2025 Volume: 01 Issue: 07 www.ijrpa.com ISSN 2456-9995 Review Article

International Journal Research Publication Analysis

Page: 01-13

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

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Article Received: 21 November 2025
Article Revised: 11 December 2025
Published on: 31 December 2025

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DOI: https://doi-doi.org/101555/ijrpa.1622

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 referred to as pharmacology. More specifically, it is the study of the interactions that occur between a living organism and chemicals that affect normal or abnormal biochemical function.[1][2] If substances have therapeutic properties, they are categorized as pharmaceuticals. The field encompasses drug composition and properties, functions, sources, synthesis and drug design, molecular and cellular mechanisms, organ/systems mechanisms, signal transduction/cellular communication, molecular diagnostics, interactions, chemical biology, therapy, and medical applications, and antipathogenic capabilities. The two principal areas of pharmacology are pharmacodynamics and pharmacokinetics. Pharmacodynamics deals with the effects of a drug on biological systems, and pharmacokinetics deals with the effects of biological systems on a drug. In broad terms, pharmacodynamics discusses the chemicals with biological receptors, and pharmacokinetics discusses the absorption, distribution, metabolism, and excretion (ADME) of chemicals from the biological systems.[3] Humans have been aware of their need on nature for a healthy existence from the beginning of time, and they have used a range of plant resources as medicine to treat a wide range of illnesses.[4] By investigating a variety of biologically active natural products, this indigenous knowledge, that has been handed 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.[5] This work is based on the anti-inflammatory activity of Garcinia indica, *Punica granatum, Musa paradisiaca* belonging to the family Clusiaceae, Lythraceae and Musaceae respectively. They have the pharmacological activity such as the antioxidant, anticancer, and antiviral, anti-inflammatory activity due to the presence of the chemical constituents such as garcinol, Ellagitannins, flavonoids, polyphenols and many others.

The study is focused on evaluating the anti-inflammatory activity of the polyherbal extract using the bovine blood which possess similarity the human blood. These herbs possess traditional background of being used as drug for the treatment of various inflammatory conditions as a monotherapy. Here we study the combination effect of herbs towards the inflammatory responses in human body.

INFLAMMATION

The biological reaction of bodily tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, comprises inflammation. Heat, discomfort, redness, swelling, and loss of function are the five cardinal signs. As inflammation is a universal reaction, it is considered as an innate immunity mechanism, while adaptive immunity is pathogen-specific.[6] It is a component of how bodily tissues react biologically to harmful stimuli like viruses, damaged cells, or irritants.[7] Immune cells, blood vessels, and chemical mediators are all involved in inflammation, which is a defensive reaction. Inflammation assists to remove injured cells and tissues, initiate tissue repair, and eradicate the initial cause of cell injury. [8] There are two types of inflammation: acute and chronic. The body's first reaction to damaging stimuli is acute inflammation, which is set 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 leads to progressive change in the type of cells present at the site of inflammation, such as mononuclear cells. In addition, cytokines and helper T cells (Th1 and Th2) used to categorize inflammation into Type 1 and Type 2.[9]

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. 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 prominent treatments like aspirin and ibuprofen, are the most prevalent class of anti-inflammatory drugs. Doctors frequently prescribe these medications to treat pain and inflammation caused 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 effectively reduce 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. [10]

Garcinia indica

Garcinia indica, a fruit-bearing tree with culinary, medicinal, and industrial applications, is a member of the mangosteen family (Clusiaceae). It may be referred as kokum. It grows mainly in the Western Ghats of India, especially in the states of Kerala, Goa, Karnataka, and Maharashtra. It is considered as an endemic species of India's woodlands and Western Ghats.[11] The plants can be observed all across the world, including Western Polynesia, Africa, and tropical Asia.[12] 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 potential in the treatment and prevention of chronic illnesses that are not communicable.[13] 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 range of physiologically active metabolites.[14] [15] These substances have been linked to biological processes like antiviral, antioxidant, and anticancer properties.[16]

Plant profile:

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 [17]

Musa paradisiaca

Formerly a hybrid of Musa acuminata and Musa balbisiana, Musa × paradisiaca is both a species and a cultivar that humans grew and tamed relatively early. Most of cultivated plantains and bananas are either polyploid cultivars of M. acuminata alone or of this

hybrid.[18] The fruits, along with other parts of the plant such the stem, peel, pulp, and leaves, serve in traditional medicine to cure a variety of human ailments.[19]

Plant profile:

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

Vernacular name:

- Hindi: Kela
- Kannada: Balehannu
- Malayalam: Kadalivala, Vazha [20]

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.[21] Pomegranate peel is rich in catechins, prodelphinidins, condensed tannins, and polyphenols.[22] The peel's greater phenolic content produces extracts that can be used as food preservatives and dietary supplements.[23]

Plant profile:

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

Vernacular name:

- Hindi: Anar, Anardana
- Tamil: Madhulai
- Malayalam: Maathalam [24]

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.[25] They go by Tellicherry, Cutch, and Thalassery. 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.[26]

Comparison of the human blood with bovine blood:

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

Plant profile:

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

Vernacular name

Hindi: Bakra

Bengali: Khass

Tamil: Puliyaa [27] [28] [29]

AIM AND OBJECTIVE

AIM

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

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

2.MTERIALS AND METHODS

EXTRACTION OF DRUG

Extraction of Kokum Peel

70% ethanol 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.[30]

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 shook 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. [31]

2.3. Pomegranate peel extraction

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. [32]

Drugs:

1. Test drug: polyherbal extract of peels of *Garcinia indica*, *Musa paradisiaca*, *Punica granatum*

Standard drug: Aspirin powder

Membrane stabilizing activity or hemolysis caused by hypotonic solution:

With a few minor adjustments, this test was conducted using the procedure outlined in Shinde et al. (1999) [33]. 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.

% 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.[34]

Impact on Protein Denaturation: With a few minor adjustments, protein denaturation was carried out as outlined in Elias et al. (1988) [35]. 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.[36]

Proteinase Inhibitory Activity: Proteinase inhibitory activity was performed according to the method of Sakat et al. 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. The control was a phosphate buffer solution.

% 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.[37]

3. CONCLUSION

Using bovine blood as a model system, the current study was conducted to assess the in vitro anti-inflammatory activity of a polyherbal extract made from the peels of Garcinia indica, Musa paradisiaca, and Punica granatum. Since inflammation is a major contributing factor to many chronic illnesses, finding safer and more effective herbal substitutes is crucial from a therapeutic standpoint.

The polyherbal extract's capacity to stabilize erythrocyte membranes, prevent protein denaturation, and reduce proteinase activity all demonstrated significant anti-inflammatory activity. The bioactive phytoconstituents found in the chosen plant materials, such as flavonoids, polyphenols, tannins, and other antioxidant compounds, may be responsible for these effects.

The results of this study indicate a synergistic effect when used in combination and validate the traditional use of these plants in inflammatory conditions. According to the findings, the polyherbal formulation has anti-inflammatory properties that are as promising as those of conventional medications like aspirin.

To verify the effectiveness, safety, and mode of action of this polyherbal extract, more in vivo research, toxicity assessments, and clinical studies are necessary. All things considered, the study offers a scientific foundation for the creation of herbal anti-inflammatory formulations with fewer side effects.

4. ACKNOWLEDGEMENT

I would like to sincerely thank my esteemed mentor for all of their helpful advice, unwavering support, and helpful suggestions during this project. Their knowledge and assistance were vital to this study's successful conclusion.

I am grateful to the department head and all of the pharmacology faculty members for providing the facilities and academic support needed to complete this research project.

Additionally, I want to thank the lab personnel for their collaboration and support throughout the experimental processes. The completion of the in vitro studies was made possible by their technical assistance.

Lastly, I want to express my sincere gratitude to my friends and family for their unwavering patience, moral support, and encouragement during the project.

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