

## Original Research Article

**Evaluation of Pharmacological Activity of Hydroethanolic Extract *Coccinia grandis* Linn. Leaves**Tandrima Majumder<sup>1\*</sup>, K. Nagaraju<sup>1</sup>, K. Sreelekha<sup>1</sup>, M. Nikitha<sup>1</sup><sup>1</sup>Anurag Pharmacy College, Kodad, Telangana-508206**\*Corresponding author**

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**Abstract:** *Coccinia grandis* L. Voigt, commonly called the ivy gourd, is a perennial herb or a vine found extensively from Africa to Asia. It is also known as *Coccinia indica*. The leaves of this plant were used to treat diabetes. The studies showed that the leaves of the plant possess antioxidant properties and produce analgesic, anti-pyretic and anti-inflammatory effect in rats. In the methodology, hydroethanolic extract of the dried leaves of *Coccinia grandis* L. i.e. was prepared and evaluated for solubility and phytochemical studies and finally in-vitro haemolytic, anti arthritic & anti inflammatory activity was carried on.

**Keywords:** *Coccinia grandis* L., haemolytic activity, anti-inflammatory, anti-arthritic.

**INTRODUCTION**

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. Medicinal plants are the “backbone” of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis [1-2]. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed by UNESCO, 1996 [3]. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and anti-malarial medications, contain ingredients from plants. Substances found in medicinal plants are known as the active principles. These compounds have been extracted and used in different forms such as infusions, syrups, decoctions, infused oils, essential oils and creams.

*Coccinia grandis* L. Voigt, commonly called the ivy gourd, is a perennial herb or a vine found extensively from Africa to Asia. It is also known as *Coccinia indica*. The plant belongs to the family Cucurbitaceae. The fruits, roots, stem of this plant were used traditionally to treat diseases like leprosy, jaundice, asthma, bronchitis, skin eruptions, burns, tongue sores, ear ache, indigestion, eye infections, nausea, insect bites and fever. The tender green fruits

were cooked and eaten, sometimes also raw. The leaves of this plant were used to treat diabetes [4-5]. The fruits of the plant were used in treatment of diabetes [6]. The studies showed that the leaves of the plant possess antioxidant properties [7] and produce analgesic, anti-pyretic and anti-inflammatory effect in rats [8]. Several biochemical studies have been performed to reveal the medicinal uses of the plant-parts. The leaf constrain also found as hypoglycemic, hypolipidemic and antioxidant activity [9]. The fruit of this plant is ovoid in shape berry type which changes green to red color when become ripen. *Coccinia grandis* contain important raw material for drug production like bioactive compounds such as secondary metabolite like alkaloids, glycoside and saponin, b- amyryne, lupeol, cucubbitacin, cephalandrol, cephalandrine and flavonoids.

**Vernacular names [9-10]**

Marathi: Tindora (Tindori, Tindora);

Oriya: Parwal, Kundru, Tondi;

Malayalam: Tendli

Konkani : Ghiloda, Kundri, Kowai, Kovai, Kovakkai;

Tamil: Kovakka;

Telugu: Dondakaya;

Kannada: Tondekayi.

Chinese: Hong Qua

Japanese: Yasai, karasuuri

Malay: Pepasan, Kovakka, Kovai

Spanish: Pepino, cimaron

## MATERIALS AND METHODS

To get the desired result, methods are needed for separation, purification and identification of many different constituents present in plants. Thus advances in our understanding of phytochemistry are directly related to the successful exploitation of known techniques, and the continuing development of new techniques to solve outstanding problems as they appear. As a result of modern extraction, and isolation techniques and pharmacological testing procedures, new plant drugs usually find their way into medicine as purified substances rather than in the form of galenical preparations [11].

### Collection of plant materials

The leaves of the climber plant of *Coccinia grandis* were collected from the village Kodad, Dist. Suryapeta, Telangana-508206. The leaves were authenticated by Department of Pharmacognosy, Anurag Pharmacy College.

### Drying and Grinding

The collected plant parts leaves were separated from undesirable materials or plants or plant parts. They were dried under sun for three weeks. The plant parts were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 200 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 1000 ml of 1:1 equimolar amount of hydroethanolic solvent. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper. (Bibby RE200, Sterilin Ltd., UK). The hydroethanolic extract was evaporated at the normal room temperature and the thick concentrate was stored in the dessicator. It rendered a gummy concentrate of greenish color. The extracts were then subjected to solubility studies and phytochemical screening. Six different solvents were taken and solubility studies were carried out. Results are shown in Table No. 1.

Testing of different chemical groups that are present in the extract, represent the preliminary studies. The chemical group tests, which are performed as follows in each test 10% (w/v) solution of extract in methanol was taken unless otherwise mentioned in individual test.[12] Results are tabulated in the Table No. 2.

### Test for Alkaloids

#### Mayer's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent was added.

### Dragendroff's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendroff's reagent was added.

### Tests for Glycosides

A small amount of an alcoholic extract of the fresh or dried plant material was taken in 1 ml of water. Then, a few drops of aqueous sodium hydroxide were added. A yellow color is considered as an indication for the presence of glycosides.

### Test for Steroids

#### Salkowski Reaction

To 2 ml of extract, add 2ml chloroform and 2 ml conc. Sulphuric acid. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

### Test for Saponins

1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. One centimeter layer of foam indicates the presence of saponins.

### Test for Aminoacids

Heat 3 ml of test solution and 3drops 5% Ninhydrin Solution in boiling water bath for 10 mins. Purple or bluish colour appears indicates the presence of amino acids.

### Tests for reducing sugar

#### Benedict's test

0.5 ml of aqueous extract of the plant material was taken in a test tube. 5 ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously.

### Fehling's Test (Standard Test)

2 ml of an aqueous extract of the plant material was added to 1 ml of a mixture of equal volumes of Fehling's solutions A and B & was boiled for few minutes.

### Tests for tannins

#### Ferric Chloride Test

5 ml solution of the extract was taken in a test tube. Then 1 ml of 5% Ferric chloride solution was added.

### Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. Immediate development of a red color indicates the presence of Flavonoids.

### **IN-VITRO PHARMACOLOGICAL ACTIVITIES** **Haemolytic Activity**

Haemolytic activity of any compounds is an indicator of general cytotoxicity towards normal healthy cells. Usually, saponins (a group of phytochemical) present in the plants showed haemolytic activity by creating changes in the erythrocyte membrane. In vitro haemolytic assay by spectroscopic method provides an easy and effective method for the quantitative measurement of hemolysis. This method provides the evaluation of the effect of different concentrations of biomolecules on the human erythrocytes. Many plants contain chemical substances that might have a hemolytic or anti-hemolytic effect on human erythrocytes. Several reports indicate that the membranes of human erythrocytes from blood types have varying stability as determined from the mean corpuscular fragility [13-15].

#### **Preparation of erythrocytes suspension**

Five millilitres of blood was collected from a healthy individual (blood group O positive) in a tube containing heparin. The blood was centrifuged at 1500 rpm for three minutes in a laboratory centrifuge. Plasma (supernatant) was discarded and the pellet was washed three times with sterile phosphate buffer saline solution (pH 7.2±0.2) by centrifugation at 1500 rpm for 5 min. The cells were resuspended in normal saline to 0.5%.

#### **Haemolytic Activity [16]**

In vitro haemolytic activity was performed by spectrophotometer method [17]. A volume of 0.5 ml of the cell suspension was mixed with 0.5 ml of the plant extracts (250 and 500µg/ml concentrations in phosphate buffer saline). The mixtures were incubated for 30 min at 37°C in an incubator. The mixture was centrifuged at 1500 rpm for 10 min in a laboratory centrifuge. The free hemoglobin in the supernatant was measured in UV-Vis spectrophotometer at 540 nm. Two controls were prepared without extracts; negative control received sterile phosphate buffer saline, while positive control received 0.1% Triton X-100. The average value was calculated from triplicate assays. Each experiment was performed in triplicates at each concentration. The level of percentage hemolysis by the extracts was calculated according to the following formula:

$$\% \text{ Haemolysis} = \frac{A_t - A_n}{A_c - A_n} \times 100$$

Here:  $A_t$  = absorbance of test sample.

$A_n$  = absorbance of the Negative control

$A_c$  = absorbance of the Positive control

All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD). Results were analyzed statically by using Microsoft Excel 2007 (Roselle, IL, USA).

### **Anti-inflammatory Activity by HRBC Membrane Stabilising Method**

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. [18]

#### **Preparation of Suspension (10% v/v) of Human Red Blood cell**

The blood sample was collected from healthy human volunteer who has not taken any NSAID for 2 weeks prior to the experiment and transferred to heparinized centrifuge tube. Blood samples were centrifuged at 3000 rpm at room temperature for 15 min. The supernatant (plasma and leucocytes) were carefully removed while the packed red blood cell was washed with fresh normal saline (0.85% w/v NaCl). The process of washing and centrifugation were repeated five times until the supernatants were clear. Then, Human erythrocytes suspension (10% v/v) were prepared as reported by Oyedapo *et al.*, [19].

#### **Preparation of drug**

**Standard drug** Diclofenac Sodium, (50, 100, 250 and 500 µg/ml) and hydroethanolic, ethanolic extract of same concentrations (50, 100, 250 and 500 µg/ml) was prepared in isosaline (0.85% NaCl) to final the concentration.

#### **Assay of Membrane stabilizing activity**

The HRBC membrane stabilizing activity assay was carried out using 10% (v/v) Human erythrocyte suspension while Diclofenac Sodium was used as standard drugs. The assay mixtures consisted of 2 ml of hyposaline (0.25% w/v) sodium chloride, 1.0 ml of 0.15 M sodium phosphate buffer, pH 7.4, 0.5 ml of 10% (v/v) human erythrocyte suspension, 1.0 ml of drugs (standard and extracts) and final reaction mixtures were made up to 4.5 ml with isosaline. To determine the anti-inflammatory activity by HRBC membrane stabilization method, the following solutions were used.

**Test solution** (4.5ml) consists of 2ml of hypotonic saline (0.25% w/v), 1ml of phosphate buffer (pH7.4), 1ml of test extract (250µg/ml and 500 µg/ml) in normal saline and 0.5ml of 10% w/v human red blood cells in isotonic saline.

**Test control** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (pH 7.4) and 1ml of isotonic saline and 0.5ml of 10%w/v human red blood cells in isotonic saline.

**Standard solution** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (7.4pH) and 1ml of Diclofenac Sodium of various concentrations (250µg/ml and 500 µg/ml) and 0.5ml 10%w/v human red blood cells in isotonic saline. Drug was omitted in the blood control, while the drug control did not contain the erythrocyte suspension. The reaction mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant solution was measured spectrophotometrically at 560 nm. Each experiment was carried out in triplicate and the average was taken. The percentage inhibition of haemolysis or membrane stabilization was calculated using the following equation [20-23].

$$\% \text{ Inhibition of haemolysis} = 100 \times (A_1 - A_2 / A_1)$$

Where:  $A_1$  = Absorption of hypotonic buffered saline solution alone

$A_2$  = Absorption of test sample in hypotonic solution

All tests were conducted in triplicate. Data are reported as means  $\pm$  standard deviation (SD). Results were analyzed statically by using Microsoft Excel 2007 (Roselle, IL, USA).

### **In-Vitro Antiarthritic Activity**

Rheumatoid arthritis (RA) is a systemic autoimmune multisystem disease characterized by pain, synovial membrane inflammation, peripheral joint inflammation, morning stiffness, destruction of articular tissues and restricted joint movement [24-26]. Arthritis can cause severe disability and ultimately affects a person's ability to carry out everyday tasks, restricts the quality of life [27]. The various proinflammatory molecules including reactive oxygen species, prostaglandins, leukotrienes and cytokines released by macrophages are involved in the cause of this disorder [28-29]. The regulation of these mediators secreted by macrophages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like cox and Lox are the potential target for chronic inflammatory conditions [30] Even though various categories like immunosuppressants, NSAIDs, steroidal anti-inflammatory drugs are being used till now, they offer only temporary relief and produce severe side effects including gastrointestinal bleeding renal morbidity and cardiovascular toxicity.

The in-vitro antiarthritic activity was studied using bovine serum protein denaturation method [85] and Egg Albumin Denaturation Method [31-32]

### **Bovine Serum Protein Denaturation Method Preparation of Reagents 5% Bovine Serum Albumin (BSA):**

Dissolved 5gm of BSA in 100 ml of water.

### **Phosphate Buffer Saline PH 6.3:**

Dissolved 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na HPO<sub>4</sub>), 0.24 g of potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) in 800 ml 24 distilled water. The pH was adjusted to 6.3 using 1N HCl and make up the volume to 1000 ml with distilled water.

### **METHOD**

Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0.05ml of test solution of various concentrations. Test control solution (0.5ml) consists of 0.45ml of bovine serum albumin (5%w/v aqueous solution) and 0.05ml of distilled water. Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution. Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0.05ml of Diclofenac sodium of various concentrations (50, 100, 250 and 500µg/ml).

### **Procedure**

0.05 ml various concentrations (50, 100, 250 and 500µg/ml) of test drugs Hydroethanolic extract and standard drug Diclofenac sodium (50, 100, 250 and 500µg/ml) were taken respectively and 0.45 ml (5% w/v BSA) mixed. The samples were incubated at 37°C for 20 minutes and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 255 nm. The control represents 100% protein denaturation. The results were compared with Diclofenac sodium. The percentage inhibition of protein denaturation can be calculated as

### **Percentage Inhibition =**

$$100 - \left[ \frac{\text{Abs. of test solution} - \text{Abs. of product control}}{\text{Abs. of test control}} \times 100 \right]$$

Each experiment was done in triplicate and the average was taken.

### **Egg Albumin Denaturation Method**

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations (250µg/ml and 500µg/ml) of three extracts. A similar volume of double-distilled

water served as the control. Next, the mixtures were incubated at  $37 \pm 2^\circ\text{C}$  in a BOD incubator for 15 minutes and then heated at  $70^\circ\text{C}$  for five minutes. After cooling, their absorbance was measured at 660 nm by using the vehicle as a blank. Diclofenac sodium in the concentrations of  $250\mu\text{g/ml}$  and  $500\mu\text{g/ml}$  was used as the reference drug and treated similarly for the determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times [V_c - V_t / V_c]$$

Where,  $V_t$  = absorbance of the test sample,  $V_c$  = absorbance of control.

All tests were conducted in triplicate. Results were analyzed statically by using Microsoft Excel 2007 (Roselle, IL, USA).

## RESULTS AND DISCUSSIONS

The dried leaves of *Coccinia grandis* L. was macerated in 1:1 Hydroethanolic solvent for seven days and gummy extracts were obtained. The yield was found to be 6%.

The extracts were subjected to Solubility and Phytochemical studies. Solubility studies results shows that all the extract was completely soluble in all the solvents i.e. Ethanol, Chloroform, Diethyl ether, Dioxane, Dimethyl Sulphoxide, and also in water.

**Table-1: Solubility Studies of Hydroethanolic Extract (HE) of *Coccinia grandis* L. leaf extract**

Sl. No.	Solvents	Inferences
1.	Ethanol	HE
		Soluble
2.	Chloroform	Soluble
3.	Diethyl Ether	Soluble
4.	Dioxane	Soluble
5.	Dimethyl Sulphoxide	Soluble
6.	Water	Soluble

Phytochemical Studies reveals the presence of the chemical constituents which are the pharmacophores responsible for the presence of the pharmacological studies. The study revealed the

presence of carbohydrates, proteins and amino acids, flavanoids, saponins and alkaloids and tannins in the hydroethanolic extract of *Coccinia grandis* leaves but steroids are absent in the extract.

**Table-2: Phytochemical Screening of Hydroethanolic Extract of *Coccinia grandis* L.**

Sl.No	Phytochemicals	Tests	Observation	Results
1.	Carbohydrates	Benedict's Test	Brick color precipitate was formed	+
		Fehling's Test		+
2.	Proteins	Biuret Test	Pink colour Appears	+
3.	Amino Acids	Ninhydrin Test	Purple colour appears	+
4.	Steroids	Salkowski Test	No red or fluorescence appeared	-
5.	Tannins	$\text{FeCl}_3$ Test	Deep blue-black appeared	+
6.	Flavanoids	Shinoda Test	Purple colour appeared	+
7.	Saponins	Foam Test	Foam is observed	+
8.	Alkaloids	Mayers Test	Precipitate appears	+

### In-Vitro Haemolytic Activity

Hemolytic assays were performed because compounds possessing potent biological activity may not be useful in pharmacological preparations if they possess hemolytic effect. In addition, these data also may reveal some information about the mechanism of cytotoxicity. *In vitro* hemolytic activity on human erythrocytes of various concentrations extracts obtained from leaves of *Coccinia grandis* was performed. The total hemolysis was obtained using Triton X-100 (0.1%) and 0% hemolysis was obtained with buffer. Each concentration shows the mean of hemolysis percentage repeated in three experiments.

None of the concentration test of *Coccinia grandis* L. extract possesses any hemolytic activity against human erythrocytes. The effect of crude extracts of this species on blood erythrocyte membrane showed no changes in hemolytic activity when concentration varied from  $250\mu\text{g/ml}$  to  $500\mu\text{g/ml}$ . The hemolytic percentage 0.231% and 0.452% were obtained for a dose of  $250\mu\text{g/ml}$  and  $500\mu\text{g/ml}$  respectively. These values were statistically considered not significant when we compared with zero value ( $p > 0.05$ ).

**Table-3: Haemolytic Activity of the *C. grandis* leaves extract**

Sl. No.	Concentration (µg/ml)	% Haemolytic
1	50	0.225
2.	100	0.293
3.	250	0.354
4.	500	0.367

The erythrocyte model has been widely used as it presents a direct indication of toxicity of injectable formulations as well as general indication of membrane toxicity. Another advantage of erythrocytes model is that blood is readily available and that cells are easy to isolate from the blood; moreover, its membrane has similarities with other cell membrane [87]. Erythrocytes have been used as a model system by a number of workers for the study of interaction of drugs with membranes [33-35]. Hemolysis is due to red blood cells destruction which resulted from lysis of membrane lipid bilayer. This hemolysis relates to concentration and potency of extract. Furthermore the hemolytic activity of each extract is related to their chemical composition. *Coccinia grandis* has no effect and not affected stability of the erythrocyte membrane; these data suggested the non-toxic effect of the extract thus making it suitable for the preparation of drugs involved in the treatment of various diseases.

#### **In-Vitro Anti-inflammatory Activity by HRBC Membrane Stabilisation Method**

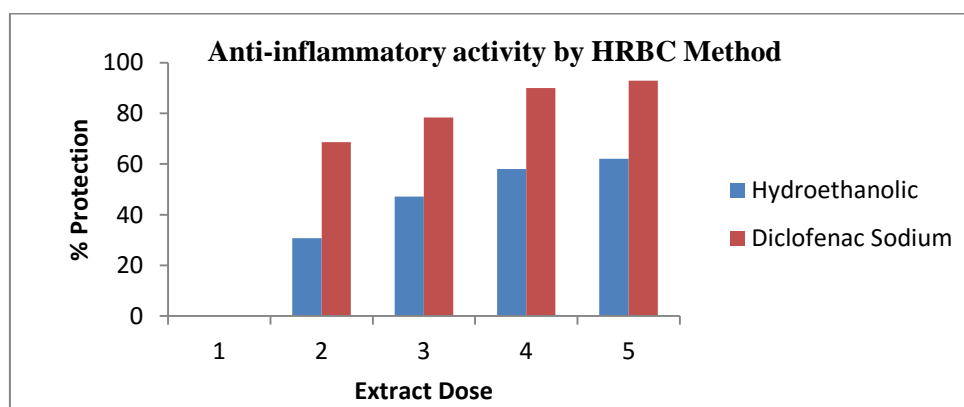
During inflammation, lysosomal hydrolytic enzymes are released into the site which causes damage of the surrounding organelles and tissues with

attendance variety of disorders [40]. Various methods were employed to screen and study drugs, chemicals, herbal preparations that exhibit anti-inflammatory properties or potentials. These techniques include uncoupling of oxidative phosphorylation (ATP biogenesis linked to respiration), inhibition of denaturation of protein, erythrocyte membrane stabilization, lysosomal membrane stabilization, fibrinolytic assays and platelet aggregation [36-39]. In the present study, stabilization of erythrocyte membranes exposed to both heat and hypotonic induced lyses was employed due to its simplicity and reproducibility.

The hydroethanolic extract of the leaves of *C. grandis* was studied for in vitro anti-inflammatory activity by HRBC membrane stabilization method. Four different concentration of the extract was used and hydroethanolic extract showed 30.7, 47.11, 58 and 62% protection of HRBC in hypotonic solution respectively. The results were compared with standard Diclofenac Sodium which showed 68.85, 78.41, 89.8 and 92.92 % protection at 50, 100, 250 and 500µg/ml concentrations. The extracts have shown positive response and dose-dependent response.

**Table-4: In-Vitro Anti-inflammatory Activity by HRBC method of the *C. grandis* leaves extract**

Sl.No.	Concentration (µg/ml)	Hydroethanolic	Diclofenac Sodium
1.	50	30.7	68.65
2.	100	47.11	78.41
3.	250	58	89.98
4.	500	62	92.92

**Fig-1: Anti-inflammatory activity by HRBC Method**

**In-Vitro Anti-arthritis Activity**

Denaturation of protein is one of the cause of rheumatoid arthritis was documented. Production of auto- antigen in certain arthritic diseases may be due to denaturation of protein. The mechanism of denaturation probably involves alteration in electrostatic hydrogen, hydrophobic and disulphide bonding. From the result of the present study, it can be stated that the hydroethanolic extract of *Coccinia grandis* leaves are capable of controlling the production of auto-antigen and thereby it inhibits the denaturation of proteins and

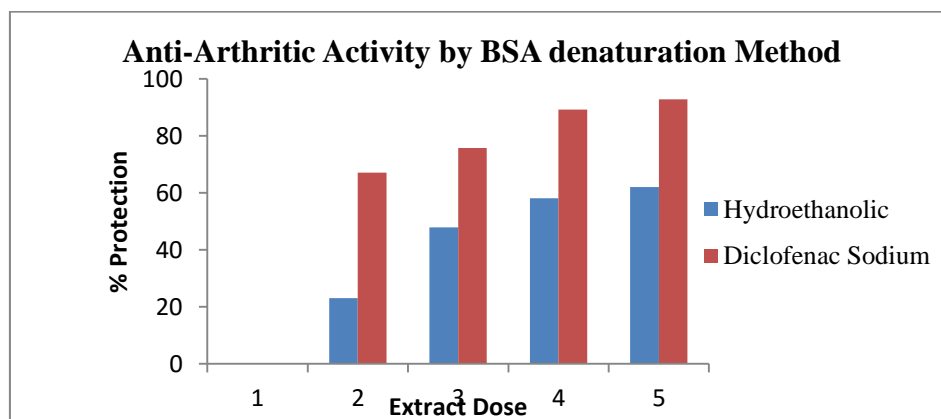
its effect was compared with the standard drug Diclofenac Sodium.

**BSA Denaturation Method**

The hydroethanolic extract showed 23, 47.87, 58.12, 62% protective activity and for standard Diclofenac sodium it was found to be 67.14, 75.69, 89.2, 92.8% protection at 50,100,250 and 500µg/ml. The extracts have shown positive and dose dependent response.

**Table-5: In-Vitro Anti-arthritis Activity *C. grandis* leaves extract by BSA Denaturation method**

Sl.No.	Concentration (µg/ml)	Hydroethanolic	Diclofenac Sodium
1.	50	23	67.14
2.	100	47.87	75.69
3.	250	58.12	89.2
4.	500	62	92.8



**Fig-2: Anti-Arthritis Activity by BSA denaturation Method**

**Egg-Albumin Denaturation Method**

The percentage protection of *C. grandis* leaves extract showed good protective activity against denaturation. The hydroethanolic extract showed 11.57,

28.69, 28.68, 32.58% protection while Standard Diclofenac Sodium showed 27.78, 37.41, 45.84 and 69.77 % protection against egg albumin denaturation at the 50, 100, 250 and 500 µg/ml concentrations. [40-41].

**Table-6: In-Vitro Anti-arthritis Activity *C. grandis* leaves extract by Egg Albumin Denaturation method**

Sl.No.	Concentration (µg/ml)	Hydroethanolic	Diclofenac Sodium
1	50	11.57	27.78
2.	100	28.69	37.41
3.	250	28.68	45.84
4.	500	32.58	69.77

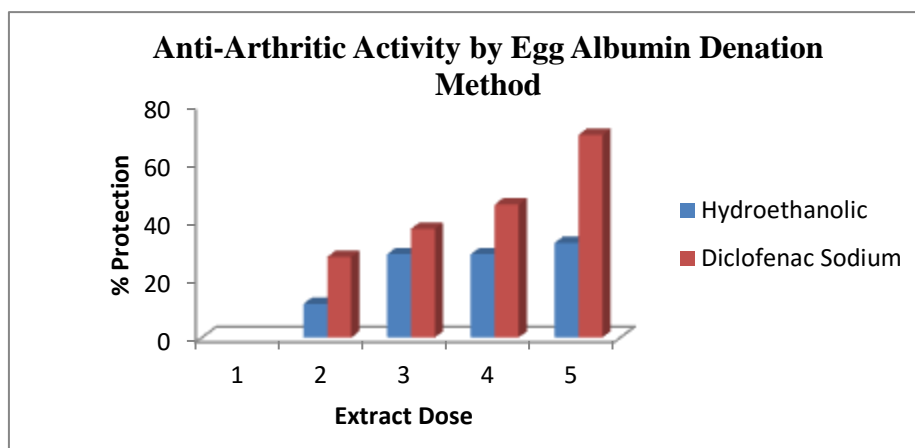


Fig-3: Anti-Arthritic Activity by Egg Albumin Denatation Method

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