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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF POLYHERBAL EXTRACT USING BOVINE BLOOD

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ABSTRACT

The present study investigates the *in vitro* anti-inflammatory activity of a polyherbal extract composed of ***Garcinia indica***, ***Musa paradisiaca***, *and* ***Punica granatum*** peels. These plants, belonging to the families *Clusiaceae*, *Musaceae*, and *Lythraceae* respectively, are traditionally recognized for their therapeutic potential, including antioxidant, anticancer, antiviral, and anti-inflammatory properties. The research aimed to evaluate the synergistic effects of these combined herbal extracts using bovine blood, which closely resembles human blood in composition. The plant materials were collected, authenticated, and extracted through maceration and decoction using ethanol and water. Preliminary phytochemical screening revealed the presence of key bioactive constituents such as flavonoids, phenolics, glycosides, terpenoids, and saponins, which are known contributors to anti-inflammatory activity. The *in vitro* evaluation was conducted using assays for membrane stabilization, protein denaturation inhibition, and proteinase inhibitory activity.Results indicated that the polyherbal formulation exhibited significant anti-inflammatory activity, likely due to the combined effects of its phytoconstituents. These findings support the traditional use of these plants in inflammatory conditions and suggest their potential as a natural alternative to conventional anti-inflammatory agents.

KEYWORDS: *Garcinia indica, Musa paradisiaca, Punica granatum*, anti-inflammatory activity, polyherbal extract, phytochemical screening, bovine blood.

1. INTRODUCTION

The study of drugs and pharmaceuticals, including their origin, composition, pharmacokinetics, pharmacodynamics, therapeutic use, and toxicology, is known as

pharmacology. More precisely, it is the study of how chemicals interact with living things to influence normal or aberrant biochemical processes.[1] [2] Substances are classified as medicines if they possess therapeutic qualities. Molecular and cellular mechanisms, organ/systems mechanisms, signal transduction/cellular communication, molecular diagnostics, interactions, chemical biology, therapy, and medical applications, drug composition and properties, functions, sources, synthesis and drug design, and antipathogenic capabilities are all included in this field. Pharmacodynamics and pharmacokinetics are the two primary subfields of pharmacology. Pharmacokinetics examines how biological systems affect a drug, while pharmacodynamics examines how a drug affects biological systems. Pharmacokinetics is the study of the absorption, distribution, metabolism, and excretion (ADME) of chemicals from biological systems, while pharmacodynamics is the study of chemicals with biological receptors.[3] The first known medical treatment is the use of medicinal herbs, which has been practiced throughout history in every society.[4] Humans have been aware of their reliance on nature for a healthy existence since the beginning of time, and they have used a range of plant resources as medicine to treat a wide range of illnesses.[5] By investigating different biologically active natural products, this indigenous knowledge, which has been passed down from generation to generation in various parts of the world, has greatly aided in the development of traditional medical systems and provided a scientific foundation for their traditional uses.[6] The anti-inflammatory properties of Garcinia indica, Punica granatum, and Musa paradisiaca—which belong to the families Clusiaceae, Lythraceae, and Musaceae, respectively—are the basis for this work. Because they include chemical components as garcinol, ellagitannins, flavonoids, polyphenols, and many more, they have pharmacological action such as antioxidant, anticancer, antiviral, and anti-inflammatory properties.

The goal of the study is to assess the polyherbal extract's anti-inflammatory properties using bovine blood, which is similar to human blood. These herbs have customary background of being a medication used as a monotherapy to treat a variety of inflammatory disorders. Here, we examine how a mix of herbs affects the body's inflammatory reactions.

1.1. INFLAMMATION

The biological reaction of bodily tissues to damaging stimuli, such as pathogens, damaged cells, or irritants, includes inflammation. Heat, discomfort, redness, swelling, and loss of function are the five cardinal indications. Since inflammation is a universal reaction, it is

regarded as an innate immunity mechanism, while adaptive immunity is pathogen-specific.[7] It is a component of how bodily tissues react biologically to damaging stimuli like viruses, damaged cells, or irritants.[8] Immune cells, blood vessels, and chemical mediators are all involved in inflammation, which is a defensive reaction. Inflammation serves to remove injured cells and tissues, start tissue repair, and eradicate the original cause of cell injury. [9] There are two types of inflammation: acute and chronic. The body's first reaction to damaging stimuli is acute inflammation, which is brought on by an increase in the flow of plasma and leukocytes—particularly granulocytes—from the circulation into the damaged tissues. The local vascular system, the immune system, and different cells in the damaged tissue are all involved in a sequence of biochemical processes that spread and develop the inflammatory response. Chronic inflammation, often referred to as prolonged inflammation, involves the simultaneous destruction and healing of tissue and causes a progressive change in the type of cells present at the site of inflammation, such as mononuclear cells. Depending on the kind of cytokines and helper T cells (Th1 and Th2) involved, inflammation has also been categorized as Type 1 or Type 2.[10]

An anti-inflammatory drug is one that lowers fever, edema, and inflammation. Approximately half of analgesics are anti-inflammatory medications, sometimes known as anti-inflammatories. Unlike opioids, which block pain by affecting the central nervous system, these medications relieve pain by blocking mechanisms of inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, antileukotrienes, and monoclonal antibodies are examples of common anti-inflammatory medications.[11] Anti-inflammatory drugs are pharmaceuticals that assist lessen pain and inflammation brought on by a number of ailments, including musculoskeletal illnesses and arthritis. Nonsteroidal anti-inflammatory drugs (NSAIDs), which include popular treatments like aspirin and ibuprofen, are the most prevalent class of anti-inflammatory drugs. Doctors frequently use these medications to treat pain and inflammation brought on by a variety of medical conditions. NSAIDs and other anti-inflammatory medications function by inhibiting the cyclooxygenase (COX) enzyme, which is essential for the synthesis of prostaglandins. Prostaglandins are substances that cause pain, fever, and inflammation. Anti-inflammatory medications successfully lessen these symptoms by blocking the COX enzyme. COX-1 and COX-2 are the two primary forms of COX enzymes. COX-1 protects the stomach lining and performs various tasks, whereas COX-2 is mostly involved in the inflammatory process. Effective pain and inflammation management

requires an understanding of the various kinds of anti-inflammatory medications and their particular applications.[12]

1.2. *Garcinia indica*

Garcinia indica, a fruit-bearing tree with culinary, medicinal, and industrial applications, is a member of the mangosteen family (Clusiaceae). It is sometimes referred to as kokum. It grows mostly in the Western Ghats of India, specifically in the states of Kerala, Goa, Karnataka, and Maharashtra. It is regarded as an endemic species of India's woodlands and Western Ghats.[13] The plants are found all across the world, including Western Polynesia, Africa, and tropical Asia.[14] They have drawn a lot of attention in the past few decades, and extracts from various plant parts of the Garcinia species—such as Garcinia brasiliensis, G. cambogia, G. gardneriana, G. pedunculata, and G. mangstana—have shown promise in the treatment and prevention of chronic illnesses that are not communicable.[15] Additionally, it was discovered that their chemical compositions are rich in bioactive compounds such as hydroxycitric acid (HCA), bioflavonoids, garcinol, xanthochymol, and guttiferone isoforms, and that they include a variety of physiologically active metabolites.[16] [17] These substances have been linked to biological processes like antiviral, antioxidant, and anticancer properties.[18]

Plant profile:

Table 1: Plant profile of Kokum (*Garcinia indica*) [19]

Kingdom	Plantae
Division	Magnoliophyte
Class	Magnoliopsida
Order	Malpighiales
Family	Clusiaceae
Genus	Garcinia
Species	Indica

Vernacular name

- Hindi Name: Kokum, Amlaveta, Vishambila
- Malayalam: Punampuli
- Tamil: Murgal, puli [20]

Figure 1: *Garcinia indica* fruit.

1.3. *Musa paradisiaca*

Originally a hybrid of Musa acuminata and Musa balbisiana, Musa × paradisiaca is both a species and a cultivar that humans grew and tamed relatively early. The majority of cultivated plantains and bananas are either polyploid cultivars of M. acuminata alone or of this hybrid. [21] The fruits, together with other parts of the plant such the stem, peel, pulp, and leaves, are used in traditional medicine to cure a variety of human ailments.[22]

Plant profile:

Table No. 2: Plant profile of Banana (*Musa paradisiaca*) [21]

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Zingiberales
Family	Musaceae
Genus	Musa
Species	Paradisiaca

Vernacular name:

- Hindi: Kela
- Kannada: Balehannu
- Malayalam: Kadalivala, Vazha [23]

Figure 2: Musa *paradisiaca* fruit

1.4. *Punica granatum*

The pomegranate (Punica granatum) is a fruit-bearing, deciduous shrub that ranges in height from 5 to 10 meters (16 to 33 feet) and belongs to the family Lythraceae, subfamily Punicoideae. abundant in mythical and symbolic connections throughout numerous cultures.[24] Pomegranate peel is rich in catechins, prodelphinidins, condensed tannins, and polyphenols.[25] The peel's greater phenolic content produces extracts that can be used as food preservatives and dietary supplements.[26]

Plant profile:

Table No. 3: Plant profile of Pomegranate (*Punica granatum*) [27]

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Myrtales
Family	Lythraceae
Genus	Punica
Species	Granatum

Vernacular name:

- Hindi: Anar, Anardana
- Tamil: Madhulai
- Malayalam: Maathalam [28]

Figure 3: *Punica granatum* fruit.

1.5. *Capra aegagrus hircus*

A subspecies of goat that was domesticated from the wild goat is the domestic goat (Capra aegagrus hircus). As members of the goat-antelope subfamily Caprinae, the goat and sheep are closely related members of the Bovidae family. Goats come in more than three hundred different breeds.[29] The name comes from Kerala's Malabar region. Meat, milk, and food are the main uses. The majority of them are entirely white. Some are admixtures, brown, or black. Horns are somewhat bent and pointed upward and outward. occasionally curled downward and backward, making contact with the flesh. medium-sized ears that reach up to the nose and are oriented downward and outward. [30]

Comparison of the human blood with bovine blood:

Table No. 4: Comparison of the human blood with bovine blood [31]

Parameter	Unit	Goat	Man
Total Plasma N	mg%	1128.0	1120.0
Urea N	mg%	22.31	17.1
Non-Protein Nitrogen (NPN)	mg%	48.52	35.6
Amino N	mg%	9.60	6.40
Sugar	mg%	59.1	112.0
Inorganic Phosphorus	mg%	7.70	—
Hemoglobin	gm%	9.26	13-17
Cell Volume (Hematocrit)	vol%	28.8	45.6

Animal profile:

Table.No.5: **Animal profile *of Goat (Capra Aegagrus hircus)*** ***[32]***

Kingdom	Animalia
Phylum	Chordata
Class	Mammalia
Order	Artiodactyla
Family	Bovidae
Genus	*Capra*
Species	*Aegagrus*
Subspecies	*Hircus*

Vernacular name:

- Hindi: Bakra
- Bengali: Khass
- Tamil: Puliyaa [33]

2.AIM AND OBJECTIVE

2.1. AIM

- To evaluate the in vitro anti-inflammatory activity of polyherbal extract using bovine blood.

2.2. OBJECTIVE

The present work is planned with the following objectives;

- Identification, authentication, collection and drying
- Phytochemical screening
- Maceration using ethanol and decoction using water
- Invitro study of anti-inflammatory activity.

3. MATERIAL AND METHODS

1. PLANT COLLECTION AND AUTHETIFICATION

- The peels of the plant *Garcinia indica* (Clusiaceae), *Musa paradisiaca* (Musaceae), *Punica granatum* (Lythraceae) were collected from Kasaragod.
- The plant material was taxonomically identified by the botanist, Dr. Biju P, Associate Professor, Department of Botany, Government College Kasaragod.

2. EXTRACTION OF DRUG

2.1. Extraction of Kokum Peel

- 70% ethanol reddest was used in the maceration process for extraction. A fine, dry powder of kokum peel (Garcinia indica) weighing up to 2g was steeped in 70% ethanol for an hour. Until all of the simplicia powder was immersed, more ethanol solvent was applied. After that, it was let to stand for four full days with sporadic stirring. Every 24 hours, the macerate was gathered. A rotary evaporator operating at 30 rpm and 40°C was used to evaporate all of the collected macerate until a thick extract was produced.[34]

Figure. No 4: Extraction of Kokum peel

2.2. Extraction of Banana peel

The aqueous extract was made by dissolving 5 grams of dried peel powder from ripe bananas (Musa paradisiaca) in 25 milliliters of distilled water. The alcoholic extract was made using a similar procedure. Ethanol, an organic solvent, was heated to 100°C for 30 minutes in order to create alcoholic extract. After that, an aqueous alcoholic extract was created by combining the two extracts. To prevent evaporation, cotton plugs were placed on top of the conical flasks containing the extract. The extract was shaken for a whole day at 250 revolutions per minute (rpm) in an orbital shaker. After being shaken all night, they were filtered twice: once using

muslin cloths and once using filter paper. The resulting extracts were kept in storage at 4°C. [35]

Figure. No 5: Extraction of Banana peel

2.3. Extraction of Pomegranate peel

The dried peel was ground into a coarse powder. Using a stirrer, 2 grams of peel powder and 100 milliliters of distilled water were combined. Using a heating mantle, the mixture was heated to 60–80 degrees Celsius for 15–20 minutes. Whatman No. 1 filter paper was used to filter the boiling mixture. The filtered extract was then further reduced in volume to 5 milliliters. [36]

Figure. No 6: Extraction of Pomegranate peel

3. PHYTOCHEMICAL STUDIES

PRELIMINARY PHYTOCHEMICAL SCREENING OF PEEL EXTRACTS

3.1. Chemical tests for alkaloids

A small portion of the dried alcoholic extract was shaken (acidified) with dilute hydrochloric acid and filtered.

The acidified filtrate was tested with the following reagents, to detect the presence of alkaloids.

i. Mayer's Test

The acidified extract (two ml) was treated with 1 ml of Mayer's reagent

(Potassium mercuric iodide), shaken, and noted for the presence of a creamy precipitate.

ii. Hager's Test

The acidified extract (two ml) was treated with 1 ml of Hager's reagent (saturated picric acid solution) and observed for the presence of yellow precipitate.

iii. Wagner's Test

The acidified extract (two ml) was treated with a few ml of Wagner's reagent (solution of iodine in potassium iodide) and observed for the presence of a reddish-brown precipitate.

iv. Dragendorff's Test

The acidified extract (two ml) was treated with a few ml of Dragendorff's reagent (Potassium bismuth iodide) and observed for the presence of orange-red precipitate.

3.2. Chemical test for glycosides

A small portion of the extract was hydrolyzed with dilute hydrochloric acid for a few hours in a water bath and the hydrolysate was later subjected to the following tests to detect the presence of glycosides.

i. Legal's Test

The residue (dry extract) left after evaporation was dissolved in a few milliliters of pyridine. Two milliliters of freshly prepared sodium nitro prusside solution was added to it and then made alkaline with sodium hydroxide solution. It was observed for the formation of pink red color.

ii. Baljet's Test

The few ml of the extract was treated with 1ml sodium picrate solution and a yellow to orange color reveals the presence of cardiac glycosides.

iii. Liebermann Burchard's Test

The five mi of the hydrolysate taken in a test tube was evaporated, the residue taken in dry chloroform (one ml) and then it was mixed with two ml of specially distilled acetic anhydride followed by a few drops of concentrated sulphuric acid through the sides of the test tube. It was then observed for the development of a deep red color in the lower portion and green color in the upper portion which changed to blue and violet.

iv. Bontrager's Test

A little of the residue obtained from the hydrolysate was mixed with water and shaken with an equal volume of chloroform. The chloroform layer was separated to which dilute ammonia solution was added and shaken well and noted whether any pink color was present in the ammoniacal layer.

v. Modified Bontrager's Test

The residue obtained was treated with ferric chloride and dilute HCl, for the oxidative hydrolysis of C-glycoside. Then it was extracted with chloroform. The chloroform layer was separated, and dilute ammonia solution was added and shaken. The ammoniacal layer was observed for pink in colour.

3.3. Chemical test for phenolic compounds and tannins

i. Ferric chloride Test

A small quantity of the extract diluted with water was treated with dilute ferric chloride solution (5%) and observed for the presence of blue color.

ii. Gelatin Test

The extract dissolved in water was filtered. To the filtrate, 2% solution of gelatin containing 10% sodium chloride was added. Noted for the presence of milky white precipitate.

iii. Lead acetate Test

The extract dissolved in water was treated with a 10% lead acetate solution. Noted for the presence of bulky white precipitate.

iv. Decolorization Test

The extract dissolved in water was treated with a dilute potassium permanganate solution. Noted for the decolorization of potassium permanganate.

3.4. Chemical test for flavanones and flavonoids

i. Aqueous sodium hydroxide Test

Aqueous sodium hydroxide solution was added to the few ml of the extract and the presence of yellow coloration of the solution was noted. The filter paper was wetted with a small quantity of alcoholic solution of the extract. That filter paper was exposed to ammonia vapors and noted the yellow color.

3.5. Chemical test for carbohydrates

A small quantity of ethanolic extract was mixed with water or alcohol and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates.

i. Molisch's Test

The filtrate (2ml) was treated with a few drops of Molisch's reagent and 2 ml of concentrated sulphuric acid was added through the sides of the test tube without shaking. Observed for the presence of a violet ring at the junction of two solutions.

ii. Benedict's Test

The filtrate (a few drops) was treated with two ml of Benedict's reagent. Then the mixture was heated in a boiling water bath for two min and the presence of red precipitate was noted.

iii. Fehling's Test

The filtrate (1ml) was treated with 1 ml each of Fehling's solutions A and B and boiled in a water bath for half an hour, then observed for the presence of red residue at the bottom of the test tube.

3.6. Chemical test for proteins and amino acids

i. Millon's Test

The extract (two ml) was treated with a few drops of Millon's reagent (1gm of mercury+ 9ml of fuming nitric acid) and observed for the presence of white precipitate, which on warming turned into a red-colored solution.

ii. Biuret Test

The extract (two ml) was treated with one drop of 2% copper sulphate solution. To this 1ml of 95% ethanol was added followed by an excess of potassium hydroxide solution and Observed for the presence of violet-colored solution.

iii. Ninhydrin Test

The extract (a few ml) was treated with two drops of ninhydrin solution and heated in a water bath and then the presence of violet colour was noted.

3.7. Chemical test for terpenoids

i. Salkowski's Test

The extract (a few ml) was dissolved in chloroform. An equal volume of concentrated sulphuric acid was added to it and noted for the appearance of red colour in the chloroform layer and greenish-yellow fluorescence in the acid layer.

3.8. Chemical test for sterols

A small amount of the alcoholic extract was refluxed with a solution of alcoholic potassium hydroxide until saponification was observed. The mixture was diluted and extracted with solvent ether. The ethereal extract was evaporated, and the residue was subjected to

i. Liebermann-Burchard Test

The residue was taken with dry chloroform (1 ml) and then it was mixed with 2 ml of specially distilled acetic anhydride followed by a few drops of concentrated sulphuric acid through the sides of the test tube and observed for the development of a deep red colour in the lower portion and green colour in the upper portion which changes to blue and violet.

ii. Salkowski's Test

The residue was dissolved in chloroform and an equal volume of concentrated sulphuric acid was added to it and observed for the red colour in the lower layer.

3.9. Chemical test for saponins

i. Foam or Froth Test

A small quantity of extract was diluted with 20 ml of distilled water in a graduate cylinder. The suspension was shaken for 15 minutes and waited to see if any froth was formed.

To 10 ml aqueous extract of the plant, 25 ml of absolute alcohol was added with constant stirring. Filtered and the precipitate formed was dried in air and examined for swelling properties.[37] [38]

4. ANTI-INFLAMMATORY STUDY

Drugs:

1. Test drug: polyherbal extract of peels of *Garcinia indica*, *Musa paradisiaca*, *Punica granatum*
2. Standard drug:Aspirin (acetyl salicyclic acid) laboratory reagent, fortune chemicals[39]

Procedure:

4.1. Hypotonic solution –induced haemolysis or membrane stabilizing activity:

With a few minor adjustments, this test was conducted using the procedure outlined in Shinde et al. (1999) [40]. The test sample was made up of 0.030 ml of stock erythrocyte (RBC) suspension combined with 5 ml of hypotonic solution (50 mM NaCl in 10 mM Sodium Phosphate Buffer at pH 7.4) that included 100–500 µg/ml of Herbal Preparation (HP 4). The control sample was made up of just hypotonic buffered solution combined with 0.030 milliliters of RBC suspension. At quantities of 100 and 200 µg/ml, the common medication acetylsalicylic was handled similarly to the test. Three duplicates of the experiment were conducted. The solutions were centrifuged for 10 minutes at 3000 rpm after being incubated for 10 minutes at room temperature. The absorbance of the supernatant was then determined

using spectrophotometry at 540 nm. The proportion of membrane stabilization or hemolysis inhibition was computed.

% Inhibition of Haemolysis = 100 [$A_1 – A_2 / A_1$]

Where, A_1 = Absorbance of hypotonic buffered solution alone

A_2 = Absorbance of test /standard sample in hypotonic solution.[41]

4.2. Effect on Protein Denaturation:

With a few minor adjustments, protein denaturation was carried out as described (Elias et al., 1988) [42]. One milliliter of conventional acetylsalicylic acid (100 and 200 µg/ml) or various quantities of Herbal Preparation (HP-4) ranging from 100 to 500 µg/ml was combined with one milliliter of egg albumin solution (1 mM) to create the test solution, which was then incubated at 27 ±1˚C for fifteen minutes. The reaction mixture was kept at 70˚C in a water bath for ten minutes in order to induce denaturation. The turbidity was measured spectrophotometrically at 660 nm after cooling. The percentage inhibition of denaturation was computed using the drug-free control. Every experiment was carried out three times, and the average was calculated.

% Inhibition of Protein Denaturation = [A_0-A_1/A_0]100

Where, A_0= Absorbance of control

A_1= Absorbance of sample.[41]

4.3. Proteinase Inhibitory Activity

The method of Sakat et al. [43], which Gunathilake et al. [44] adapted, was used to measure proteinase inhibitory activity. In short, 0.06 mg of trypsin, 1 mL of 20 mM Tris-HCl buffer (pH 7.4), and 1 mL of test sample (0.02 mL extract 0.980 mL methanol) made up the reaction solution (2 mL). After 5 minutes of incubation at 37 C, 1 mL of 0.8% (w/v) casein was added, and the mixture was then incubated for an additional 20 minutes. Two milliliters of 70% perchloric acid were added to stop the reaction after the incubation period. After centrifuging the mixture, the absorbance of the supernatant was measured at 210 nm using buffer as a blank. A phosphate buffer solution was employed.

% Inhibition of Denaturation = 100 [A_1-A_2/A_1]

Where, A_1= Absorption of the control sample

A_2 = Absorption of the test sample.[43] [44]

5. PHYTOCHEMICAL SCREENING OF THE EXTRACTS

5.1. Phytochemical screening of *Garcinia indica*

The phytochemical studies of dried peel of *Garcinia indica* were carried out. The ethanolic extract of *Garcinia indica* shows the presence of alkaloids, glycosides, phenolics, flavones and flavonoids, carbohydrates, terpenoids, sterols and saponins.

Table. No 6: Phytochemical screening of *Garcinia indica*

Sl. No	Phytoconstituents Test	Result
1	Alkaloids	+
2	Glycosides	+
3	Phenolics	+
4	Flavones and flavonoids	+
5	Carbohydrates	+
6	Proteins and Amino acids	-
7	Terpenoids	+
8	Sterols	+
9	Saponins	+
10	Gums and Mucilage	-

Figure. No 7: Phytochemical screening of *Garcinia indica*

5.2. Phytochemical screening of *Musa paradisiaca*

The phytochemical studies of dried peel of *Musa paradisiaca* were carried out. The hydroalcoholic extract of *Musa paradisiaca* shows the presence of

Table.No 7: Phytochemical screening of *Musa paradisiaca*

Sl. No	Phytoconstituents Test	Result
1	Alkaloids	+
2	Glycosides	+
3	Phenolics	+
4	Flavones and flavonoids	+
5	Carbohydrates	+
6	Proteins and Amino acids	-
7	Terpenoids	+
8	Sterols	+
9	Saponins	+
10	Gums and Mucilage	+

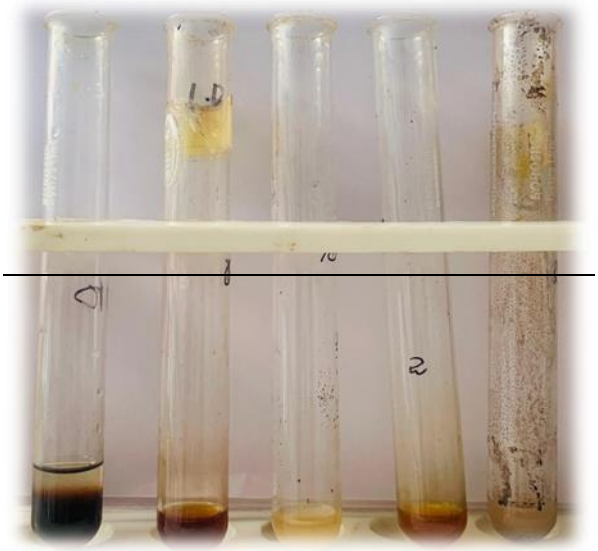

Figure. No 8: Phytochemical screening of *Musa paradisiaca*

5.3. Phytochemical screening of *Punica granatum*

The phytochemical studies of dried peel of *Punica granatum* were carried out. The aqueous extract of *Punica granatum* shows the presence of alkaloids, glycosides, phenolics, flavones and flavonoids, carbohydrates, terpenoids, sterols and saponins.

Table. No 8: Phytochemical screening of *Punica granatum*

Sl. No	Phytoconstituents Test	Result
1	Alkaloids	+
2	Glycosides	+
3	Phenolics	+ +
4	Flavones and flavonoids	+
5	Carbohydrates	+ +
6	Proteins and Amino acids	-
7	Terpenoids	+
8	Sterols	+
9	Saponins	+
10	Gums and Mucilage	-

Figure. No 9: phytochemical screening of *Punica granatum*

5.4. Hypotonic solution –induced hemolysis or membrane stabilizing activity

Table. No 9: Hypotonic solution –induced hemolysis or membrane stabilizing activity

Treatment	Concentration (µg/ml)	Absorbance at 540nm	% inhibition of hemolysis
Control	-	0.671	-
Pomegranate (P)	100	0.438	34.72
	200	0.428	36.21
	300	0.415	38.15
Kokum (K)	100	0.562	16.24
	200	0.531	20.86
	300	0.524	21.90
Banana (B)	100	0.572	9.9
	200	0.527	21.46
	300	0.519	22.65
Standard (Aspirin)	100	0.429	36.06
	200	0.420	37.4
	300	0.413	38.45
Combination (P: K: B) 300:200:200	100	0.421	37.25
	200	0.417	37.85
	300	0.414	38.30

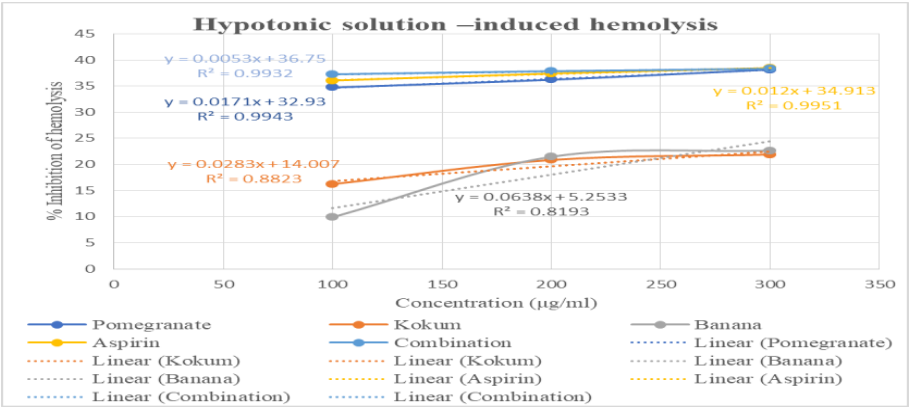

Figure. No 10: Hypotonic solution –induced haemolysis or membrane stabilizing activity line graph.

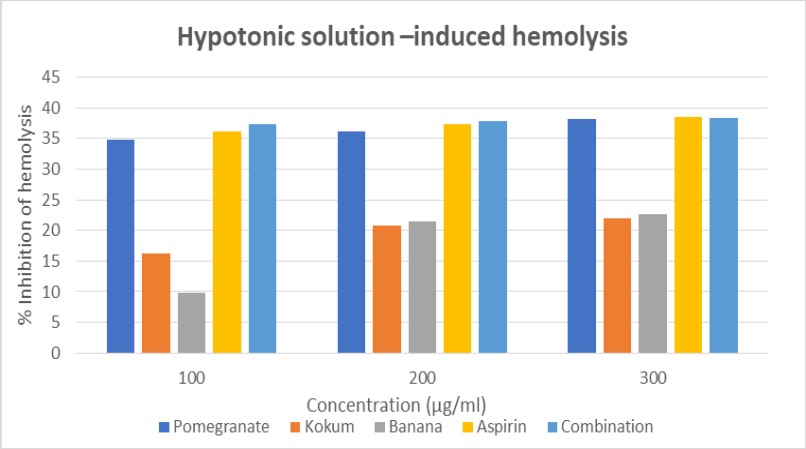

Figure. No 11: Hypotonic solution –induced haemolysis or membrane stabilizing activity bar graph.

5.5. Effect on Protein Denaturation

Table. No 10: Effect on Protein Denaturation

Treatment	Concentration (µg/ml)	Absorbance at 660nm	% inhibition of protein denaturation
Control	-	0.036	-
Pomegranate (P)	100	0.026	27.78
	200	0.017	52.78
	300	0.011	69.44
Kokum (K)	100	0.029	19.44
	200	0.027	25.00
	300	0.025	30.33
Banana (B)	100	0.034	5.56
	200	0.030	16.67
	300	0.029	19.44
Standard (Aspirin)	100	0.013	63.89
	200	0.010	72.22
	300	0.006	83.33
Combination (P: K: B) 300:200:200	100	0.011	69.44
	200	0.008	77.78
	300	0.007	80.50

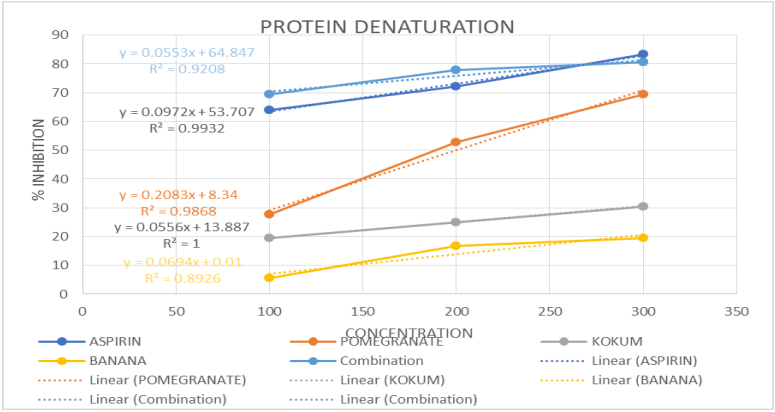

Figure. No 12: Effect on Protein Denaturation line graph

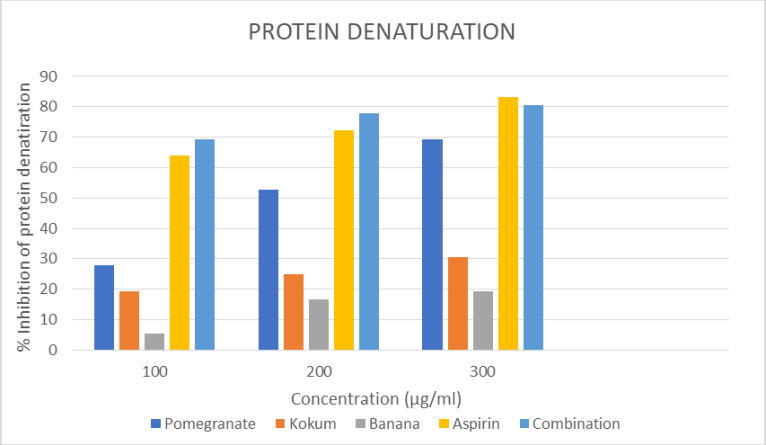

Figure. No 13: Effect on Protein Denaturation Bar graph

5.6. Proteinase Inhibitory Activity

Table. No 11: Proteinase Inhibitory Activity

Treatment	Concentration (µg/ml)	Absorbance at 660nm	% inhibition of protein denaturation
Control	-	0.820	-
Pomegranate (P)	100	0.605	26.21
	200	0.446	45.60
	300	0.287	65
Kokum (K)	100	0.608	25.85
	200	0.456	44.39
	300	0.297	63.78
Banana (B)	100	0.613	25.24
	200	0.459	44.02
	300	0.305	62.80
Standard (Aspirin)	100	0.592	27.80
	200	0.439	46.46
	300	0.282	65.60
Combination (P: K: B) 300:200:200	100	0.590	28.04
	200	0.437	46.70
	300	0.283	65.48

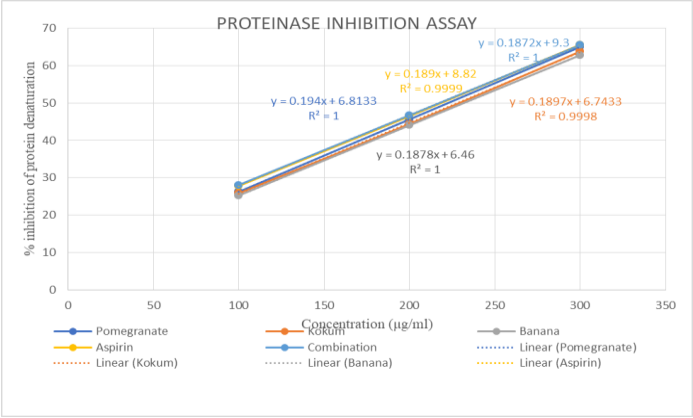

Figure. No 14: Proteinase Inhibitory Activity line graph.

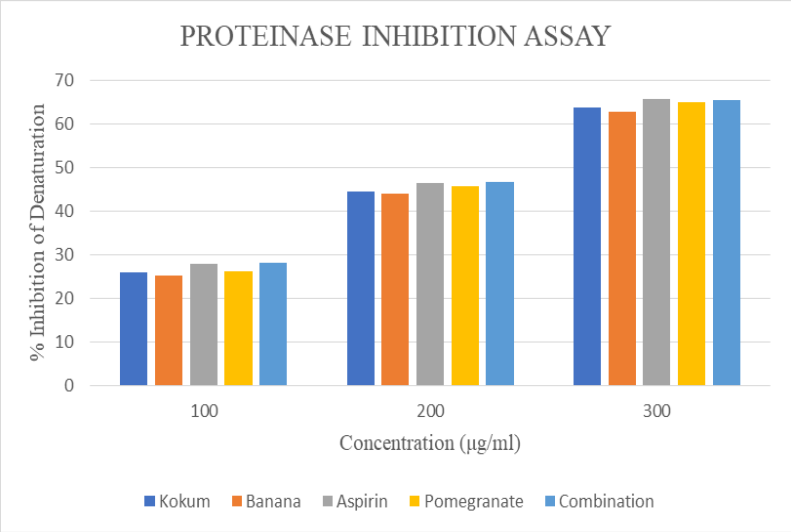

Figure. No 15: Proteinase Inhibitory Activity bar graph

6. DISCUSSION

Anti-inflammatory activity of the polyherbal extract prepared from the peels of Garcinia indica, Musa paradisiaca, and Punica granatum is evaluated using in-vitro methods such as hypotonic solution–induced hemolysis, protein denaturation, and proteinase inhibitory assays. Since they are closely related to membrane stabilization, preventing protein denaturation, and inhibiting proteolytic enzymes implicated in inflammatory processes, these models are frequently employed to evaluate anti-inflammatory potential.

Phytochemical analysis of the different peel extracts in this study reveals the presence of phenolics, flavonoids, glycosides, terpenoids, sterols, and saponins. The pharmacological effect of the polyherbal formulation may be attributed to these phytoconstituents, which are known to have strong anti-inflammatory and antioxidant qualities.

By comparing the results of the control, standard drug (aspirin), individual extracts, and the polyherbal combination, the following observations are noted:

- When compared to the control group, the polyherbal extract considerably stabilizes the erythrocyte membrane in a concentration-dependent manner, according to the hypotonic solution–induced hemolysis assay.
- Red blood cell membranes are well protected against lysis by the combination extract's membrane stabilizing function, which is similar to that of the common medication aspirin.
- Erythrocyte membrane stabilization implies that lysosomal membrane rupture is prevented, which in turn prevents the release of inflammatory mediators like phospholipases and proteases.

- The polyherbal extract significantly inhibits heat-induced protein denaturation in the protein denaturation assay, with increasing doses showing more inhibitory efficacy.
- The polyherbal formulation's suppression of protein denaturation is comparable to that of the conventional medication, indicating that it may have a role in reducing inflammation brought on by denatured proteins, as is the case with diseases like arthritis.
- The polyherbal extract significantly inhibits the activity of proteolytic enzymes in the proteinase inhibitory experiment, suggesting less tissue damage during inflammatory circumstances.
- A synergistic impact among the phytoconstituents is suggested by the combination extract's increased activity when compared to separate plant extracts.

The combination of phenolic chemicals and flavonoids, which are known to suppress inflammatory mediators and stabilize biological membranes, may be responsible for the polyherbal formulation's reported anti-inflammatory efficacy. The chosen plant extracts work in concert to improve the formulation's overall efficacy.

Thus, the present study demonstrates that the polyherbal peel extract possesses significant *in-vitro anti-inflammatory activity* comparable to the standard drug. These findings support the traditional use of the selected plants in inflammatory conditions. However, further *in-vivo studies and toxicity evaluations* are necessary to confirm its safety and therapeutic potential.

7. SUMMARY AND CONCLUSION

The peels of *Garcinia indica*, *Musa paradisiaca*, and *Punica granatum* are collected, authenticated, dried, powdered, and subjected to extraction using suitable solvents. The extracts are evaluated for their *in-vitro anti-inflammatory activity* using standard experimental models such as hypotonic solution–induced hemolysis, protein denaturation, and proteinase inhibitory assays. Aspirin is used as the standard drug for comparison.

Important bioactive components such phenolics, flavonoids, glycosides, terpenoids, sterols, and saponins are present in the peel extracts, according to preliminary phytochemical screening. These phytoconstituents are thought to be the cause of the pharmacological activity seen in this investigation because of their well-established anti-inflammatory and antioxidant qualities.

The polyherbal peel extract shows notable effectiveness in a concentration-dependent manner, according to the results of the in-vitro anti-inflammatory tests. When compared to the control group, the formulation exhibits effective membrane stabilizing function, inhibition of protein denaturation, and suppression of proteolytic enzyme activity. It is discovered that the polyherbal extract's activity is similar to that of the common medication aspirin. When compared to separate extracts, the combination extract shows increased activity, indicating a synergistic interaction between the phytoconstituents.

The current study shows that the polyherbal peel extract has strong anti-inflammatory properties in vitro. It may work by stabilizing biological membranes, preventing protein denaturation, and inhibiting inflammatory enzymes. The idea of adding value to agricultural waste is further supported by the use of fruit peels.

Thus, the results of this study indicate that the polyherbal formulation could be a viable natural anti-inflammatory medication. To verify its safety, effectiveness, and therapeutic usefulness, however, more in-vivo research, toxicity assessment, active component isolation, and pharmacological studies are needed.

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