

In vitro anti-inflammatory activity of *Garcinia hanburyi* extract

J. Narayanan*, V. Chitra

ABSTRACT

Aim: *Garcinia hanburyi* belongs to the family Clusiaceae. In the present study, *G. hanburyi* has been selected to test for the anti-inflammatory activity by proteinase inhibitory activity, membrane stabilization, and albumin denaturation assay at various concentrations. **Materials and Methods:** The whole plant was dried, extracted to obtain Xanthone rich extract. Phytochemical studies of extract showed the presence of phenols, alkaloids, steroids, resins, and saponins. The natural sources such as plants are an abundant source having various medicinal properties. **Results and Discussion:** The results showed that the extract from *G. hanburyi* at a concentration from 100 to 500 µg/ml protects protein denaturation. Significant proteinase inhibitory activity was seen at the concentration of 400 µg/ml and 500 µg/ml. Heat-induced hemolysis of erythrocyte was considerably inhibited at the concentrations of 400 µg/ml. **Conclusion:** The results which are obtained from the present study shows that the extract of *G. hanburyi* can be a promising resource of anti-inflammatory agents.

KEY WORDS: Anti-Inflammatory, *Garcinia hanburyi*, Human red blood cell, Membrane stabilization, Xanthones

INTRODUCTION

Inflammation is an adaptive response which involves an increase in the vascular permeability, increase in the protein denaturation and causes membrane alteration. Damage can be caused due to physical agents, chemical agents, stress, and microbes. Inflammation is caused from the response to stress. This response is characterized by pain, heat, swelling, and redness around the inflamed region.

Inflammation forms the basis of several processes such as physiological and pathological. The response of tissue in case of injury is the release of kinins, histamine, and prostaglandins. The inflammatory cascade is made up of a complex network consisting of physiological, behavioral, and immunological events.

This causes vasodilation and an enhanced permeability of the capillaries leading to an increase in the blood flow to the site of injury.^[1] The inflammatory mediators also behave as chemical messengers which causes attraction of body's defensive cells by a process known

as chemotaxis. Acute and chronic inflammation is the two types of inflammation. Response of the body, when there is an initial harmful stimuli, leads to acute inflammation. It is from the increased movement of leukocytes and plasma from blood to the site of tissue injury. This leads to inflammatory response through various biochemical events. Chronic inflammation or prolonged inflammation causes a change in the nature of cells present at the inflammatory region. This leads to a destruction of the tissue and proceeds to heal the damaged site.^[2] The management of inflammation can be done using various methods. Utilization of natural sources to treat various cases of inflammation is a better therapeutic use than with synthetic drugs. It offers various advantages such as less adverse effects, higher potency, and avoids many of the limitations of synthetic drugs.^[3]

Garcinia hanburyi belongs to the family Clusiaceae. It is an evergreen tree with gray smooth bark and exudes a yellow gum resin.^[4] The plant has an emetic and purgative action. It has been used in traditional Thai medicine. The plant is proven to show various activities such as anti-tumor and cytotoxic activity.^[5]

The literature survey was conducted and it was seen that there has been no evaluation of the anti-inflammatory

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Department of Pharmacology, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, Tamil Nadu, India

*Corresponding author: J. Narayanan, Department of Pharmacology, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, Tamil Nadu, India. Phone: +91-9600094814. E-mail: akashnara07@gmail.com

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potential of *G. hanburyi*. The aim and core objective of the study was for the screening of the extract of *G. hanburyi* for the anti-inflammatory potential. Herbal sources, phytomedicines are essential natural resources to be used as a therapeutic tool. Many parts of the world still utilize traditional medicine to treat various diseases and disorders. Synthetic anti-inflammatory agents including Nonsteroidal Anti-inflammatory Drugs often have undesirable side effects and sometimes fatal effects too. Natural anti-inflammatory compounds can be used for long-term treatment and with lesser side effects. Therefore, the present investigation was conducted to determine anti-inflammatory activity of *G. hanburyi* by albumin denaturation assay, anti-proteinase activity, and membrane stabilization.

MATERIALS AND METHODS

Plant Materials

The plant materials are procured from Rajesh Chemicals, Mumbai. It is authenticated by Prof. P. Jayaraman, PARC - Plant Anatomy Research Center, Tambaram. The registration number of the certificate is PARC/2017/3577.

Preparation of Extract of *G. hanburyi*

Extracts were prepared by the maceration method at 60°C for 72 h using ethanol 70% and ethyl acetate. The extracted samples were evaporated using water bath. The thick extract obtained is made into various concentrations for the study (5, 25, 100, and 200 µg/l)

Preliminary Phytochemical Analysis

The plant extracts were used for preliminary screening of active constituents such as proteins, steroids, carbohydrates, phenols, alkaloids, resins, and saponins.^[6] The phytochemical analysis was performed using procedures from the literature to investigate the constituents in the plant Table 1.^[7,8]

In Vitro Anti-inflammatory Activity Determination

Inhibition of albumin denaturation

The protein denaturation assay for *G. hanburyi* was performed by the method of Muzushima^[9] and Sakat *et al.* The procedure was conducted with a few slight modifications.^[10] The reaction consisted of a mixture with 100 µl of the test solutions, 100 µl of BSA (Bovine Serum Albumin 5%). pH was adjusted with the help of glacial acetic acid. The test tubes are then incubated for 20 min at 37°C. It was heated for 10 min at 70°C. The tubes were allowed to be cooled for 10 min, and the turbidity measurement was carried out at 660 nm. Distilled water and the sample were used as a blank. Aspirin was used as a positive control.

% inhibition of protein denaturation was calculated by:

$$\% \text{ inhibition} = \frac{\left(\begin{array}{l} \text{Absorbance of Control} \\ - \text{Absorbance of Sample} \end{array} \right)}{\text{Absorbance of control}} \times 100$$

Anti-proteinase Activity

Proteinase inhibitory action was performed by the method of Oyedepo and Femurewa.^[11] The procedure was conducted with slight modifications. The reaction utilized a mixture with 0.06mg of trypsin, 1 ml of 20 mM Tris-HCl buffer. 1 ml of test extracts was prepared with different concentrations. The reaction mixture was incubated for 5 min at 37°C. 1 ml of casein (0.8%) was added and incubated for 20 min. 2 ml of perchloric acid (70%) was added for the reaction termination. The suspension obtained was centrifuged, and absorbance of the supernatant was taken 210 nm. Buffer was utilized as blank.

% inhibition of proteinase inhibitory activity calculated using:

$$\% \text{ inhibition} = \frac{\left(\begin{array}{l} \text{Absorbance of Control} \\ - \text{Absorbance of Sample} \end{array} \right)}{\text{Absorbance of control}} \times 100$$

Membrane Stabilization

Red blood cells (RBCs) suspension preparation^[10,12]

Fresh human blood was collected and taken in a centrifuge tube with heparin. The test tubes were centrifuged for 10 min at 3000 rpm. It was washed for 3 times with normal saline, and the volume of blood was measured. They are made up to 10% suspension using normal saline.

Heat-induced Hemolysis^[10,13]

Heat-induced hemolysis was performed using 1ml of test extracts along with 10% prepared RBC suspension. Aspirin was used a standard. Incubation was done at 56°C for a period of 30 min. Absorbance was read for the supernatant at 560 nm.

Percentage inhibition of Hemolysis calculated using:

$$\% \text{ inhibition} = \frac{\left(\begin{array}{l} \text{Absorbance of Control} \\ - \text{Absorbance of Sample} \end{array} \right)}{\text{Absorbance of control}} \times 100$$

Hypotonicity-induced Hemolysis^[14]

Varying concentrations of the extract ranging from 100 to 500 µg/ml along with the reference sample and control were used. Diclofenac sodium was the standard used. It is mixed along with 2 ml of hyposaline, 1ml of phosphate buffer along with 0.5 ml of human RBC (HRBC) suspension. The mixtures are then incubated for 30 min at 37°C. It is then centrifuged at 3000 rpm and the resulting supernatant was obtained. The

supernatant was decanted and then absorbance was read at 560 nm.

% hemolysis protection was estimated by:

$$\% \text{ Protection} = 100 - (\text{OD sample} / \text{OD control}) \times 100.$$

Statistical Analysis

The results are expressed by Mean±standard deviation. GraphPad was used for statistical analysis by Dunnett's Multiple Comparison method and One-Way ANOVA.

RESULTS

Inhibition of Albumin Denaturation

Investigation was performed to analyze the anti-inflammatory activity of the plant extract. It was found that the ethyl acetate extract of *Garcinia hanburyi* (EAGH) was effective in inhibiting protein denaturation [Table 2]. The maximum inhibitory value of 57.14% at a concentration of 400 µg/ml. The standard used for the comparison of anti-inflammatory activity showed a percentage inhibition of 68.57% at 100 µg/ml compared with that of the control.^[15]

Proteinase Inhibitory Action

The ethyl acetate extract of the plant showed significant anti-proteinase activity when it was studied at different concentrations [Table 3]. A maximum inhibitory value of 48.71% was seen at 500 µg/ml. Aspirin used as the standard showed a maximum percentage inhibition of 64.10% at 100 µg/ml compared with that of the control.^[16]

Membrane Stabilization

Heat-induced hemolysis

Different concentrations of the extract were utilized to inhibit the heat-induced hemolysis [Table 4]. A maximum inhibitory value of 65.62% was seen at 500 µg/ml. Aspirin used as the standard showed a maximum percentage inhibition of 71.87% at 100 µg/ml compared with that of the control.^[17]

Table 1: Preliminary phytochemical analysis of *G. hanburyi*

Phytoconstituents	<i>G. hanburyi</i>
Tannins	+
Flavonoids	+
Terpenoids	+
Carbohydrates	+
Saponins	+
Steroids	-
Phlobatannins	-
Glycosides	+
Coumarins	-
Alkaloids	+
Proteins	+
Anthraquinones	-
Anthocyanins	+
Emodins	-

G. hanburyi: *Garcinia hanburyi*

Hypotonicity-induced Hemolysis

The results of the hemolysis assay showed that the extract at a concentration of 500 µg/ml had a percentage inhibition of 65.62% [Table 5]. This showed that the extract is useful for protection of the erythrocyte membrane. The erythrocyte membrane is lysed with the hypotonic solution for this activity. Diclofenac was used as the standard. It showed the maximum inhibition of 71.87% at 100 µg/ml compared with that of the control.^[18]

DISCUSSION

In the present study, the anti-inflammatory activity of *G. hanburyi* which included *in vitro* assays such as albumin denaturation assay, anti-proteinase activity, and membrane stabilization assay. For several years, effective novel anti-inflammatory drugs were introduced such as valdecoxib and celecoxib. Most of the drugs were removed because of their serious cardiovascular systemic side effects. There has been a rise in discovering new anti-inflammatory medications which are the need of the hour. These discoveries should be focusing on the aim to reduce the side effects of the drugs as well as improved therapeutic index.

Protein denaturation has been a documented cause for inflammation. Various other inflammatory drugs which include phenylbutazone and salicylic acid have been shown for thermal induction of protein denaturation. The extracts can be used for inhibition for release of lysosomal contents. This is from the neutrophils at the inflammatory sites which include bactericidal enzymes and proteinases. These constituents lead to tissue inflammation and damage.^[19]

Proteinases have been involved in arthritic reactions. A source of proteinases is neutrophils which carries lysosomal granules.^[20] These are involved in an important role for the development of tissue damage which arises during inflammatory reactions.^[9] Various studies have implicated the role of polyphenols and flavonoids which prevent these inflammatory mediators.^[21] The presence of these phytoconstituents in the plant undertaken for the study may be responsible for the anti-inflammatory properties.

These findings of the study support the use of traditional medicine for the inflammatory disorders as well as showing a promising potential to be used as an anti-inflammatory agent. The plant by the help of the *in vitro* analysis appears to be an avenue to explore as a source of anti-inflammatory drugs. The results of the current study will lead to the strengthening of the process for the utilization of botanicals as a therapeutic agent. The compounds present in the plant as well as isolated products may not be served as a drug but can be utilized for the development of novel class of therapeutic agents.

Table 2: % Inhibition of heat-induced protein denaturation

Groups	Concentration (µg/ml)	Absorbance	% inhibition
Control		0.35±0.04	-
EEGH	100	0.24±0.06**	31.42
EEGH	200	0.19±0.02**	45.71
EEGH	300	0.20±0.04**	42.85
EEGH	400	0.13±0.04**	62.85
EEGH	500	0.15±0.05**	57.14
ASPIRIN	100	0.11±0.06**	68.57

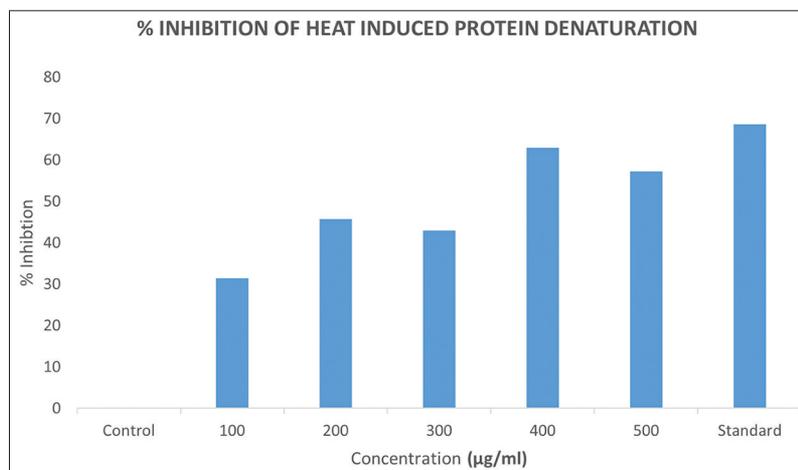
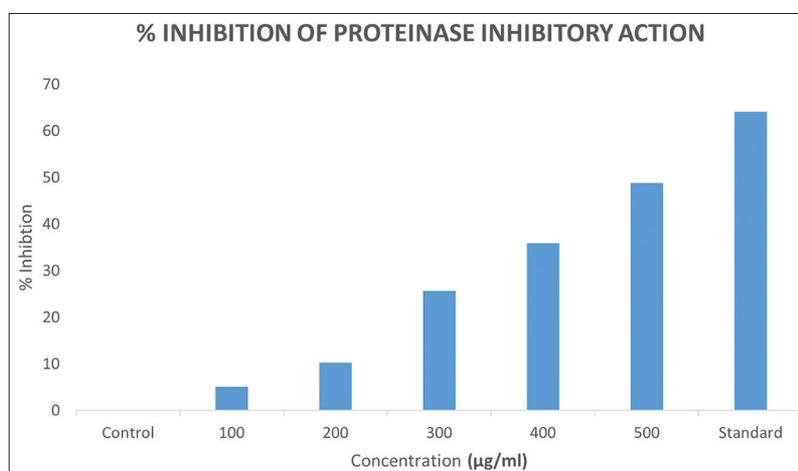


Table 3: Percentage inhibition of proteinase inhibitory action

Groups	Concentration (µg/ml)	Absorbance	% inhibition
Control		0.39±0.05	-
EEGH	100	0.38±0.09**	5.1
EEGH	200	0.37±0.02**	10.2
EEGH	300	0.29±0.07**	25.64
EEGH	400	0.25±0.01**	35.89
EEGH	500	0.20±0.03**	48.71
Aspirin	100	0.14±0.01**	64.10

RBC: Red blood cells



CONCLUSION

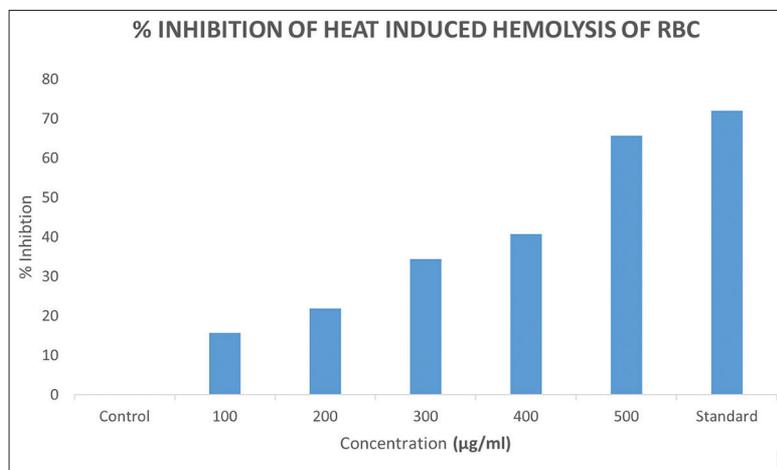
The results which are obtained from the anti-inflammatory studies using the EAGH have shown that the extract has a therapeutic potential to be used as an anti-inflammatory agent. The EAGH extract inhibits various inflammatory parameters such as inhibition of albumin denaturation,

proteinase action, heat-induced, and hypotonicity-induced hemolysis. Therefore, the EAGH can be more thoroughly investigated for its anti-inflammatory activities which are related to various diseases and disorders. Further studies should be conducted to select EAGH as a therapeutic agent for their anti-inflammatory activity with the help of *in vivo* and *in vitro* studies.

Table 4: Percentage inhibition of heat-induced hemolysis of RBC

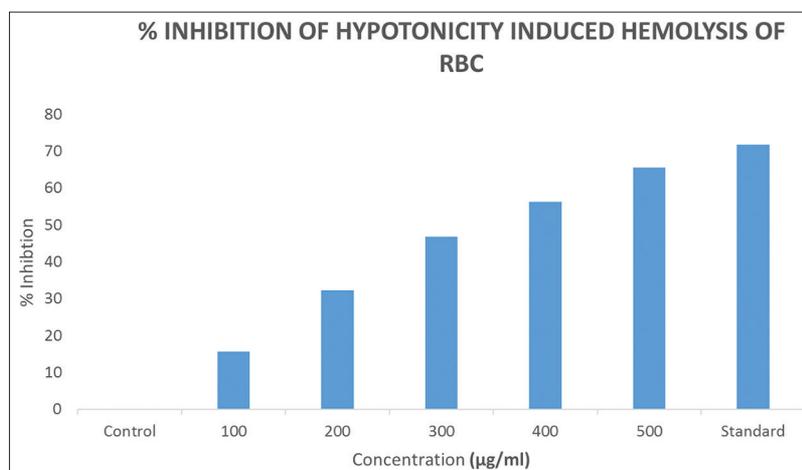
Groups	Concentration ($\mu\text{g/ml}$)	Absorbance	% inhibition
Control		0.32 \pm 0.01	-
EEGH	100	0.27 \pm 0.06**	15.6
EEGH	200	0.25 \pm 0.07**	21.8
EEGH	300	0.21 \pm 0.05**	34.37
EEGH	400	0.19 \pm 0.01**	40.62
EEGH	500	0.1 \pm 0.03**	65.62
Aspirin	100	0.09 \pm 0.06**	71.87

RBC: Red blood cells

**Table 5: Percentage inhibition of hypotonicity-induced hemolysis of RBC**

Groups	Concentration ($\mu\text{g/ml}$)	Absorbance	% inhibition
Control		0.32 \pm 0.03	-
EEGH	100	0.27 \pm 0.02**	15.62
EEGH	200	0.21 \pm 0.09**	32.25
EEGH	300	0.15 \pm 0.04**	46.87
EEGH	400	0.14 \pm 0.07**	56.25
EEGH	500	0.11 \pm 0.02**	65.62
DICLOFENAC	100	0.09 \pm 0.01**	71.87

RBC: Red blood cells



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