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Comprehensive Phytochemical Profiling and Assessment of Antioxidant and Anti-inflammatory Activities of Manila Tamarind (Pithecellobium dulce) Extracts: Qualitative and Quantitative Analysis

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KEYWORDS

Antioxidant and anti-inflammatory, manilla tamarind, phosphomolybdate assay, phytochemicals, phytochemicals screening, and protein denaturation.

ABSTRACT:

BACKGROUND: The bioactive constituents extracted from different sections of the plantsare used as therapeutic drugs in alternative medicine for treating diseases. The presentstudy was intended to evaluate the chief phytochemicals present in PITHECELLOBIUM dulce, which was called Manilla tamarind, and its antioxidant and anti-inflammatory property.

METHODS: Aqueous extracts of Manilla tamarind was prepared in various fraction basedon their polarity and screened for their major bioactive compounds. Their anti-oxidant propertywas evaluated by the phosphomolybdate assay and the anti-inflammatory property was evaluated by invitro percentage inhibition of protein denaturation.

RESULTS: Qualitative screening of aqueous extracts of Pithecellobium dulce in various concentrations ensured the presence of alkaloids, saponins, terpenoids, etc. Quantitative analysis of percentage inhibition of protein inhibition showed **92.5%** inhibition and positive results on antioxidant properties.

CONCLUSION: The obtained results showed the presence of various phytochemicals and their various property (i.e.) both anti-oxidant and anti-inflammatory implying they have natural therapeutic potential and can be consumed for improving health status.

1. Introduction

Manilla tamarind is an edible natural fruit that has been used for cooking in some regions. Studies have concluded that the hydro-alcoholic fruit extract of Manilla tamarind (HAEPD) can be used safely in enemy tests and clinical trials in 2010. Bark and pulp are used as a traditional remedy to prevent inflammation of the gums, toothache, and bleeding. Bark extracts are used to fight diarrhoea and constipation. The leaf extract is used to treat gallstones and prevent diarrhoea (Saleh et al., n.d.)

FIG 1 & FIG 2
RIPED FRUIT OF MANILLA TAMARIND
(*Svan_organic_form*, n.d.)

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Herbal plants synthesize a variety of secondary metabolites with the ability to fight against oxidative stress-caused disease. The antioxidant property of plants is because of the presence of organic substances.

Phytochemical is defined as a chemical that acts as a bioactive nutrient found in plants and their products like fruits and vegetables that have desirable health benefits beyond basic nutrition and protect against degenerative diseases(Jimenez-Garcia et al., 2018). These edible plants could decrease malnutrition, especially in low-income groups.

A lot of undiscovered and non-staple plants supply better nutritive value in the human diet; so, incorporating neglected herbs in the diet may improve the intake of nutrient-dense organic compounds (Memariani et al., 2020). The word phytochemical comes from the Greek word" PHYTON" meaning" PLANTS" that they used as traditional medicine (Craig, 1997).

Consuming a diet rich in secondary metabolite - a non-nutritive organic compound in green plants has a positive effect. Green leafy vegetables as well as other vegetables and Dry fruits have a high content of these phytochemicals (secondary metabolite) especially phenolic compounds that have proved to have a role in the protection and treatment of various degenerative diseases (McCarty, 2004).

Different Phytochemicals from different foods can interact with each other. The total intake of nutritional load (dietary intake load) of phytochemicals in the everyday diet has a vital role in human metabolism & its load is called, "he **phytochemical index (PI),** which can be defined as the percent of dietary calories derived from foods rich in phytochemicals" (Schmidt et al., 2008).

Medicinal plants that are rich in bioactive components are used in botanical therapeutics, and domestication of crops that have drastically increased the bioactive content and diminished others (Gupta et al., 2016). One of the major problems of the world is Infectious diseases and every year because of these diseases worldwide almost 57 million people (about twice the population of Texas) die(Koche, 2018).

In the past 30 years, the pharmacology industry had produced a variety of new antibiotic drugs but with a limitation with their toxic effect and MDR (global emergence of multi-drug resistance that limits the effectiveness (Zaynab et al., 2019). Based on their diversity, role, and their structure; phytochemicals were classified into main two categories they are primary and secondary metabolite:

PRIMARY METABOLITE:

The monomers of carbohydrates, proteins, nucleic acids materials like purines and pyrimidine, and plant pigments like chlorophylls come under the first category of classification (Zaynab et al., 2019). Their main functions are the growth, development, and reproduction of cells and molecules signalling to trigger a defence response thus protecting them from all pathogens and insects creating a defensive mechanism (*PHYTONUTRIENTS*, n.d.)

SECONDARY METABOLISM:

Plant chemicals such as alkaloids and their derivatives, terpenes and their derivatives, flavonoids and their classifications, lignin and their by-products, plant steroids, and their derivatives, curcumins and their interconnected pigments, saponins, and their derivatives, a wide group of phenolic and glucosides are considered secondary metabolite.

These organic Secondary metabolites are produced or derived from the primary metabolite itself or any of the substrates or by-products of primary metabolite by undergoing various pathways. Plants with the help of environmental change and mutation managed to synthesize secondary metabolites (Waterman PG. Roles for secondary metabolites in plants. Ciba Found Symp. 1992, 1992).

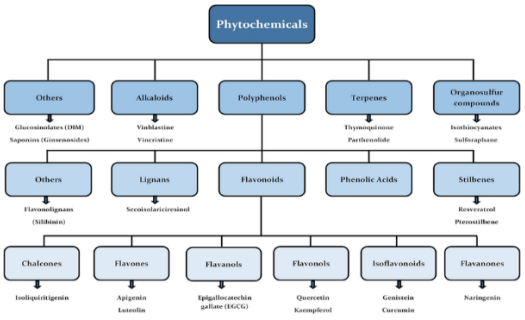

FIG 3 CLASSIFICATION OF PHYTOCHEMICALS

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("Classification of Phytochemicals," n.d.)

Some of the main classes of phytochemicals are discussed below:

ALKALOIDS - Alkaloids are often found especially in nitrogen having organic compounds present in the flora of valuable medicinal properties and help treat diseases like malaria, diabetes, cancer, cardiac dysfunction, and platelet aggregation (Michel et al., 2016).

TERPENOIDS - 30,000 lipid-soluble compounds are coming under the class of terpenoids. Based on their isoprene units which are synthesized by plants they are classified as hemiterpene; monoterpenes; isoprene units, diterpenes, SesterPenes, triterpenes, and tetraterpene. They also show a significant effect on insect CNS; most monoterpenes show a neurotoxin property on insects by interacting with the octopaminergic cycle (vertebrates' noradrenergic system) (Kennedy & Wightman, n.d.)

POLYPHENOL - The phenolic ring is the monomer of polyphenol and phenolic acids. Phenolic alcohols are grouped under polyphenols, they are further as classified Hydroxycinnamic and hydroxybenzoic acids. In our diet, most of the phytochemicals are polyphenol compounds that help atherosclerosis by reducing the oxidation of LDL (low-density lipoprotein). (Abbas et al., 2017)

ORGANOSULFATE COMPOUNDS - There are two diverse types of Organo sulfate compounds, they are glycosylating and s- methyl cysteine sulfoxide (Stoewsand, 1995). Their main ability is that they prevent carcinogenesis by the compound N-nitroso diethylamine and among the strongest was disulphide which is derived from the cruciferous plant (Balasubramanian RAJKAPOOR, 2005).

FLAVANOIDS - FLAVANOIDS are secondary metabolites with a two-phenyl ring and a heterocyclic in their 15–carbon skeleton structure. They are further divided into major groups like flavanol, quercetin, kaempferol, myricetin, flavones, isoflavones, flavones, and anthocyanin, etc. they all have their subclasses and a wide range of health benefits (Hossain et al., n.d.)

PHYTOSTEROLS - These are compounds found in the plant's cell membrane with a chemical structure and function like cholesterol because of this reason it was also called sterols. Studies have shown that they are more than 200 diverse types of these phytosterols but classified as **sterols** and **stanols.** The prior has a double bond and the latter lacks the double bond. Their leading role is their ability to reduce the risk factor of cancer by 20% and another role like controlling in and out of various biomolecules through lipid bilayers and the role of hydrophobic and hydrophilic ends which controls the fluidity of membranes that proves their role in preventing cardiovascular disease, diabetes, cancer, and liver disorder (Suryamani et al., 2022).

LYCOPENE - It is a carotenoid that has a powerful antioxidant that got the spotlight of scientists in recent years. They are found in fruits and vegetables with colours ranging from pink to red like tomatoes, watermelon, and grapefruits. They are shown to lower the risk of prostate glands. They have sub-classes like **zeaxanthin** and **lutein**. They help mainly with eye and age-related disorders that affect optical functions (Rao et al., 2006).

ELLAGIC ACID is known for its cancer-reducing property and cholesterol-reducing properties with antioxidant and anti-inflammatory properties. The main food sources are Raspberries, Strawberries, Blackberries, Grapes, Pomegranates, walnuts, pecans

RESVERATROL is known for its cardiovascular and cognitive health in association with increased cerebral blood flow. They are found in

1. peanuts
2. Pistachios
3. blueberries
4. dark chocolate

PHYTOESTROGEN - Other than cardiovascular health and cancer, it helps in reducing the risk of osteoporosis. They are mimics of the hormone oestrogen which favour women with menopausal symptoms. They are found in soy, Broccoli, Oranges, Carrots, Coffee, and Legumes.

CAROTENOIDS - These are plant pigments that take control of the colors. The brightest color in all fruits and vegetables is due to these carotenoids. It is said that more than 600 types of carotenoids are found in nature and they can only be absorbed only with fat sources and covert to vitamin A

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1. pumpkins
2. carrots
3. spinach
4. kale
5. tomatoes
6. oranges
7. yams(Rao et al., 2006).

The purpose on which the study was conducted is to evaluate the phytochemicals property of the traditional crop Manilla tamarind. The botanical name of manila tamarind is Pithecellobium dulce. It is also called madras thorn, monkey pod tree, and kodukaaipuli in Tamil. This species is a flowering plant in the pea family, Fabaceae and it originated from the Pacific Costis and highlands of Mexico, central Asia, and South America (*PHYTONUTRIENTS*, n.d.).

DESCRIPTION:

MANILLA TAMARIND tree heights about 10-15 m. it has spines throughout its trunk which is 9 m in girth and its leaves are bipinnate. The flower of this tree is greenish–white, fragrant, and 12 cm (about the length of the long edge of a credit card) in length but it looks short because it was coiled around itself. It is dispersed through birds(*National Plant System*, n.d.). Its antioxidant and anti-inflammatory properties are studied in this research.

ANTIOXIDANT:

"Anti-oxidant is defined as a substance that inhibits oxidation and helps to counteract the deterioration of other compounds in other words it helps the cells to protect themselves from free radicals (reactive oxygen species) that cause damage". Antioxidants can prolong the shelf life and help in managing to keep the nutritional quality of sugar and fat-containing foods, as well as counteract the consequences of damage caused by ROS in the human body

FREE RADICALS – **"**It is defined as any molecule or a group of molecules that hold a single unpaired electron in their valance orbital that is incapable of independent existence. All the free radicals are highly reactive and unstable". So, to become they contribute an electron or receive an electron from nearby molecules, thus having the role of oxidant or reductant. The most important free radicals are hydroxyl radicals, nitric oxide, superoxide, hydrogen peroxide, etc. They are highly reactive species capable of damaging the nucleus cell membrane, Deoxyribonucleic acid, protein molecules, carbohydrates structure, and lipid layers. The major target for ROS are lipids, proteins, and nucleic acids. These are produced both inside and outside the body. **ROS** (REACTIVE OXYGEN SPECIES) from internal sources is produced from normal essential metabolism and rays like UV rays & x-rays, the ozone layer of the earth, smoking, and air pollution due to automobiles and industrialization and their chemicals contribute to the external source(Halliwell et al., 1995). It is a continuous process that happens not only because of enzymatic reactions but also of non-enzymatic reactions. Antioxidants fight against all these enzyme inhibitors, peroxide decomposers, hydrogen donors, singlet oxygen quenchers, synergist radical scavengers, and electron donor & metal-chelating agents. Antioxidants of both enzymatic and non-enzymatic classes exist to detoxify Reactive oxygen species in the intracellular environment and extracellular environment. A molecule of antioxidants is stable enough to donate or accept an electron from a roaming free radical and reduce its destructive power. These super cool antioxidants slow down and prevent free radicals from causing cell damage through their properties. That's why antioxidants can in one piece interact with free radicals & discontinue chain reactions before vibrant molecules are damaged. Although there are many enzymes system exclusive to the human body that can scavenge free radicals, there are some important principal substance, especially vitamins(comes under micronutrient) which have antioxidants property include vitamin E also called α-tocopherol, vitamin C (ascorbic acid), and B-carotene and major phytochemicals ought to be taken in diet.

In the previous few eras, treatment with drugs has undergone great progress, particularly in metropolitan republics. Research analysis has proved that nutrition shows a fantastic role in the inhibition of chronic diseases through relating them. Functional foods are considered not only necessary to live but also as a source of psychophysical well-being because they help in anticipation and decreasing the risk of factors for various diseases. Diet can be considered effective if it aids one or more specific functions in the body such as the effective care of a nutritious diet, which contributes state of health & well-being or reduces the risk of illness.

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Examples: Whole Foods. Broccoli, carrots, and tomatoes due to their high physiologically active content (sulforaphane, B-carotene, and lycopene, respectively).

"Nutraceuticals" was founded in 1979 by Stephen DeFelice. It is argued that anything derived from the diet may provide health assistance for hindrance & cure of diseases Nutraceuticals enclose any type of nutritional supplement, dietary complements, and hereditarily engineered "designer" diets, pharmaceutical merchandises. , as well as processed foodstuffs such as breakfast cereal, broths, & beverages. Nutraceuticals are something with non-toxic food supplements with logically proven health aids in both management and anticipation. Consumption of dietary and plant-derived antioxidants adding to endogenous (from inside) antioxidant defence systems, is a suitable alternative. Edible parts of plants are considered one of the major sources of antioxidants. Indigenous foods, spices, and herbs are rich sources of natural antioxidants; a high-performance diet that includes a high level of antioxidants in a functional diet is one strategy that gains value (Lobo et al., n.d.). They play a remarkable role in programmed cell death or apoptosis. "Apoptosis is showed by the stimulation of the caspases, a family of cysteine proteases, trailed by specific caspase-mediated morphological changes like membrane blebbing, nuclear DNA fragmentation, cell shrinkage, chromatin thread condensation, and apoptotic body formation "(Johnstone et al., 2002)

ANTI-INFLAMMATORY:

If your body comes in contact with an irritating foreign agent such as viruses or toxic chemicals or you are experiencing damage that will activate the innate and acquired resistant system. This exempt system of immunity directs its main responders: inflammatory cells and cytokines; is a stimulant for inflammatory cells**.** These immunity cells trigger an inflammatory reaction to set up bacteria and further inflammatory agents or begin to heal damaged soft tissue. The consequence can be aches, bulges, scratches, or soreness and may also affect invisible physique structures. There exist two kinds of inflammations -acute and chronic inflammation.

ACUTE inflammation: A reaction to unexpected physical injury, such as a wounding finger. To restore cuts, your body guides inflammatory cells to the wound. These immunity cells begin the healing process.

Chronic inflammation: The human body's response by sending inflammatory cells with or without external risk. For illustration, rheumatoid arthritis inflammatory cells, and joint tissues can lead to inflammation that comes & go and can bring serious injury to joints with aching and paralysis(*Inflammation*, 2021).

The anti-inflammatory property of some main phytochemicals:

I. Phenolic compounds mediate the signalling process and help regulate the redox (both oxidation and reduction) system and balance the immune reaction by constraining inflammation of cytokines synthesis (Houghton, 2019).

II. Polyphenol hydroxybenzoic acid-derived compounds, especially; EA also called ellagic acid is more common in fruits, such as raspberries plus strawberries, along with in mushrooms, & nuts. Without a doubt, EA has a strong anti-inflammatory activity; made known to restore inflammation & adiposity (Houghton, 2019)

III. Another one called flavonoid from plants called quercetin (33 ′, 4 ′, and 5,7pentahydroxyflvanone) shows anti-inflammatory oxidant scavengers, chemopreventive and protecting neurons kinds of stuff. The average dose is from "50 to 800 mg" / day, depending on dietary routines. The anti-inflammatory effect of quercetin may vary from cell to cell for both animals and humans (Marín et al., 2013).

IV. "Curcumins is a genuine polyphenol with a yellow coloured stain that is obtained from Curcuma longa botanical name of turmeric. The phenolic compound impact diverse bioactive effects like anti-inflammatory, oxidant scavengers, anti-bacterial, and anticancer functions."(Wang et al., 2009)

V. Cinnamaldehyde is a chemical compound separated from the bark of Cinnamomum Cassieres. By tradition, this plant is regularly used to relieve signs of feebleness, nevertheless, more recently, this bioactive complex was helpful in cancer, CVD & ulcerative colitis. This act is recognized for its anti-

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inflammatory properties, in addition to the unregulated RNA regulatory function (Qu et al., 2019).

2. Objectives

1. To qualitatively analyse the phytochemical constituents present in Manila Tamarind extracts, including phytosterols, terpenoids, glycosides, coumarins, saponins, sterols, and phlobotannins.
2. To quantitatively assess the anti-inflammatory activity of Manila Tamarind extracts using the protein denaturation method, with the inhibition of protein denaturation serving as a measure of anti-inflammatory potential.
3. To quantitatively evaluate the antioxidant activity of Manila Tamarind extracts using the phosphomolybdate method, with the antioxidant effect determined by the reduction of Mo (VI) to Mo (V) and expressed as a percentage compared to a standard antioxidant (ascorbic acid).
4. To correlate the observed pharmacological activities with the phytochemical composition of Manila Tamarind extracts, elucidating potential mechanisms underlying their anti-inflammatory and antioxidant effects.
5. To provide valuable insights into the medicinal potential of Manila Tamarind as a natural remedy for inflammation and oxidative stress-related disorders, contributing to the development of novel therapeutic agents with enhanced efficacy and minimal side effects

3. Methods

3.1 PREPARATION OF PLANT EXTRACT:

It involves the separation of medically active parts of plant tissue from inactive or inert compounds by using selective solvents in a standard extraction procedure. The purpose of the standard extraction procedure for crude drugs is to reach the therapeutic part and eliminates the inert material by treatment with a selective solvent known as the menstruum. Menstruum is a substance that dissolves a solid or holds it in suspension, Herbal preparation-the solvent used was methanol. The plant mixture was prepared by mixing each extract of 25mg dissolved in 10 ml of methanol and boiled. It was centrifuged at 2500 rpm for 10 minutes.

3.2 QUALITATIVE ANALYSIS OF PHYTOCHEMICAL SCREENING OF MANILLA TAMARIND

To assess the chemical configuration of the various extracts, qualitatively. A preliminary plant phytochemical assay was conducted according to standard methods. (Sahreen et al., 2010)

3.2.1 TEST FOR GLYCOSIDES:

KELLER KILLANIS TEST: TO 1 ml of each extracted plant and 1 ml of glacial acetic acid are added and allowed to cool. After cooling two drops of FeCl3 were added and 2 ml Of H2So4 concentration near the test tube wall was carefully removed. The development of a reddish-brown ring at the intersection of two layers records the presence of glycosides.

FIGURE 4

KELLER KILLANIS TEST SHOWING THE PRESENCE OF GLYCOSIDES

3.2.2 TEST FOR TERPENOIDS:

Each sample was taken from 0.5 mg in a test tube and 2ml of chloroform and conc.H2So4 (concentrated sulphuric acid) was added to the sample for the formation of a brown layer between the other two layers indicating the occurrence of terpenoids.

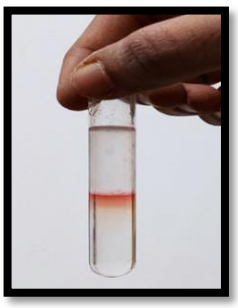

FIGURE 5

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TEST FOR TERPENOIDS SHOWING THE PRESENCE OF TERPENOIDS

3.2.3 TEST FOR SAPONINS:

FROTH FORMATION: In 2 ml of the sample is mixed with 2 ml of distilled water in a test tube. After this accumulation, the test sample was vigorously mixed for about 15 minutes. The formation of a soapy layer indicates the presence of saponins in the experimental sample**.**

-

FIGURE 6

TEST FOR SAPONINS SHOWING THE PRESENCE OF SAPONIN

3.2.4 ASSESSMENT OF COUMARINS:

For the coumarins assay, 1 mg of the PLANT sample was allowed to react with 1 ml of 10% sodium hydroxide. The formation of yellow colour in the test tube indicates the presence of coumarins.

FIGURE 7

ASSESSMENT OF COUMARINS SHOWING THE PRESENCE OF COUMARINS

3.2.5 TEST FOR PHYTOSTEROLS:

SALKOWSKI'S EXPERIMENT: the plant sample extract was mixed with chloroform and filtered. The filtrates with 5-6 drops of concentrated sulphuric acid. There is a yellow-golden colour at the bottom with a layer separating the above is seen and it gives a positive test result

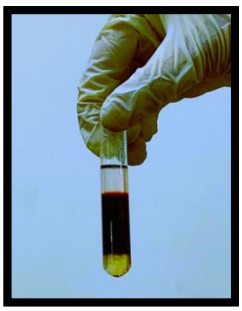

FIGURE 8

TEST FOR PHYTOSTEROLS SHOWING THE PRESENCE OF PHYTOSTEROL

3.2.6 TEST FOR PHLOBOTANNINS:

In 1 ml of each extract, a few drops of 10% ammonium solution are added. There is a colour change to pink indicating the presence of phlobotannins in the plant samples.

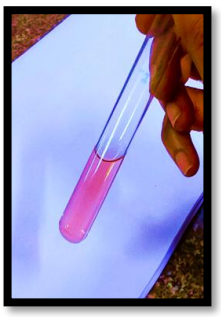

FIGURE 9

TEST FOR PHLOBOTANNINS SHOWING THE PRESENCE OF PHLOBOTANNINS

3.2.7 TEST FOR QUINONES:

A dose of 1 ml of each plant sample was permissible to act in response to 1 ml Of concentrated

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sulphuric acid. The appearance of red colour indicates the presence of Quinone.

FIG 10

TEST FOR QUINONES SHOWING THE PRESENCE OF QUINONES

OBSERVATION:

PHYTOSTEROLS	+
TERPENOIDS	+
GLYCOSIDES	+
COUMARINS	+
SAPONIN	+
STEROLS	+
PHLOBOTANNIS	+

TABLE – 1

3.3 QUALITATIVE ANALYSIS OF PHYTOCHEMICALS WITH THE TYPE OF TESTS THAT SHOWS THE PRESENCE OF COMPOUNDS

3.3.1 INVITRO ASSAY TO INVESTIGATE ANTI-INFLAMMATORY ACTIVITY OF HERBAL EXTRACTS BY PROTEIN DENATURATION METHOD:

Materials required:

- ✓ PBS- phosphate buffer saline
- ✓ Egg ALBUMIN (EGG WHITE)
- ✓ Acetylsalicylic acid (aspirin)
- ✓ Plant extract
- ✓ Micropipette and tips
- ✓ Eppendorf tube
- ✓ Water bath

PRINCIPLE:

Protein denaturation results from the loss of biological physical and chemical properties of protein molecules. Protein denaturation has been linked to the development of inflammatory diseases such as *rheumatoid arthritis, diabetes, and cancer*. Therefore, the ability of substances to inhibit protein breakdown or denaturation may also prevent inflammatory disorders (Barrera-Necha et al., 2003)

PROCEDURE:

1) Denaturation of protein was performed as pronounced in(César Escalona-Arranz et al., n.d.) With slight modification. Eight different test tubes were taken and All test tubes are filled with 1 ml of PBS

2) Test solution which is the plant extract consisting of 1ml with different concentrations from 100 – 500 µl is taken in different test tubes.

3) Standard salicylic acid with a concentration of 200 µl and 500µl is taken in 2 aliquots.

4) Now in both standard and test samples, 1 ml of egg white is added

5) Blank is prepared by adding PBS AND EGG WHITE.

6) The mixture is now incubated at room temperature at 37°c for 10 minutes

7) Denaturation of egg white is brought by keeping the reaction mix in a water bath at 45- 70°c for about 10 minutes.

8) Later cool the Turbidity was measured at 550 nm.

9) % inhibition of protein breakdown or denaturation was deliberated from control (standard - no drug was added).

10) Each experiment with sample and test was done in triplicate and the average was taken.

	DISTILLED WATER	**PBS (ml)**	**EGG ALBUMIN (ml)**	**PLANT EXTRACT (µl)**	**ASPRIN (µl)**	**OD VALUE**
BLANK	**500**	**1.4**	**1**	**-**	**-**	**0.0**
S1	**400**	**1.4**	**1**	**100**	**-**	**0.37**
S2	**300**	**1.4**	**1**	**200**	**-**	**0.48**
S3	**200**	**1.4**	**1**	**300**	**-**	**0.77**
S4	**100**	**1.4**	**1**	**400**	**-**	**0.85**
S5	**-**	**1.4**	**1**	**500**	**-**	**0.99**
STANDARD	**300**	**1.4**	**1**	**-**	**200**	**0.40**

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STANDARD	**-**	**1.4**	**1**	**-**	**500**	**0.96**

TABLE 2

OVERVIEW OF QUANTITATIVE ASSESSMENT BY PROTEIN DENATURATION

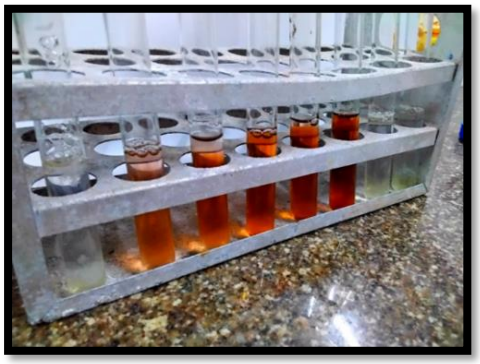

FIG 11

QUANTITATIVE ASSESSMENT BY PROTEIN DENATURATION AT INCUBATION

STATISTICAL ANALYSIS:

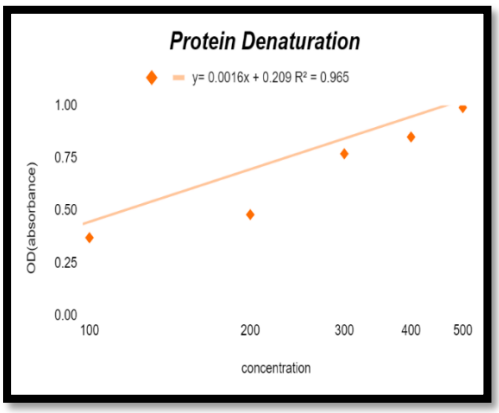

FIG 12

CALIBRATION CURVE FOR PROTEIN DENATURATION ASSAY

CALCULATION:

After the experiment is carried out the percentage for protein denaturation inhibition is calculated by the next equation.

"% Inhibition of denaturation = (1-D/C) × 100

Here;

D is the absorbance of the test sample

C is the absorbance of standard (standard reference drug).

% Inhibition of denaturation = (1-D/C) × 100

= **(**1-0.77/0.44) × 100

= 92.5 % of inhibition.

The inhibitory effect of different concentrations of an herbal preparation on protein showed significant inhibition of denaturation of egg albumin in a concentration-dependent manner".

3.3.2 ANTIOXIDANT ASSAY BY PHOSPHOMOLYBDATE METHOD

AIM:

To evaluate the anti-oxidant property by phosphomolybdate assay.

PRINCIPLE:

The basic goal of measuring antioxidant capacity using a phosphomolybdate analysis comprises the reduction of Mo (VI) to Mo (V) by a selected plant extract containing antioxidant compounds.

PROCEDURE:

a) The antioxidant activities of various extracts of manilla tamarind were tested by the phosphomolybdate complex Method.

b) 100 ml in an aliquot of sample solution at various concentrations combined through 1 mL of actively prepared reagent "(0.6 M sulfuric acid, 28 mm sodium phosphate and 4 mm of ammonium moly)".

c) Methanol solution (0.3 mL) was added to the extract.

d) The test tube was enclosed and placed under a 95 ° C water bath for 90 minutes

e) Samples are brought to room atmospheric temperature

f) The absorption was dignified at 695 nm against negative. The results are explained by absorption.

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g) The equivalent of ascorbic acid is calculated using the standard AA graph.

h) The test was performed in triplicates and values were expressed as equal to ascorbic acid per mg per g of extract(IN Vitro Anti-inflammatory Activity of Ficus Racemosa L. Bark Using Albumin Denaturation Method, 2019).

CALCULATION:

	DISTILLED WATER (ml)	REAGENT (ml)	PLANT EXTRACT (µl)	ASCORBIC ACID (µl)	OD VALUE
BLANK	1	1	-	-	0.0
S1	0.5	1	1500	-	0.48
S2	0.4	1	1600	-	0.59
S3	0.3	1	1700	-	0.68
S4	0.2	1	1800	-	0.86
S5	0.1	1	1900	-	0.98
STD	0.5	1	-	1500	0.62
STD	0.4	1	-	1600	0.96

TABLE – 3

OVERVIEW OF QUANTITATIVE ASSAY BY PHOSPHOMOLYBDATE METHOD

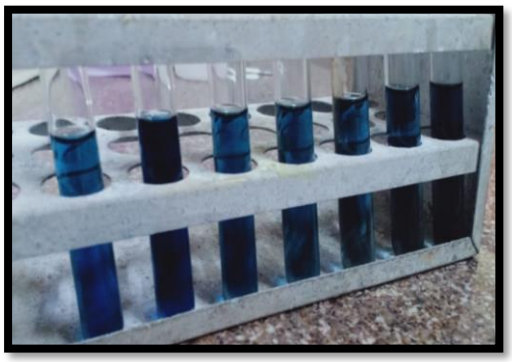

FIG 13

QUANTITATIVE ASSAY BY PHOSPHOMOLYBDATE METHOD AT INCUBATION

The antioxidant capacity was estimated using

"Antioxidant effect=control OD-sample OD /control OD"

= (0.96-0.59/0.96) * 100

= 37% of antioxidant effect

The antioxidant capacity was estimated to be 37 %. The in vitro evaluation of antioxidant properties showed a significant amount of antioxidant propert**ies.**

STATISTICAL ANALYSIS:

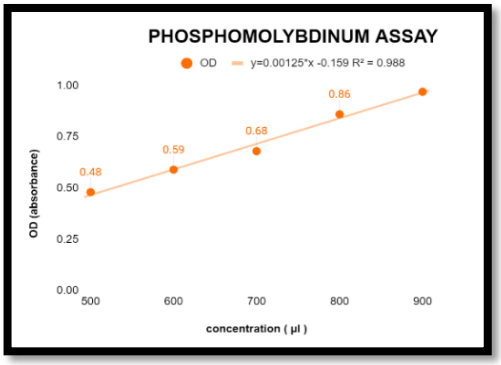

FIG 14

CALIBRATION CURVE FOR PHOSPHOMOLBDATE ASSAY

4. Results

The quantitative analysis of phytochemicals has shown a positive test for phytosterols, coumarins, Quinone, phlobotannins, saponins, terpenoids, and glycosides. The evaluation of anti-inflammatory properties through the protein denaturation method showed a significant inhibition of denaturation giving a positive result with = 92.5 % of inhibition. The ability to reduce Phosphomolybdate increases with increasing concentration other has been a direct relationship between volume and absorption. A remarkably high decrease in strength was seen in the concentration and estimated to be 37 %.

5. Discussion

The present study aimed to comprehensively analyse the phytochemical profile of Manila Tamarind (Pithecellobium dulce) extracts and evaluate their antioxidant and anti-inflammatory properties. The qualitative analysis of phytochemical constituents revealed the presence of phytosterols, terpenoids, glycosides, coumarins, saponins, sterols, and phlobotannins, which are known to possess various pharmacological activities.

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In the evaluation of anti-inflammatory activity using the protein denaturation method, the Manila Tamarind extract exhibited significant inhibition of protein denaturation in a concentration-dependent manner. Protein denaturation is associated with the development of inflammatory diseases such as rheumatoid arthritis, diabetes, and cancer. Therefore, substances capable of inhibiting protein denaturation hold promise in the prevention and management of inflammatory disorders. The observed inhibition rate of 92.5% underscores the potential anti-inflammatory efficacy of the Manila Tamarind extract.

Furthermore, the antioxidant activity of the Manila Tamarind extract was assessed using the phosphomolybdate method. The results demonstrated a notable antioxidant effect, with the extract showing a 37% capacity compared to ascorbic acid. Antioxidants play a crucial role in neutralizing free radicals and reducing oxidative stress, thereby protecting cells from damage and preventing various chronic diseases.

The observed pharmacological activities of Manila Tamarind extract can be attributed to its rich phytochemical composition. Phytosterols, terpenoids, and glycosides are known for their anti-inflammatory and antioxidant properties, while coumarins, saponins, and phlobotannins possess diverse biological activities including anti-inflammatory, antioxidant, and anticancer effects.

The findings of this study contribute to the growing body of evidence supporting the medicinal potential of Manila Tamarind. The ability of the extract to inhibit protein denaturation and scavenge free radicals underscores its promise as a natural remedy for inflammatory and oxidative stress-related conditions(Begum & Lakshmanan, 2022; Ganesan et al., 2022). Further studies are warranted to elucidate the underlying mechanisms of action and to explore its therapeutic potential in vivo and in clinical settings.

CONCLUSION: In conclusion, Manila Tamarind extracts exhibit significant anti-inflammatory and antioxidant activities, which can be attributed to their diverse phytochemical constituents. These findings highlight the therapeutic potential of Manila Tamarind as a natural remedy for inflammation and oxidative stress-related disorders given, the anti-oxidant and anti-inflammatory properties of Manilla tamarind, incorporating this may lead to beneficiary effects. The powdered pulp may be added to milk and beverages dressing for salads and may be used instead of artificial extract for flavour and colour. This powder got an attractive colour, flavour, and aroma which will also be pleasing and palatable to everyone, especially children. This can also be incorporated into making jam, jelly, ice creams, and other sweets and confectionaries to improve their quality and increase their nutritional importance. In conclusion, Manila Tamarind extracts exhibit significant anti-inflammatory and antioxidant activities, which can be attributed to their diverse phytochemical constituents. These findings highlight the therapeutic potential of Manila Tamarind as a natural remedy for inflammation and oxidative stress-related disorders

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