cold, heat, and standard disinfectants, allowing bacteria to thrive in a wide range of conditions. *Bacillus* is a big genus that contains around 200 different species. *Bacillus* species are mostly non-pathogenic, and several have been employed for biotechnological and industrial purposes. Only a few Bacillus species have been shown to cause disease in both animals and humans. *Bacillus anthracis* and *Bacillus subtilis* are medically significant Bacillus species; *B. anthracis* is the causative agent of anthrax, a major animal illness, and *B. cereus* can cause food poisoning as well as local and systemic infections. Furthermore, *Bacillus licheniformis* has been linked to foodborne disease, and *Bacillus thuringiensis* is a significant insect pathogen (Zhang *et al*., 2019).

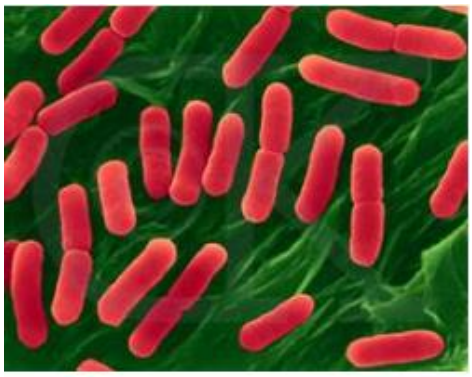
2.3.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative bacterium that is found in almost every habitat. It is an opportunistic human pathogen that can cause a variety of life-threatening acute and chronic infections, especially in people immune-compromised. It is highly pathogenic since it causes morbidity and mortality in cystic fibrosis patients. It is one of the most common nosocomial bacteria infecting hospitalized patients and is intrinsically resistant to a wide spectrum of medicines (Moradali *et al*., 2017).

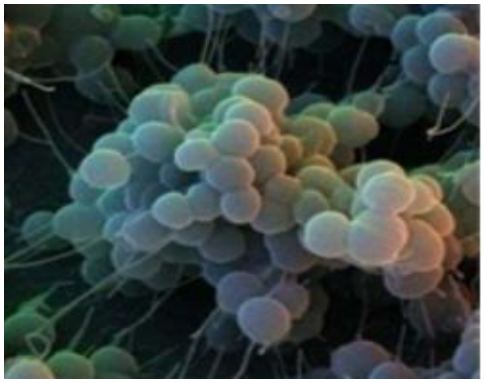
2.3.5 *Aspergillus* species

Aspergillus species can be found growing on plants and decomposing organic materials, as well as in soil, air/bioaerosols, animal systems, and freshwater and marine settings.in addition to indoor settings such as building surfaces, air, and household appliances. As well as drinking water and dust also contain *Aspergilli.* The Aspergillus genus contains a vast range of species that can grow on a variety of organic substrates and adapt to a wide range of environmental conditions (Khan *et al*., 2018). They generate asexual conidia that are easily airborne and stress-tolerant, as well as sexual ascospores that are environmentally persistent Despite the fact that the *Aspergillus* genus has hundreds of species, only a few have significant effects on human or

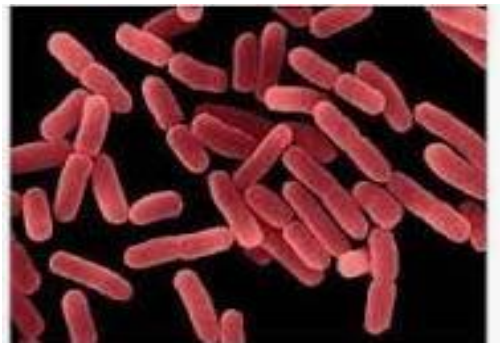
animal health. *Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger, and Aspergillus terreus,* cause infections (Paulussen *et al*., 2017).



Escherichia coli

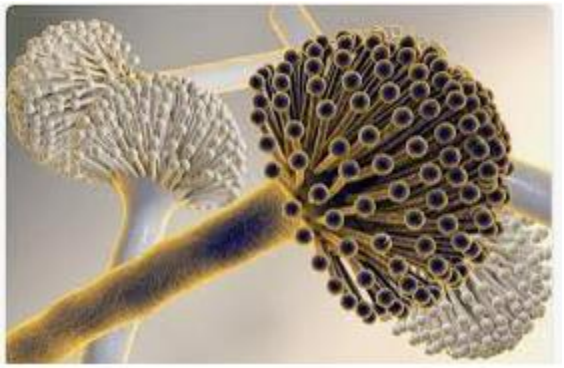


Staphylococcus aureus



Bacillus subtilis

Pseudomonas aeruginosa



Aspergillus niger

Figure 1: Pathogenic microorganisms

(https://sphweb.bumc.bu.edu/otlt/mphmodules/ph/ph709_infectiousagents/PH709InfectiousAgents4.html)

2.4 ANTIMICROBIAL ACTIVITY

Antimicrobials have a critical role in lowering the global burden of infectious illnesses. However, the growth and spread of multidrug-resistant (MDR) strains in pathogenic bacteria has become a huge public health hazard because there are fewer, or even no, effective antimicrobial medicines available to treat pathogenic bacteria-caused infections (Manandhar *et al*., 2019).

Bacterial infections are a leading cause of chronic illness and death. Because of their cost-effectiveness and potency, antibiotics have long been the chosen treatment for bacterial illnesses. Several studies have shown that widespread antibiotic use has resulted in the evolution of multidrug-resistant bacterial species (Aslam *et al.,* 2018). Antimicrobial susceptibility testing can be utilized in drug development, epidemiology, and therapeutic outcome prediction. The utilization of antimicrobial testing methodologies for the *in vitro* examination of extracts and pure medicines pare way to develop antibacterial agents (Ouedrhiri *et al.,* 2017).

2.4.1 METHODS TO TEST ANTIMICROBIAL ACTIVITY:

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similar to the disk diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested (Balouiri *et al*., 2016).

The advantages of this method are simple, inexpensive, and a good indicator. The historical viewpoint on traditional approaches such as disk diffusion, Epsilometer test (Etest), and microdilution that paved the way for current AST is discussed. Several new techniques, like microfluidic-based optical and electrochemical AST, have been thoroughly examined (Ginwala *et al.,* 2019).

2.4.2 BROTH DILUTION METHOD

Plant are evaluated for their antimicrobial potential using the broth dilution method, in which the minimum inhibitory concentration against the series of bacterial and fungal species can be determined. The MIC test is generally simple to prepare for and perform, which naturally improves reproducibility. The MIC test can be carried out on a modest scale without the use of excessive antimicrobial agents. Experimental antimicrobials, such as biologically produced antibacterial peptides, require this. The MIC test is a simple technique to evaluate a formulation's antimicrobial properties across a variety of parameters, such as bacteria species or surfactant blends (Kowalska-Krochmal *et al.,* 2021).

2.4.3 GROWTH CURVE ASSAY

The time-kill kinetics assay is used to study the activity of an antimicrobial agent a bacterial strain and can be used to determine the bactericidal or bacteriostatic activity of an agent over time.

2.5 INFLAMMATION

Inflammation is the main component of the innate immune response, which is activated when tissue homeostasis is threatened. The goal of the inflammatory response is to neutralize infectious pathogens and start the tissue repair process. Pain (dolor) mediated by bradykinin and prostaglandins, heat (calor) as a result of increased blood flow, redness (rubor) as a result of blood vessel dilation, and swelling (tumor) as a result of fluid buildup in the extravascular space are the four cardinal symptoms. The end result is a loss of function (Ganz *et al.,*2019).

There are three stages to the inflammatory response. The vascular permeability rises in the first phase, resulting in fluid exudation from the blood into the interstitial space. The infiltration of leukocytes from the circulation into the tissue characterizes the second phase. The creation of granuloma and tissue healing is part of the third phase. Histamine, leukotrienes, nitric oxide, prostaglandins, bradykinin, cytokines, serotonin, platelet-activation factor, lipoxins, and growth factors are all inflammatory mediators that are released either from plasma or by inflammatory cells (Bauer *et al*., 2019).

Inflammation can be either acute or persistent. Acute inflammation is a quick reaction that tries to eliminate invading germs while also assisting wound healing. It is characterized by the exudation of fluids and plasma proteins, as well as the migration of neutrophils into the damaged location. The influx of macrophages and lymphocytes during chronic inflammation causes fibrosis and tissue necrosis. Inflammation that is chronic can linger for weeks, months, or even years (Bektas *et al.,* 2018).

2.5.1 INFLAMMATION AND DISEASES

The inflammation arises as a result of a microbial infection or mechanical injury, it is beneficial. It is defined as a group of immune responses that are preserved and engaged in the repair and recovery of damaged tissues. Long-term inflammation or inflammatory pathway dysregulation, on the other hand, has the ability to damage or injure tissues and lead to the development of a number of chronic diseases (Chen *et al*., 2018).

Immune cells interact with intestinal bacteria in the colon and regulate proinflammatory effector cells as well as anti-inflammatory pathways. Inflammatory bowel illnesses, such as Crohn's disease and ulcerative colitis, can develop as a result of uncontrolled bacterial colonization, dysregulation of the homeostatic balance, disruption of the epithelial barrier, and irregulated activation of immune effector cells. In developing countries, acute enteritis is said to be the second-largest cause of death. Every day, the number of persons suffering from chronic inflammatory illnesses of the intestine and chronic enteritis rises substantially (Klionsky *et al*., 2021).

Inflammation is considered to be one of the key factors in the development of several other disease conditions, like depression, diabetes, cancer, obesity, osteoporosis, cardiovascular disease, rheumatoid arthritis, Parkinson’s disease, and asthma (Nakkala *et al*., 2021).

Cytokines, or low-molecular-weight regulating proteins, are expressed at all stages of the immune response. The primary acute phase inflammatory indicators are fibrinogen and C-reactive protein. They play a role in coronary artery disease, atherosclerosis, and other cardiovascular disorders such as stroke, myocardial infarction, peripheral vascular disease, and atherothrombosis (Aksentijevich *et al.,* 2020).

2.5.2 ANTI-INFLAMMATORY ACTIVITY

Inflammation is a complex combination of protective and reparative reactions to tissue injury caused by mechanical, autoimmune, or viral stresses. Inflammation can be acute or persistent. During the acute phase of inflammation, neutrophils, macrophages, and dendritic cells all contribute to cytokine generation, which spreads the inflammatory events. Many diseases, such as atherosclerosis, arthritis, cancer, and ischemic heart disease, have an inflammatory genesis, despite the fact that inflammation has a beneficial effect. Several pathways are involved in the production and release of pro-inflammatory mediators (Alonso *et al.*, 2019).

Since ancient times, plants have played a significant part in human health care. Plants create a variety of biologically active chemicals as a defense mechanism against pathogens and environmental stress. These tiny organic compounds are produced during secondary metabolism and have a variety of biological functions. Anti-inflammatory properties are highlighted among the several functions (Nunes *et al.*,2020).

2.5.2.1 PROTEIN DENATURATION ASSAY

The denaturation of proteins is one of the causes of inflammation. Protein denaturation is known to be inhibited by a number of anti-inflammatory medications. Under some experimental conditions, denaturation was induced by incubating medications with bovine serum albumin, and the results were compared to the control. The method was used, with a few minor changes noted. (Teede *et al*., 2018).

2.5.2.2 MEMBRANE STABILIZATION TEST (HEMOLYSIS ASSAY)

The hemolysis of red blood cells method was used to evaluate the membrane stability test. Heat, on the one hand, and distilled water, with minor changes, was used to promote hemolysis. The *in vitro* hemolysis assay measures the amount of hemoglobin released into the bloodstream (Kabir *et al.*, 2018). Stabilization of human red blood cells by hypotonicity induced membrane lysis. To examine *in vitro* anti-inflammatory action of extract HRBC membrane stabilization technique is incorporated by following (Samina *et al*., 2020). Human red blood cells and test materials are co-incubated in buffers with a predetermined pH to replicate extracellular, early endosomal lysis in the hemolysis experiment. The amount of hemoglobin released into the media is spectrophotometrically quantified after a centrifugation stage to pellet intact red blood

cells. The erythrocyte membrane acts as a surrogate for the lipid bilayer membrane that encloses endolysosomal vesicles in this model system (Castelletto *et al*., 2019).

2.5.2.3 SODIUM DICLOFENAC- STANDARD ANTI-INFLAMMATORY DRUG

Diclofenac is a weak organic acid that belongs to the nonsteroidal anti-inflammatory drug class. Its principal function is known to be the ability to reduce the activity of the cyclooxygenase enzyme isoforms, hence inhibiting prostaglandin generation. When taken orally, diclofenac can cause serious side effects such as gastrointestinal bleeding, stomach ulcers, renal and heart problems, and more. However, these side effects are almost non-existent in topical formulations, and their efficacy for skeletal muscle lesions may be comparable to that of oral or intramuscular injections (Kakoulidou *et al*., 2020).

2.6 *Pithocellobium dulce* –THE MEDICINAL HERB

Figure 2: Leaf of *Pithocellobium dulce*

(https://en.wiktionary.org/wiki/Pithecellobium_dulce)

Pithecellobium dulce is a fast-growing tree with a rounded or broadly spreading crown. It is normally between 10 and 15 meters tall but can be anywhere from 5 and 18 meters. Multiple boles are frequently generated; these are usually small, ranging in diameter from 30 to 50 cm to up to 100 cm. In the tropics, the tree is frequently cultivated as a decorative and shade-giving plant. Fruit and seed are also farmed in South America. The fruits can be purchased at local markets (Murugesan *et al.*,2019).

Although these trees can be found throughout India's roadways, few people are aware of their culinary uses. It looks like tamarind and is commonly known as Manila Tamarind. It is an

acerbic eatable organic fruit that is commonly used in cooking. It has a high nutritional value and a variety of health benefits (Kulkarni, *et al.*,2019).

2.6.1 BIOLOGICAL SOURCE:

- Botanical Name: *Pithecellobium dulce*
- Family Name: *Leguminosae*
- Parts used: Bark, leaves, seeds, flowers, pulp

The leaves have four leaflets and are paripinnate (2.0-3.5 cm long x 1.0-1.5 cm wide). On either side of the leaf pedicels, little thorns (2.0-15.0 mm long) are inserted, while other kinds are thornless. The leaflets are deciduous and shed in succession, despite the fact that the tree seems to be evergreen. The inflorescences are axillary panicles with spherical glomerules of small, white-greenish, slightly flamboyant flowers (1 cm in diameter). Greenish-brown to red-pinkish fruits with indehiscent pods. Pods are thin and set in a spiral of one to three whorls, about 10-15 cm long x 1-2 cm wide. There are ten seeds in each pod. Flattened, black, and gleaming seeds (1 cm in diameter). The Manila tamarind tree has many purposes. Its pods have a rich sweetish acidic pulp that is palatable. They can be consumed raw or processed into a lemonade-like soft drink. Oil is collected from the seeds and used in cooking and soap production (Sekhar *et al*., 2021).

2.6.2 HEALTH BENEFITS OF MANILA TAMARIND:

1. It is used to treat toothache, gum disease, and mouth ulcers.

2. It has antiseptic properties.

3. High vitamin C content of Manila tamarinds boosts the immune system and decreases phlegm.

4. The bark is used to treat dysentery and chronic diarrhea.

5. The plant has high thiamine content which encourages the body to convert sugars into energy, thereby improving mood and reducing stress.

6. According to a study published in the Journal of Ethnopharmacology, the anti-ulcer activity of Manila tamarind fruit was comparable to that of the conventional medicine omeprazole.

7. Fruit extracts were proved to protect the liver from oxidative stress in a study published in Evidence-Based Complementary and Alternative Medicine.

8. It also prevents hair loss and is used for the greasy scalp.

9. It reduces the signs of aging by lightening the skin (Ashok *et al*., 2020).

2.6.3 *Pithocellobium dulce* FRUIT

Figure 3: Fruit of *Pithocellobium dulce*

(https://www.amazon.in/Amazing-Store-*Pithecellobium*-Jungle-Jalebi/dp/B084Z4XQ8V)

P. dulce fruits have long been employed in Ayurveda medicine and folk cures. Tannin, olein, and glycosides are among the physiologically active chemicals found in this plant. The plant's various components yielded a total of 38 active phytocompounds, including quercetin, kaempferol, and dulcitol. The bark of this plant contains catechol-type tannins. Polyphenol classes of phytocompounds have been discovered to have anti-venom action. Their fruits are high in phenols, flavonoids, and saponins, which have been shown to help with diabetes, oxidative stress, and gastrointestinal problems. The leaves and seeds of the plant have antibacterial, antifungal, and adulticidal properties (Sneha *et al*., 2020). Fruits of Manila tamarind (*Pithecellobium dulce*) are known for their high antioxidant and antimicrobial activity, especially against skin-related problems (Shukla *et al.,* 2019).

The seed oil was utilized as an edible as well as for soap production. The bark has been known to be astringent in dysentery, and febrifuge, and contains up to 37% tannins. Polyphenols from the bark extract possess venomous action. Steroids, saponins, glycosides,

lipids, and polysaccharides have been identified in the seed and have been claimed to have anti-diabetic and antioxidant properties compound afzelin (kaemperol-3-O—L-rhamopyranoside) was discovered from *Pithecellobium dulce* leaves. Anti-inflammatory, analgesic, antioxidant, and antidiabetic properties have also been documented for the leaves. The saponin content of *Pithecellobium dulce* fruits has been investigated for anti-inflammatory activity, free radical scavenging, gastro-protective, antidiabetic, and hepatoprotective properties. Flowers' aqueous extract has been shown to have cardioprotective properties (Preethi *et al*.,2018).

Ethnic communities employ a variety of plant species to treat diseases such as diarrhoea, skin conditions, asthma, malaria, and a variety of other ailments. Higher plant natural compounds could provide a new supply of antibacterial agents. Tannins are water-soluble polyphenols that are typically found in dead or dying cells of plants. Because of the inhibitory effect of protein precipitation on several enzymes, they may play a protective role in barks and heartwood. Tannins have been shown to have antibacterial properties. Tannins hinder the growth of many fungi, yeasts, bacteria, and viruses. Barks are generally thought to be a good source of tannins (Singh *et al*., 2018). As the present study aimed to determine the phytochemical, antimicrobial, and anti-inflammatory activity of the fruit peel of *Pithocellobium dulce.*

Methodology

3. METHODOLOGY

The present study has been designed to evaluate the antimicrobial and anti-inflammatory effects of *P.dulce* peel extract. The anti-microbial potential of *Pithocellobium dulce* peel extract was determined using well diffusion, broth dilution, and time-kill kinetics assay. The anti-inflammatory effect of the *Pithocellobium dulce* peel extract was examined using protein denaturation, and membrane stabilization assays.

3.1 COLLECTING PLANT SAMPLE

The plant sample namely the fruits of Manila tamarind was purchased from the local market in Coimbatore.

3.2 PREPARATION OF *PITHOCELLOBIUM DULCE* PEEL EXTRACT

About 10g of the fresh peel of the fruit were collected, cleaned, dried in shade, grained, and extracted using methanol. The extract was evaporated in a rotary evaporator at 50℃ and concentrations ranging from 20mg were dissolved in 5µl of dimethyl sulfoxide.

3.3 PHYTOCHEMICAL ANALYSIS

The methanolic extract of the peel of *Pithocellobium dulce* was screened for the presence of phytochemicals according to the method (Conneau *et al.,* 2019).

3.3.1. DETECTION OF ALKALOIDS

DRAGENDROFF'S TEST: A Fraction of the extract was treated with Dragendroffs reagent and observed for the formation of the reddish-orange precipitate.

3.3.2. DETECTION OF PHENOLICS

FERRIC CHLORIDE TEST: A Fraction of the extract was treated with 5% Fecl3 solution and observed for the formation of deep blue colour.

3.3.3. DETECTION OF FLAVONOIDS

Concentrated H_2SO_4 test: To a small fraction of the extract, concentrated H_2SO_4 was added and observed for the formation of orange color.

3.3.4. DETECTION OF SAPONINS

Foam test: A Fraction of the extract was vigorously shaken with water and observed for persistent foam.

3.3.5. TEST FOR TANNINS

Braemers test: To a fraction of extract, a few drop of 10% ferric chloride was added. A dark green, blue, or brown color was observed, indicating the presence of tannins.

3.3.6. TEST FOR PROTEINS

Xanthoproteic test: The extract (few mg) was dissolved in 2 ml water and then 0.5 ml of conc. HNO3 was added to it. The yellow color indicated the presence of proteins.

3.3.7 TEST FOR ANTHOCYANINS

The presence of anthocyanins has been demonstrated by adding 2 mL of the plant extract with 2mL of 2 N HCl. The appearance of a pink-red color that turns purplish-blue after the addition of ammonia indicates the presence of anthocyanins.

3.3.8 TEST FOR STEROIDS

The crude plant extracts (1 mg) were taken in a test tube and dissolved with chloroform (10 mL), then added an equal volume of concentrated sulphuric acid to the test tube by the side. The upper layer in the test tube was turned red and the sulphuric acid layer showed yellow with green fluorescence.

3.4 ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY TEST

3.4.1 MICROORGANISMS

The antimicrobial activity of the plant extract was tested against the following microorganisms:

1. *Escherichia coli*
2. *Pseudomonas aeruginosa*
3. *Bacillus subtilis*
4. *Staphylococcus aureus*
5. *Aspergillus niger*

These microorganisms were procured from PSG-IMS Coimbatore.

3.4.2 WELL DIFFUSION ASSAY

Pre-screening for anti-microbial activity was done using the agar well diffusion method (Piccione *et al*., 2022). A broth suspension of each pathogen understudy was obtained by inoculating a loop of each bacterium to 3ml of nutrient Broth. The inoculated tubes were incubated in a bench-top orbital shaking incubator at 150 rpm, 37°C for 24 hours. Petri-plates containing nutrient agar medium were seeded with 0.1 ml of 24 hr culture of bacterial strains by spread plate technique. Wells were made in these agar media using a sterile cork borer and then 100µl of the DMSO suspended madras tamarind extract was added to the wells. Control wells with only DMSO were also maintained. Plates were incubated at 37 °C for 24 hours. The activity was determined by observing the formation of a zone of inhibition around each well.

3.4.3 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

The minimum inhibitory concentration (MIC) of wheatgrass extract was determined by the broth dilution method against bacterial culture. MICs are the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. 100µL of nutrients broth for bacteria and SDB for fungal was distributed from the 1st to the 6th

well of a 96-well polypropylene microtitre plate. The dilutions of leaves formulation were added to the 2nd test well of each microtitre line, and then 100µL of scaler dilution was transferred from the 2nd to the 6th well. The 1st well was considered as growth control because no leaf extract was added. We then added 100µL of a bacterial suspension to each well. Plates were incubated at 37ºC for 18 h. Then the plates were read at 600nm for growth using a Varioskan plate reader.

3.4.4 GROWTH CURVE

The growth curve of the test cultures was compared with *Pithocellobium dulce* fruit peels methanol extract and non-treated culture as control. About 1ml of methanolic extract was added to the test culture inoculated on a nutrient broth medium. Optical density was observed every 30 minutes at 540nm for both extract-treated and non-treated cultures using a UV spectrophotometer. The growth curve of the treated culture was plotted against the non-treated cultures and compared to know the significant inhibitory efficiency of *Pithocellobium dulce* fruit peel to different test pathogens.

3.5 DETERMINATION OF ANTI-INFLAMMATORY ACTIVITY OF PITHOCELLOBIUM DULCE PEEL EXTRACT BY PROTEIN ANTI-DENATURATION AND MEMBRANE STABILIZATION ASSAYS

3.5.1 PROTEIN ANTI-DENATURATION ASSAY

The protein anti-denaturation assay was carried out based on the method proposed by (Heendeniya *et al.*, 2018).

PRINCIPLE

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

REAGENTS

1. Bovine egg albumin
2. Phosphate buffer saline (pH, 6.4)
3. Standard drug – sodium diclofenac (20µg)
4. Plant extract

PROCEDURE

About 0.1ml of fresh chicken egg albumin was mixed with 1.9ml of phosphate-buffered saline (pH 6.4) and 1ml of the extract with varying concentrations. A similar volume of distilled water was used as a negative control. Then the mixture was incubated at 37^0C in an incubator for 20 min and then heated at 70ºC for 5 min. After cooling, the absorbance was measured at 660nm on the spectrophotometer. Sodium diclofenac in the final concentration of 20µg 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;

% Inhibition = Absorbance of control – Absorbance of sample / Absorbance of control x 100

3.5.2 MEMBRANE STABILIZATION ASSAY

The membrane stabilization assay was evaluated by the method of hemolysis of red blood cells based on the method proposed by (Yesmin *et al.,* 2020)

Preparation of the suspension of erythrocytes

Fresh whole blood (3ml) collected in EDTA tubes was centrifuged at 2500 rpm for 10 rpm at 4ºC. 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 40% suspension with isotonic buffer solution. The reconstituted red blood cells (supernatant resuspended) were used for the analysis.

3.5.2.1 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. Phosphate buffer (pH 6.4)
3. Methanol
4. Standard drug – sodium diclofenac (20µg)
5. Extract sample

PROCEDURE

Plant extracts of increasing concentration in isotonic buffer solution were taken in centrifugation tubes. Similarly, the standard drug sodium diclofenac was taken. The negative control contained 2 ml of distilled water. A suspension of 0.1ml of red blood cells is added to each tube and mixed gently. The tubes were incubated at 54ºC for 20 min in a water bath. After incubation, tubes were centrifuged at 2500 rpm for 10min at 4ºC and the hemoglobin content of the supernatant was estimated using the spectrophotometer at 540nm. The percentage of inhibition of hemolysis by the extract was calculated as follows:

% Inhibition of hemolysis = (1- OD sample /OD control) x 100

Where OD sample = absorbance of the sample; OD control = absorbance of the control.

3.5.2.2 HEMOLYSIS INDUCED BY HYPOTONICITY

PRINCIPLE

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

REAGENTS

1. Human red blood cell suspension

2. Standard drug- sodium diclofenac

3. Plant extract

4. Hypotonic buffer solution

PROCEDURE

The extract samples were prepared in distilled water at different concentrations obtained by double dilution. Sodium diclofenac at the same concentrations was used as a reference medicine. Distilled water was used as a negative control. To each tube, 0.1ml of a suspension of erythrocytes was added and then the mixtures were incubated for 1 hour at 37ºC. The tubes were then centrifuged at 2500 rpm for 10 min at 4ºC. 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 as;

% Inhibition of hemolysis = (1- OD sample / OD control) x 100

The concentration of the extract for 50% inhibition was determined by the dose-response curve.

Results & Discussion

4. RESULT AND DISSCUSION

Secondary metabolites found in the tissues of many plant species have the ability to fight disease-causing microorganisms. Glycosides, saponins, flavonoids, steroids, tannins, alkaloids, and terpenes are examples of these chemicals. Extracts from many plant parts, including roots, leaves, bark, flowers, fruits, and seeds, contain phytochemicals that have antibacterial or antifungal action. Plant species are frequently utilized in folklore medicine to treat multiple diseases or infections. Extracts of plants with a long history of traditional use are being examined and scientifically validated for activity against human infections using current advanced methods in order to uncover potential novel medications. Many natural phytochemicals have been proved to possess significant therapeutic effects such as antioxidant, anti-inflammatory and anticancer properties. Hence it has become inevitable to identify such therapeutically effective natural phytoconstituents from different types of plants (Karthikeyan *et al.*, 2019).

The fruits of the candidate plant of the present study, *Pithocellobium dulce* looks like tamarind and is known as manila tamarind. It is an acerbic eatable organic fruit that is commonly used in cooking. It has a high nutritional value and a variety of health benefits. *Pithocellobium dulce* is being used in traditional medicine. The bark and pulp of the Manila Tamarind are traditionally used to treat gum problems, toothaches, and bleeding. Dysentery, diarrhea, and constipation are all treated with bark extract. A leaf extract is used to treat gall bladder problems and to prevent miscarriage. The seed powder is used to treat ulcers. (Kulkarni *et al.*, 2018)

The present study is aimed to evaluate the antimicrobial, and anti-inflammatory activities and a preliminary phytochemical analysis of the methanol extract of *pithocellobium dulce* fruit peel, and the results obtained are presented in this chapter.

4.1 PHYTOCHEMICAL ANALYSIS OF *Pithocellobium dulce* FRUIT PEEL EXTRACT

4.1.1 PHYTOCHEMICAL ANALYSIS

Phytochemical screening was carried out in the methanolic leaf extracts of *pithocellobium dulce* fruit peel was carried out and the results are shown in Table 1.

Table 1

Phytochemical screening of the methanolic of extract *Pithocellobium dulce* fruit peel

Phytochemicals	Methanol
Alkaloids	+
Flavonoids	+
Tannins	+
Saponin	-
Phenols	+
Proteins	+
Terpenoids	+
Steroids	+
Glycosides	+

‘+’ presence ' -’ Absence

In the present study qualitative phytochemical analysis of the methanolic extract showed the presence of phytoconstituents namely, alkaloids, flavonoids, tannins, saponin, phenols, proteins, terpenoids, steroids, and glycosides. Shaikh *et al.,* (2020) have indicated that the phytochemical tests of aqueous extract Malaysian ‘Kundang (*Bouea macrophylla* Griffith; Family: Anacardiaceae) revealed the presence of alkaloids, flavonoids, proteins, saponins, and steroids.

Karthikeyan *et al.,* (2019) qualitatively analyzed aqueous extract, acetone extract, ethanol extract, and hexane ether extract of pomegranate peel for the presence of various active constituents like alkaloids, flavonoids, steroids, tannins, glycosides, and phenols. Behbahani *et al.,* (2019) indicated that the phytochemical analysis of an ethanolic extract of cumin essential oil revealed the presence of various active constituents of phenols, saponins, and flavonoids.

4.2 DETERMINATION OF ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *Pithocellobium dulce* FRUIT.

Pathogenic bacteria are bacteria that can cause disease. Most species of bacteria are harmless and are often beneficial but others can cause infectious diseases. Bacteria are microscopic pathogens that reproduce rapidly after entering the body. They can release toxins that damage tissues and cause illness. There are thousands of species of fungi, some of which cause disease in humans. Common fungal skin conditions include athlete's foot and ringworm. These conditions are contagious and can spread through person-to-person contact.

4.2.1 EFFECT OF METHANOLIC EXTRACT OF *Pithocellobium dulce* FRUIT PEEL EXTRACTION AGAR WELL DIFFUSION ASSAY.

Agar well diffusion method is still required for antimicrobial activity tests. This method allows rapid determination of the efficacy of a particular microorganism by measuring the diameter of the zone of inhibition.

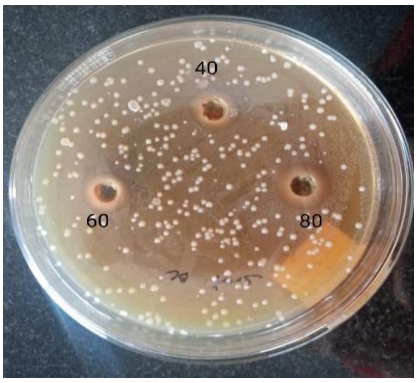
Table 2: Zone of inhibition against one fungal and four bacterial species by the methanolic extract of *Pithocellobium dulce* fruit peel.

Organisms	The concentration of the sample(µg)	Zone of inhibition (mm)
Escherichia coli	40	0.9
	60	1.3
	80	**1.7**
Staphylococcus aureus		
	40	1.2
	60	1.4
	80	**1.5**
Bacillus subtilis		
	40	0.8
	60	1
	80	**1.3**
Pseudomonas aeruginosa	40	1
	60	1.2
	80	**1.4**
Aspergillus niger	40	0.9
	60	1.2
	80	**1.5**

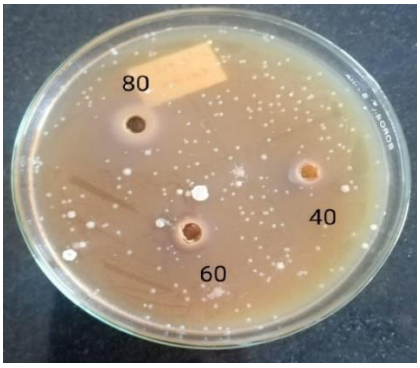
The results of the antimicrobial response for different concentrations of the fruit peel extract are summarized in Table 2. The results obtained showed that the antimicrobial activity of the fruit peel exhibited a dose-dependent effect which is evident from the zone of inhibition with

maximum inhibitory effect on all the tested species treated with 80µg of plant extract followed by 60µg and 40µg.

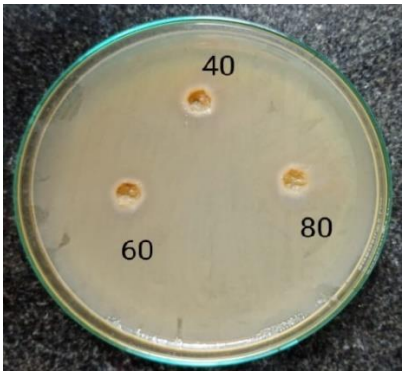
Figure 4: Zone of inhibition against one fungal and four bacterial species by the methanolic extract of *pithocellobium dulce* fruit peel.



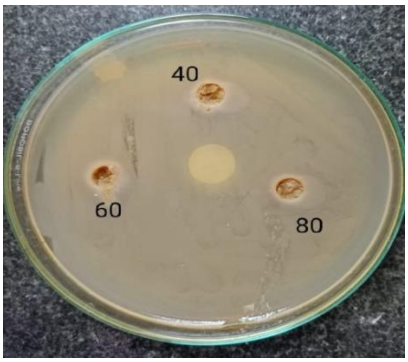
(A)*Escherichia coli*



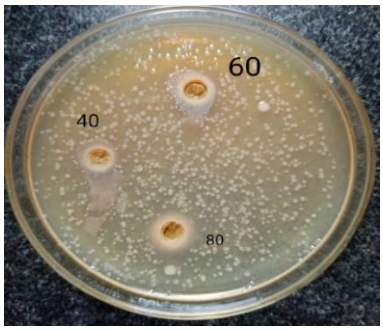
(B) *Staphylococcus aure*



(C)*Pseudomonas aeruginosa*



(D)*Basillus substi*



(E) *Aspergillus niger*

Thus, the results in the present study show that *P. dulce* fruit peel extracts exhibited significant antimicrobial activity against most of the tested microorganisms. Using the agar well diffusion method many studies tested the antimicrobial activity of plant extract. Adeyemi *et al*., (2018) study reported that the cold-water extract revealed a high degree of antifungal activity at varying doses as evident from agar diffusion analysis.

Mummed *et al.,* (2018) tested the antimicrobial activity using agar well analysis reported on medicinal plants. The susceptibility of the four bacteria evaluated to the extracts differed significantly between clinical isolates and reference strains. The mean zone of inhibition for extracts exhibiting colony growth inhibitory action at the highest dose ranged from 8.7 to 22.3mm. When compared to the test extracts, gentamicin demonstrated a significant superiority (p0.05) in the zone of inhibition. Pranati *et al*., (2019) reported observed the antibacterial activity effect of the prepared AgNPs was against all the organisms used in the study with the zone of inhibition *Pseudomonas sp.*

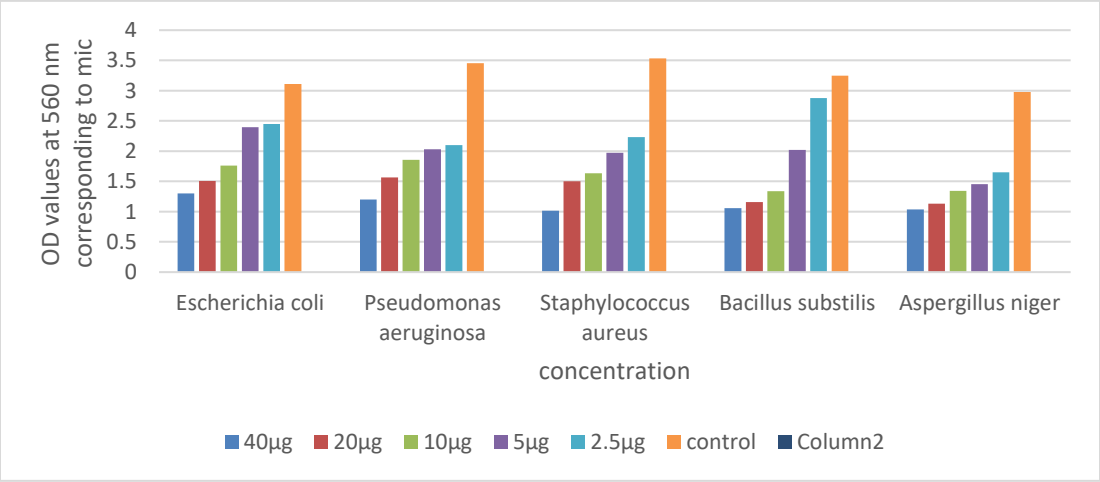
4.2.2 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION OF THE METHANOLIC EXTRACT OF *Pithocellobium dulce* FRUIT PEEL.

Methanol extracts (fruit peel extracts prepared in organic solvents) were further subjected to determination of minimum inhibitory concentration against the same pathogenic species. The results obtained were shown in Table 3. The minimum inhibitory concentration values were exhibited by the methanol extracts against most of the bacterial strains and one fungal strain tested was found to be 40µg/ml.

Table 3: Minimum inhibitory concentration against one fungal and four bacterial species by the methanolic extract of *Pithocellobium dulce* fruit peel.

Pithocellobium dulce fruit peel	Control	40µg/ml	20µg/ml	10µg/ml	5µg/ml	2.5µg/ml	1.25µg/ml
Escherichia coli	+	–	–	–	+	+	+
Psedomonas aeruginosa	+	–	–	–	+	+	+
Staphylococcus aureus	+	–	–	–	–	+	+
Bacillus subtilis	+	–	–	–	+	+	+
Aspergillus niger	+	–	–	+	+	+	+

FIGURE 5: Minimum inhibitory concentration against one fungal and four bacterial species by the methanolic extract of *Pithocellobium dulce* fruit peel.



The results of MIC assay confirmed the findings of antimicrobial assays, wherein it was reported that methanol extracts were more potent inhibitors of the microorganisms tested as that *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Aspergillus niger* were among the most sensitive strains. De Zoysa *et al.,* (2019) reported the MIC values obtained from plants exhibited antibacterial activity ranging between 0.003 and 2.4 mg/ml. Ethanol, aqueous, and hexane extracts of *E. divaricata* gave MIC values of 0.48 mg/ml, 1.2 mg/ml, and 1.6 mg/ml, respectively, against *S. aureus.* Ethanol and hexane extracts of *V. zizanioides* gave MIC values of 2.4 mg/ml and 0.003 mg/ml, respectively, against *S. aureus*. Therefore, the highest antimicrobial activity was observed for the hexane extract of *Vetiveria zizanioides.*

Romulo *et al.,* (2018) study reported *C. albicans* (MIC 128 g/mL), *S. aureus* (MIC 256 g/mL), *E. faecalis* (MIC 256 g/mL), and *P. aeruginosa* (MIC 256 g/mL) were all inhibited by the leaf extract of *Orthosiphon aristatus* (Blume) Miq. (Lamiaceae). *C. albicans* (MIC 128 g/mL), *S. aureus* (MIC 256 g/mL), and *E. faecalis* (MIC 256 g/mL) were all s usceptible to the leaf extract of *Woodfordia floribunda* Salisb. (Lythraceae). *S. aureus* (MIC 256 g/mL) and *Candida albicans* (MIC 256 g/mL) were both suppressed by *Rotheca serrata* (L.) Steane & Mabb. *(Lamiaceae)* leaf extract. Manandhar *et al.,* (2019) *O.corniculata* was shown to have high antibacterial activity in plant extracts tested. S. Typhi had a MIC of 100 mg/ml against *O. corniculata,* and MDR S. *Typhi* had a MIC of 50 mg/ml. Other gram-negative bacteria are the same way (*E. coli, K. pneumoniae, and MDR C. koseri*).

4.2.3 EFFECT OF METHANOLIC EXTRACT OF *Pithocellobium dulce* FRUIT PEEL EXTRACT ON GROWTH CURVE.

The growth curve of the test cultures treated with the methanolic extract of *Pithocellobium dulce* fruit peels and un-treated control was compared. In this analysis, about 1ml of methanolic extract was added to the test culture inoculated on a nutrient broth medium. Optical density was observed every 30 minutes at 540nm for both extract-treated and un-treated cultures using a UV spectrophotometer.

The growth of the culture of each pathogenic species treated with the extract was compared to that of untreated control and a growth curve was derived for both the treatment groups against time. The results obtained were shown in Figure 6 (a,b,c,d,e)

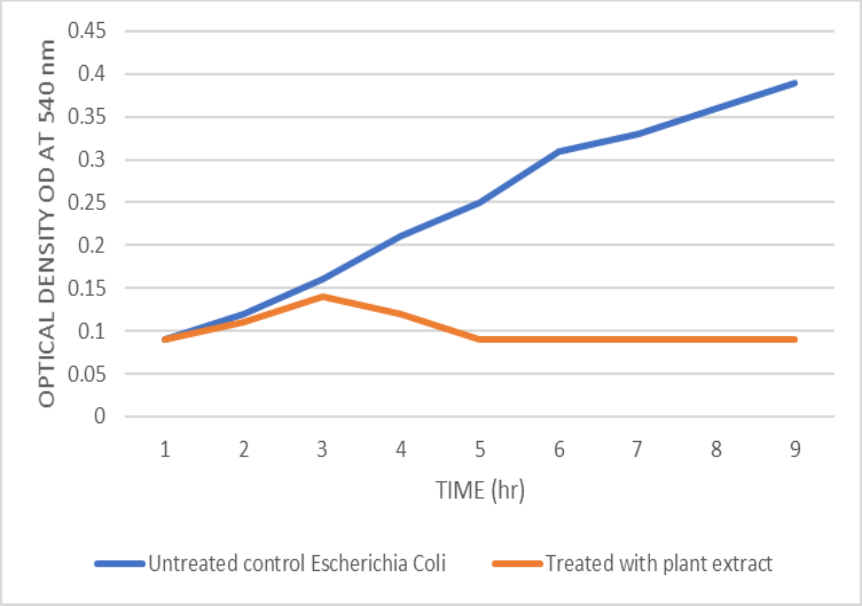


Figure 6 (a)

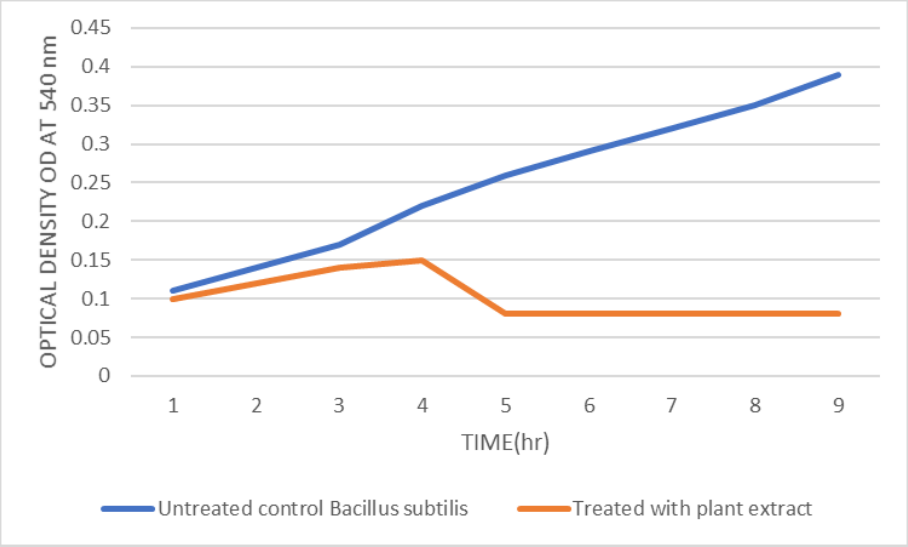


Figure 6 (b)

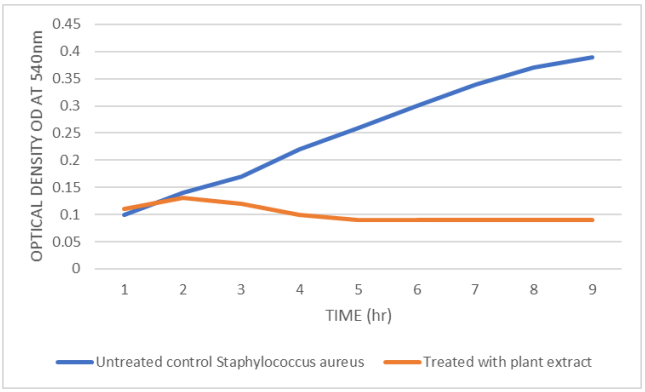


Figure 6 (c)

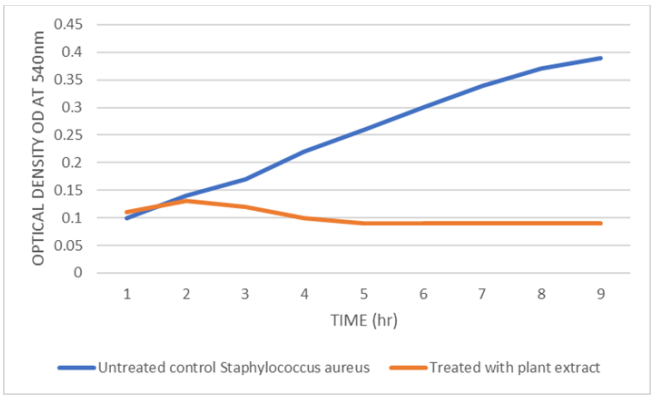


Figure 6 (d)

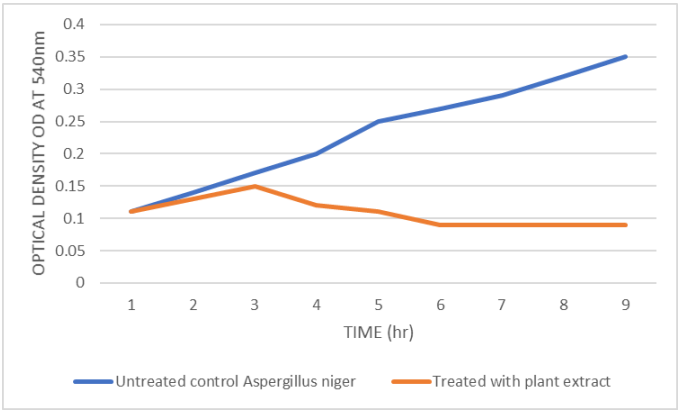


Figure 6 (e)

It was found that the manila tamarind fruit peel extract of the group growth index of bacterial and fungal species was decreased after a certain period of time around (3hrs) time after which a stationary phase was attained. In a study by Huang *et al.,* (2018) The antibacterial mechanism of *A. asiatica* essential oil showed a rapid reduction in the number of viable bacterial cells. Over the first 12 hours of the test, the number of bacteria in the treatments (1MIC) gradually decreased. Unlike the shifting trend in bacterial quantity at 1MIC, the number of viable cells in the first three hours after cultivation decreased dramatically with 2MIC treatment. Various incubation times and concentrations of the drug were shown to have different effects.

Similarly, in another study, Qais *et al*., (2019) tested the growth kinetics of methicillin-resistant Staphylococcus aureus (MRSA1) growth kinetics in the presence of different concentrations of MK (Murraya koenigii)-AgNPs.

4.3 DETERMINATION OF ANTI- INFLAMMATORY ACTIVITY OF THE METHANOLIC EXTRACT OF *Pithocellobium dulce* PEEL EXTRACT:

The anti-inflammatory activity of the leaf extract was assessed using protein denaturation and erythrocyte membrane stabilization methods.

4.3.1 EFFECT OF METHANOLIC EXTRACT OF *Pithocellobium dulce* PEEL EXTRACT ON PROTEIN DENATURATION

Denaturation of proteins is the main cause of inflammation. As part of the investigation of the mechanism of the anti-inflammatory activity, the ability of the extract to inhibit protein denaturation was studied. Protein denaturation is a physical or chemical process in which proteins lose their tertiary and secondary structures. Resulting in the loss of their biological function. One of the well-known causes of inflammatory and arthritic illness is tissue protein denaturation (Kousalya, *et al.,* 2020). In 2018, Ngoua *et al.,* reported that anti-inflammatory activity can be determined by measuring the inhibition of protein denaturation and stabilization of RBC membranes. This result indicates that *Pithocellobium dulce* extracts have maximum anti-denaturation activities compared to sodium diclofenac which was used as a standard anti-inflammation drug. The results of the protein denaturation test are shown in figure 7 and Table 4.

Hence a dose optimization study using different concentrations of the leaf extract has to be carried out to determine the optimum dose that can exhibit maximum inhibitory effect against protein denaturation.

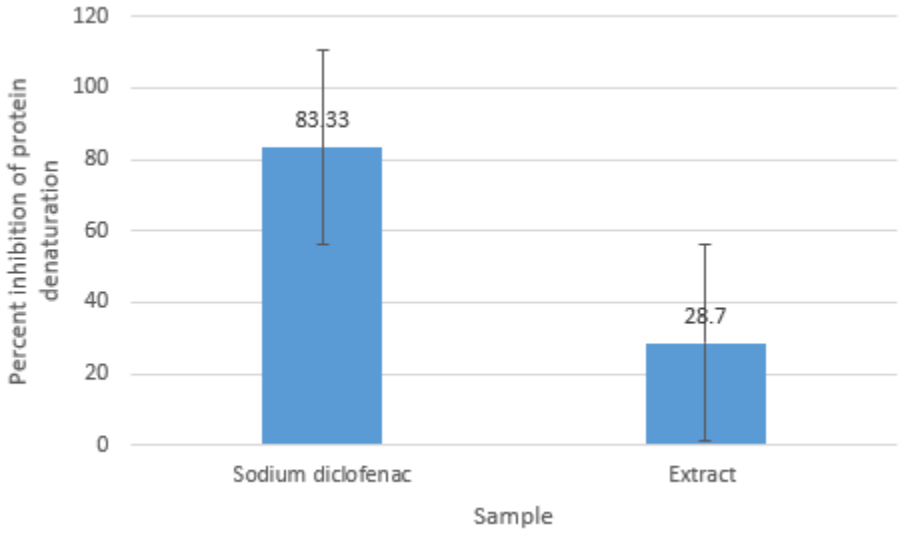


FIGURE 7: EFFECT OF *Pithocellobium dulce* AND SODIUM DICLOFENAC AGAINST PROTEIN DENATURATION ACTIVITY

Table 4: PER CENT INHIBITION OF PROTEIN DENATURATION ACTIVITY

SAMPLES	**PER CENT INHIBITION OF PROTEIN DENATURATION**
Extract	28.70±5.78
Sodium diclofenac	83.33±10.01

The values are mean ±S.D. of triplicates

Yadav *et al.*, (2020) reported the anti-inflammatory activity of ethyl acetate extract of *Malvastrum coromandelianum* in an *in vitro* model using the protein denaturation method. When compared to a reference medication, the ethyl acetate extract of *Malvastrum coromandelianum* considerably prevented the protein denaturation.

Dharmadeva *et al.,* (2018) reported that at a concentration of 100µg/ml, *Ficus racemose* showen cold and hot water extracts were significantly higher than prednisolone and ibuprofen ($P < 0.05$). Chandra *et al.,* (2018) studied the protein denaturation activity of aqueous extract of *coffea Arabica* and showed a concentration-dependent inhibition of albumin denaturation.

4.3.5 ERYTHROCYTE MEMBRANE STABILIZATION TEST

The anti-inflammatory activity of *Pithocellobium dulce* extract observed by inhibition of albumin denaturation is further confirmed by membrane stabilization test. The extracts showed good inhibition of hemolysis of red blood cells.

4.3.5.1 Hemolysis induced by heat:

The anti-inflammatory activity of *Pithocellobium dulce* extract was assessed by hemolysis induced by heat method using human red blood cells. The result showed the evaluation of membrane stabilizing activities of *Pithocellobium dulce* extract has showed good anti-hemolytic activity when compared to the reference drug, sodium diclofenac (Figure 8 and Table 5).

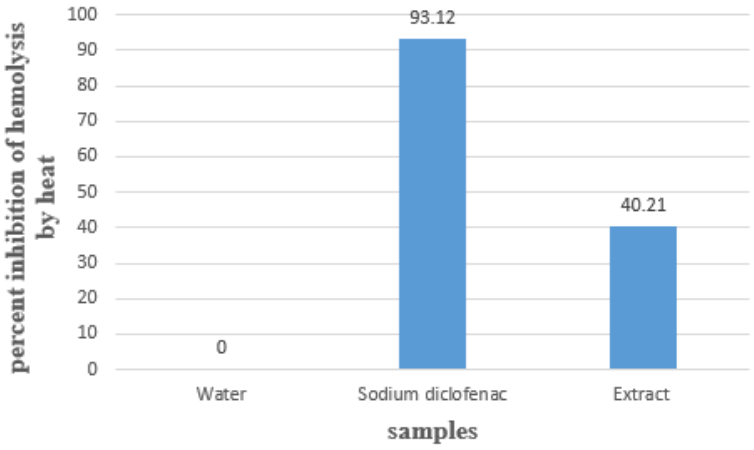


Figure 8: EFFECT OF *Pithocellobium dulce* LEAF EXTRACT AND SODIUM DICLOFENAC ON HEMOLYSIS BY HEAT

Table 5: Hemolysis induced by heat

SAMPLE	PERCENT INHIBITION OF HEMOLYSIS
Water	0.0±0.00
Sodium diclofenac	93.12±2.81
Extract	40.12±1.78

The values are mean ±S.D. of triplicates

Yesmin *et al.,* (2020) using the heat-induced anti-inflammatory test, reported that the crude ethanolic extract of *P. chaba* (500 g/ml) and positive control ASA acetylsalicylic acid (500 g/ml) showed 52.667 percent and 78 percent inhibition of red blood cell (RBC) hemolysis, respectively. In a hypo tonicity-induced anti-inflammatory test, 35.67 percent and 59 percent inhibition of RBC hemolysis were found.

In a similar study by Islam *et al.,* (2020) compared to the standard acetylsalicylic acid, all of the *Albizia richardiana* extracts showed considerable protection against lysis of human erythrocyte membrane induced by heat and hypotonic solution. MEAR-methanolic extract of *Albizia richardiana* (16.66 percent) had exceptionally substantial clot lysis, while the conventional medication streptokinase had no effect (70.94 percent).

Ngoua *et al.,*2018 reported the heat-induced hemolysis of sodium diclofenac was IC50 = 180.911 2.205 g/mL while the *Lophira procera* extracts showed very substantial anti-hemolytic activity IC50 range from 36.793 0.529 g/mL to 48.912 0.9573 g/mL

4.3.5.2 Hemolysis induced by hypotonicity:

Human red blood cell membranes are similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity of drugs. The anti-inflammatory activity of *Pithocellobium dulce* extract was also assessed by hemolysis induced by hypotonicity method using human red blood cells. The results showed the *Pithocellobium dulce* extract showed good membrane stabilizing

activities which is evident from the percent inhibition of hemolysis ranging 41.55% which is almost considerate to that of the standard drug sodium diclofenac (70.77%) as shown in Figure 9 and Table 6.

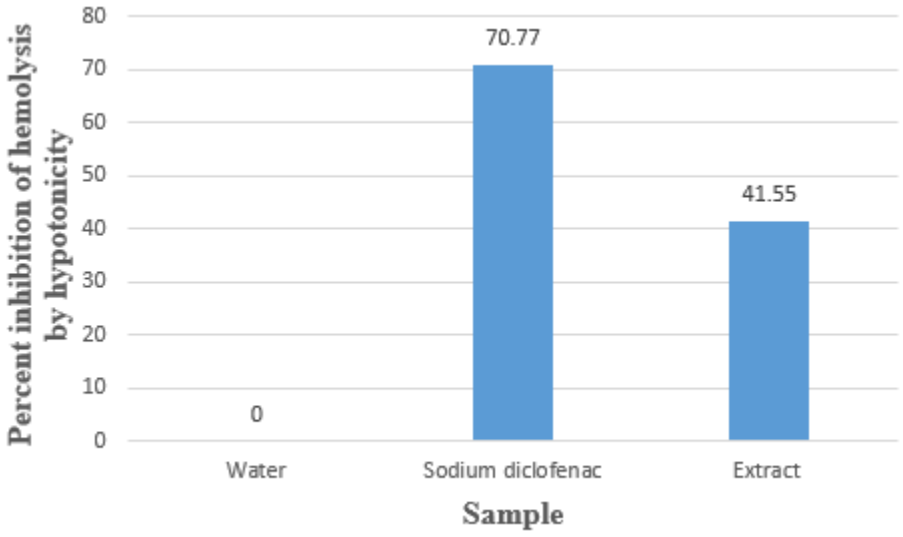


Figure 9: EFFECT OF *Pithocellobium dulce* LEAF EXTRACT AND SODIUM DICLOFENAC ON HEMOLYSIS BY HYPOTONICITY

Table 6: Hemolysis induced by hypotonicity

SAMPLE	**PERCENT INHIBITION HEMOLYSIS INDUCED BY HYPOTONICITY**
Water	0.0±0.0
Sodium diclofenac	70.77±1.25
Extract	41.55±0.85

The values are Mean ± S.D. of triplicates

In a similar study Anyasor *et al.*, (2019) compared diclofenac sodium, and different doses of MEJSL methanolic extract *J. secunda leaves* significantly (P 0.05) reduced heat-induced BSA denaturation and stabilization of erythrocyte membrane against hypotonicity-induced hemolysis in a concentration-dependent manner.

Moriasi *et al.,* (2020) reported that the inhibition of heat-induced and hypotonicity-induced HRBC hemolysis by the Phytexponent Phytexponent showed a significantly higher percentage of inhibition of heat-induced and hypotonicity-induced HRBC hemolysis.Paul *et al.,* (2021) reported that at a dose of 1000 g/ml, *A.vera* gel homogenate suppresses hypotonicity-induced (74.89%) and heat-induced (20.86 %) RBC membrane lyses, respectively, as compared to indomethacin standard (80.52 and 43.9% respectively, at 200 g/ml).

Thus, the result of the present study revealed that the methanolic extract of *Pithocellobium dulce* fruit peel exhibits significant antimicrobial activity and also protects cells from protein denaturation and lysis thereby exhibiting an anti-inflammatory effect.

Summary & Conclusion

5. SUMMARY AND CONCLUSION

Plants have been widely recognized as an important source of novel therapeutic compounds since ancient times for the treatment of various diseases and were reported in traditional medicine systems such as the Siddha and Ayurveda. The phytochemicals such as alkaloids, saponins, steroids, *tannins*, flavonoids, and amino acids, were responsible for biological activity. Antimicrobial activity is a term that refers to all active principles (agents) that stop pathogens from growing, preventing the formation of microbial colonies, and sometimes killing microorganisms. Secondary metabolites present in plants include tannins, terpenoids, alkaloids, and flavonoids, which have been shown to have antimicrobial activities *in vitro.*

A recent trend in inflammation research is to look for alternative therapeutic agents derived from natural sources that do not have the side effects associated with traditional steroids or nonsteroidal anti-inflammatory medicines. The present study was carried out to investigate the phytochemical analysis, antimicrobial, and anti-inflammatory properties of *Pithocellobium dulce* fruit peel extract. A preliminary phytochemical screening of the methanolic extracts of *Pithocellobium dulce*fruit peel revealed the presence of Alkaloids, flavonoids, tannins, phenols, proteins, terpenoids, steroids, steroids, glycosides. Saponin was absent in the methanolic extract of *P.dulce* fruit peel extract.

In agar well diffusion assay, the zone of inhibition was observed in cultures treated with *Pithocellobium dulce* fruit peel using one fungal species and four bacterial species at the dosage of 80 µg followed by 40 µg, 60 µg was observed.

The minimum inhibitory concentration for the antimicrobial properties of manila tamarind was determined and was found to be 40µg for all the tested fungal and four bacterial strains. The antimicrobial activity was further confirmed by growth curve analysis wherein manila tamarind extract inhibited the growth of bacterial and fungal species for a certain period of time around 3 hours after which a stationary phase was attained.

Following the analysis of antimicrobial activity, the fruit peel was tested for its potential effect on inflammation using.

In the growth curve, the manila tamarind fruit peel extract containing species growth was decreased, after a certain period of the time around 3 hours after which a stationary phase was attained. Protein denaturation assay, membrane stabilization methods. The methanolic extract of *P.dulce* showed good anti-denaturation activities compared to that of the standard drug sodium dielofenac which can be used for the treatment of inflammation.

Thus, the research findings of the present study revealed that the peels of the *Pihocellobium dulce* fruit peel exhibit good antimicrobial and anti-inflammatory activity which can be further explored to identify a potent lead compound from the fruit peel that may be used to treat microbial infection and inflammatory condition.

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