

## Evaluation of anti-inflammatory and anti-arthritic activity by in vitro method on ethanolic extraction of *Polianthes tuberosa*

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### Abstract

Inflammation plays an important role in various diseases with high prevalence within populations such as rheumatoid arthritis, atherosclerosis and asthma. Since ancient times, in various cultures worldwide, Inflammatory disorders and related diseases have been treated with plants or plant-derived formulations. In this study, we explored the anti-inflammatory and anti-arthritic potential of the ethanolic extract of *Polianthes Tuberosa*. The plant material was extracted using the Soxhlet method, and preliminary analysis showed it contains several bioactive compounds, including carboxylic acids, flavonoids, carbohydrates, saponins, gums, proteins, and alkaloids. To understand its anti-inflammatory effects, the extract was tested using three in vitro assays: albumin denaturation, proteinase inhibition, and human red blood cell membrane stabilization. The extract demonstrated promising activity in all assays. In the albumin denaturation test, it inhibited protein denaturation by 56% at 500 µg/mL (IC<sub>50</sub> = 86.51 µg/mL). Proteinase activity was reduced by 62% at the same concentration (IC<sub>50</sub> = 81.35 µg/mL), while HRBC stabilization showed 63% protection of cells with only 36% lysis (IC<sub>50</sub> = 274.1 µg/mL). These findings suggest that PT extract may effectively reduce inflammation and offer protective effects, likely due to its rich phytochemical composition. The study is overall findings demonstrate PT promise as a natural treatment for arthritis and inflammation.

**Keywords:** *Polianthes Tuberosa*; Anti-inflammatory; Proteinase inhibition; Albumin denaturation; HRBC stabilization

### 1. Introduction

Inflammation is a protective response to tissue injury or harmful stimuli that removes the injurious agent, clears damaged tissue, and initiates healing; when brief it is acute, and when prolonged it is chronic. *Polianthes tuberosa* (tuberose) is a widely cultivated ornamental plant that has recently gained considerable pharmacological interest, particularly for its anti-inflammatory and related biological activities.[1] The present study is therefore designed to evaluate the anti-inflammatory and anti-arthritic activity of the ethanolic extract of *Polianthes tuberosa* by in vitro methods, with a focus on protein denaturation, proteinase inhibition and human red blood cell (HRBC) membrane stabilization assays. Soxhlet extraction with ethanol is employed to obtain a concentrated plant extract, followed by qualitative phytochemical screening to correlate observed biological effects with the presence of specific classes of compounds. The findings from this work are expected to provide scientific support for the traditional use of *Polianthes tuberosa* in inflammatory conditions and to contribute baseline data for future isolation, characterization and development of novel anti-inflammatory lead molecules from this species.

*Aim:*

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The aim of the study is Evaluation of antiinflammatory and anti- arthritis by *invitro* method on ethanolic extraction of *Polianthes tuberosa*

*Objectives:*

- Soxhlet extraction & phytochemical screening of plant materials
- HRBC Membrane Stabilization Method in flower extract
- Anti-inflammatory activity of flower extract by Proteinase inhibition assay
- Anti-arthritic activity of flower extract by albumin denaturation assay

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## 2. Methodology

### 2.1. Soxhlet extraction of plant materials (flower)

#### 2.1.1. Materials Required

Ethanol was purchased from Merk, USA. Whatman No.1 filter paper was purchased from Millipore, USA.

#### Procedure

Test sample (PT) can be fresh or dried. It needs to be crushed, using a pestle and mortar, to provide a greater surface area. The test sample should be sufficient to fill the porous cellulose thimble (in our experiments we use an average of 14 g of thyme in a 25- x 80-mm thimble). All equipment should be too assembled. Build a rig using stands and clamps to support the extraction apparatus. Following this, 50g of sample PT and 100ml of ethanol added to a round bottom flask, which is attached to a Soxhlet extractor and condenser on heating mantle. The crushed plant material is loaded into the thimble, which is placed inside the Soxhlet extractor. The side arm is lagged with glass wool. The solvent is heated using the heating mantle and will begin to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process should run for a total of 4 hours. Once the extraction set up, it can be left to run without direct supervision. It is not advised to leave the equipment completely alone due to the mix of running water and an electrical appliance, so a technician or other lab user should be made aware. The equipment can be turned off.

**Percentage of Yield obtained = 13.3%**

### 2.2. Qualitative Analysis of Phytochemicals in test samples [2]

#### 2.2.1. Detection of Carboxylic acid

To 1ml plant extract, 2ml of sodium bicarbonate solution is added. Colour changes occur indicates the presence of carboxylic acid.

#### 2.2.2. Detection of Tannins

To 2ml of plant extract, 2-3ml of 10% HCL is added and boiled for 5-6 min. Formation of red colour indicates the presence of tannins.

#### 2.2.3. Detection of Steroids

To 0.5 ml extract, 5ml of chloroform is added and equal amount of conc. H<sub>2</sub>so<sub>4</sub> was added. In the upper layer formation of red colour and in the lower layer, yellow with green colour is formation indicates the presence of steroids.

#### 2.2.4. Detection of Flavanoids

To 0.5 ml extract, 4ml of 1% ammonia was added and to this 1ml of conc. H<sub>2</sub>so<sub>4</sub> was added. The formation of yellow colour indicates the presence of flavonoids.

#### 2.2.5. Detection of Glycosides

Taken 2ml of hydrolysate, 3ml of chloroform was added, shaken vigorously, then the chloroform layer gets separated. Then 10% ammonia solution was added. The formation of pink colour indicates the presence of glycosidess

#### *2.2.6. Detection of Proteins*

Bradford Method:

To 500µl of plant extract, 5ml of the Bradford reagent was added, incubated at dark for 10 to 15 min. Taken the OD at 575nm.

#### *2.2.7. Detection of Phenol (Ferric Chloride Test)*

To 50 mg of extract, 5ml of distilled water was added and a few drops of 5% ferric chloride solution were added. The formation of dark green colour indicates the presence of phenol.

#### *2.2.8. Saponin Test*

To 50 mg of plant extract, 20 ml of distilled water was added and shaken vigorously for 15 min, formation of 2 cm layer of foam indicates the presence of saponins.

#### *2.2.9. Test for Alkaloids - Mayer's test*

To a few ml of plant sample extract, two drops of Mayer's reagent are added along the sides of the test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

#### *2.2.10. Saponification test*

To 1 or 2ml of 10 N sodium hydroxide, 2ml of extract is added and boiled for 2 minutes formation of soap or fat indicates the positive test for saponification.

#### *2.2.11. Gum Test*

The 100 mg of plant extract was dissolved in 2 ml of distilled water. 2ml of absolute alcohol with constant stirring. White colour cloudy precipitate indicates gums & mucilage's.

#### *2.2.12. Detection of flavanoglycoside*

The 50 mg of plant extract was dissolved in 5ml ethanol. Added a few drops of magnesium sulfate & few drops of conc. HCL. The formation of pink colour indicates the presence of flavanoglycoside.

#### *2.2.13. Detection of Carbohydrates*

To 0.5ml of extract, 0.5 ml of Benedict reagent was added and boiled for 2 min. Color changes and precipitates are formed. It indicates the presence of carbohydrate.

#### *2.2.14. Detection of resins*

To 0.5 ml of plant extract, 3 ml of CuSO<sub>4</sub> solution is added. Shaken for about 1-2 min, the formation of green colour precipitate indicates the presence of resins.

#### *2.2.15. Biuret test*

To 2ml of extract, 1 drop of 2% CuSO<sub>4</sub> solution. Add 1 ml of 95 % ethanol add 2 to 3 sodium hydroxide pellets. Formation of pink colour indicates the test is positive.

### **2.3. HRBC Membrane Stabilization Method in flower extract**

#### *2.3.1. Principle*

Membrane stabilization is a process in which the integrity of the membranes is stabilized by using anti-inflammatory drugs.

#### *2.3.2. Materials required*

Sodium citrate, Alsever solution, Test sample and NaCl was purchased from SRL (India), and 1X PBS was from Himedia, (India). 96 well plates were from Tarson (India).

### 2.3.3. Procedure

The anti-inflammatory activity of various extracts (PT) was assessed by *in vitro* HRBC membrane stabilization method. Blood was collected from healthy volunteers. The collected blood was mixed with an equal volume of Alsever solution (dextrose 2% sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL) and centrifuged with iso saline. To 1 mL of HRBC suspension, an equal volume of test drug in five different concentrations, 500, 250, 100, 50 and 10 µg/mL and 100 µL of 1XPBS was used as negative control and 100 µL of 1% SDS as positive controls. All the assay mixtures were incubated at 37°C for 1 hour and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm. [3]

The percentage of protection can be hence calculated from the equation as given below:

$$\text{Percent of protection} = 100 - \text{Percentage of Hemolysis}$$

Here "OD of test" is optical density or the test sample's absorbance and "OD of control" is optical density or absorbance of the negative control. Here, the negative control used was Alsever's solution with blood in it and it contained no Aspirin or Methanolic extract of the plant material in it.

## 2.4. Anti-inflammatory activity of flower extract by Proteinase inhibition assay

### 2.4.1. Procedure

The proteinase inhibitory assay was performed following the method modified by Oyedepo and Famurewa. The reaction mixture (2 ml) contained 0.06 mg trypsin, 1 ml Tris-HCl buffer (20 mM, pH 7.4) and test sample (PT) at different concentrations (500, 250, 100, 50 and 10 µg/ml). The reaction mixture was incubated at 37 °C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. Perchloric acid (2 ml of 70%) was added to stop the reaction. The cloudy suspension was centrifuged and the absorbance of the supernatant was measured at 210 nm against Tris-HCl buffer as blank. The experiment was performed in triplicate. [4]

## 2.5. Anti-Arthritic activity of flower extract by albumin denaturation assay

### 2.5.1. Principle

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as a strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. Most biological proteins lose their biological function when denatured. The denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, the ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat-induced albumin denaturation.

### 2.5.2. Material Required

Acetyl salicylic acid, BSA was purchased from Sigma Aldrich, USA. 10X PBS was purchased from Himedia, India.

### 2.5.3. Procedure

Denaturation of proteins is the main cause of inflammation. Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi and Sakatet *al.* with slight modification. 500 µL of 1% bovine serum albumin was added to PT (500, 250, 100, 50 and 10 µg/mL) of test sample. This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Acetyl salicylic acid was taken as a positive control. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using:

$$\% \text{ Inhibition} = 100 - ((A1 - A2) / A0) * 100$$

Where A1 is the absorbance of the control, A2 is the absorbance of the test sample and A0 is the absorbance of the positive control. [5]

A dose response curve was plotted to determine the IC50 values. IC50 is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

### 3. Results and discussion

In this study, we explored the anti-inflammatory and anti-arthritic potential of the ethanolic extract of *Polianthes tuberosa*. The plant material was extracted using the Soxhlet method, and preliminary analysis showed it contains several bioactive compounds, including carboxylic acids, flavonoids, carbohydrates, saponins, gums, proteins, and alkaloids. To understand its anti-inflammatory effects, the extract was tested using three in vitro assays: albumin denaturation, proteinase inhibition, and human red blood cell (HRBC) membrane stabilization. The extract demonstrated promising activity in all assays. In the albumin denaturation test, it inhibited protein denaturation by 56% at 500 µg/mL ( $IC_{50}$  = 86.51 µg/mL). Proteinase activity was reduced by 62% at the same concentration ( $IC_{50}$  = 81.35 µg/mL), while HRBC stabilization showed 63% protection of cells with only 36% lysis ( $IC_{50}$  = 274.1 µg/mL). These findings suggest that PT extract may effectively reduce inflammation and offer protective effects, likely due to its rich phytochemical composition. Similarly, a study by Christy et al. (2024) explored the use of PT flower extract to synthesize zinc oxide nanoparticles (ZnO NPs) and tested their antimicrobial and anti-inflammatory properties. The nanoparticles demonstrated dose-dependent inhibition against both bacterial and fungal pathogens and showed significant anti-inflammatory effects, suggesting that PT-derived products could be effective for treating inflammatory conditions and infections.

**Table 1** The table qualitative phytochemical screening methods

| S.No. | Name of the Sample | Phytochemical compound | Result |
|-------|--------------------|------------------------|--------|
| 1.    | PT                 | Resins                 | -      |
| 2.    |                    | Carboxylic acid        | +      |
| 3.    |                    | Tannins                | -      |
| 4.    |                    | Steroids               | -      |
| 5.    |                    | Flavonoid              | +      |
| 6.    |                    | Carbohydrates          | +      |
| 7.    |                    | Glycosides             | -      |
| 8.    |                    | Saponification         | +      |
| 9.    |                    | Protein                | +      |
| 10.   |                    | Phenol                 | -      |
| 11.   |                    | Biuret                 | -      |
| 12.   |                    | Saponin                | +      |
| 13.   |                    | Gum                    | +      |
| 14.   |                    | Flavanoglycosides      | -      |
| 15.   |                    | Alkaloids              | +      |

#### 3.1. HRBC Membrane Stabilization Method in plant extract

##### 3.1.1. OD Value at 540 nm

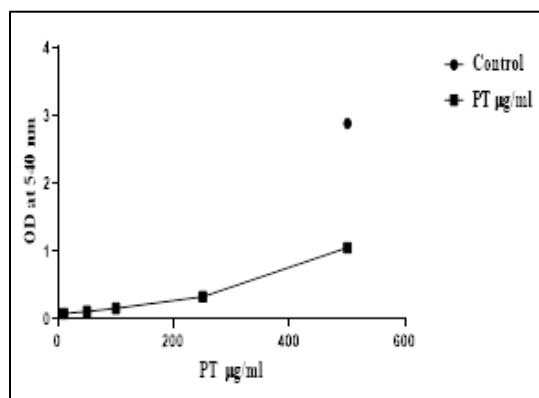
Hemolysis (%) Formula:  $\text{Test OD} / \text{Control OD} \times 100$  – Negative control

Control Mean OD value: 2.884

**Table 2** HRBC OD VALUES AT 540

| S. No | Tested sample concentration (µg/ml) | OD Value at 540 nm (in triplicates) |       |       |
|-------|-------------------------------------|-------------------------------------|-------|-------|
| 1     | Positive control                    | 2.883                               | 2.886 | 2.883 |
| 2     | Negative control                    | 0.067                               | 0.055 | 0.057 |

|   |           |       |       |       |
|---|-----------|-------|-------|-------|
| 3 | 500µg/ml  | 1.050 | 1.092 | 1.021 |
| 4 | 250 µg/ml | 0.320 | 0.324 | 0.360 |
| 5 | 100µg/ml  | 0.164 | 0.160 | 0.169 |
| 6 | 50µg/ml   | 0.121 | 0.121 | 0.107 |
| 7 | 10 µg/ml  | 0.089 | 0.086 | 0.081 |

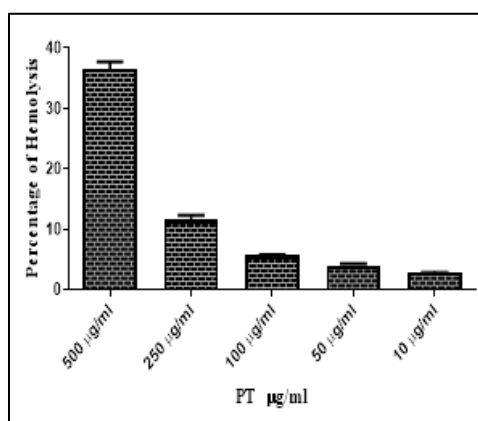


**Figure 1** CONTROL VS PT FOR HRBC TEST

### 3.1.2. % Hemolysis

**Table 3** % OF HEMOLYSIS

| S. No | Tested sample concentration (µg/ml) | Hemolysis (%)<br>(in triplicates) |        |        | Mean Value (%) |
|-------|-------------------------------------|-----------------------------------|--------|--------|----------------|
| 1.    | Control                             | 100                               | 100    | 100    | 100            |
| 2.    | 500µg/ml                            | 36.348                            | 37.804 | 35.342 | 36.498         |
| 3.    | 250 µg/ml                           | 11.036                            | 11.174 | 12.423 | 11.544         |
| 4.    | 100µg/ml                            | 5.6268                            | 5.4881 | 5.8002 | 5.638          |
| 5.    | 50µg/ml                             | 4.1358                            | 4.1358 | 3.6504 | 3.974          |
| 6.    | 10 µg/ml                            | 3.0263                            | 2.9223 | 2.7489 | 2.899          |



**Figure 2** percentage of hemolysis



Figure 3 HRBC test

### 3.2. Anti-inflammatory activity of flower extract by Proteinase inhibition assay

#### 3.2.1. OD Value at 210 nm

Control Mean OD value: 0.764

**Table 4** Proteinase inhibition assay OD values

| S. No | Tested sample concentration (µg/ml) | OD Value at 210 nm (in triplicates) |       |       |
|-------|-------------------------------------|-------------------------------------|-------|-------|
| 1.    | Control                             | 0.764                               | 0.769 | 0.760 |
| 2.    | 500 µg/ml                           | 0.239                               | 0.260 | 0.229 |
| 3.    | 250 µg/ml                           | 0.280                               | 0.280 | 0.294 |
| 4.    | 100 µg/ml                           | 0.328                               | 0.316 | 0.316 |
| 5.    | 50 µg/ml                            | 0.369                               | 0.363 | 0.375 |
| 6.    | 10 µg/ml                            | 0.403                               | 0.462 | 0.434 |

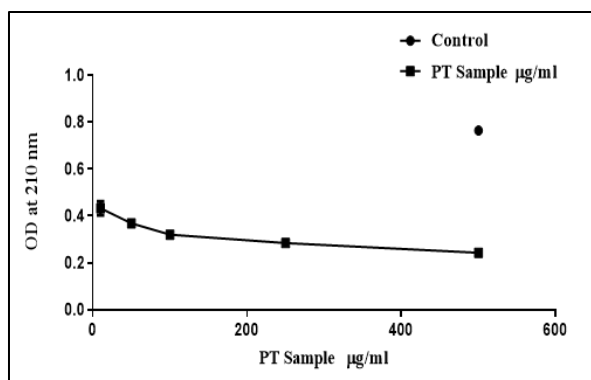
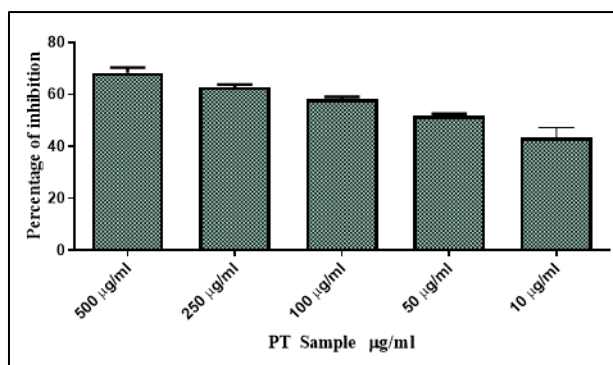


Figure 4 Control vs PT Proteinase inhibition assay

#### 3.2.2. Inhibition percentage (%)

**Table 5** inhibition percentage

| S. No | Tested sample concentration (µg/ml) | Inhibition percentage (%) (in triplicates) |        |       | Mean Value (%) |
|-------|-------------------------------------|--------------------------------------------|--------|-------|----------------|
| 1.    | 500 µg/ml                           | 68.71                                      | 65.968 | 70.02 | 68.2373        |
| 2.    | 250 µg/ml                           | 63.35                                      | 63.350 | 61.51 | 62.74          |
| 3.    | 100 µg/ml                           | 57.06                                      | 58.638 | 58.63 | 58.1152        |
| 4.    | 50 µg/ml                            | 51.70                                      | 52.486 | 50.91 | 51.7016        |
| 5.    | 10 µg/ml                            | 47.25                                      | 39.528 | 43.19 | 43.3246        |



**Figure 5** Inhibition percentage



**Figure 6** Proteinase inhibition assay

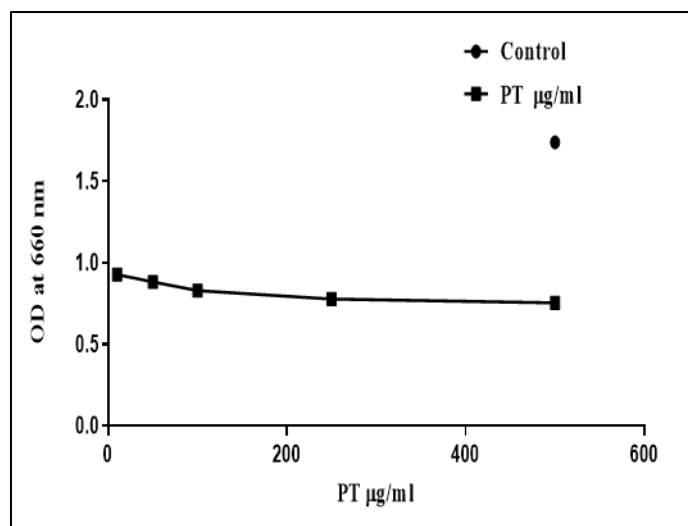
### 3.3. Anti-inflammatory activity of plant extract by albumin denaturation assay

#### 3.3.1. OD Value at 660 nm

Control Mean OD value: 1.739

**Table 6** Albumin denaturation assay OD values

| S. No | Tested sample concentration ( $\mu\text{g/ml}$ ) | OD Value at 660 nm (in triplicates) |       |       |
|-------|--------------------------------------------------|-------------------------------------|-------|-------|
| 1.    | Control                                          | 1.764                               | 1.732 | 1.722 |
| 2.    | 500 $\mu\text{g/ml}$                             | 0.726                               | 0.766 | 0.769 |
| 3.    | 250 $\mu\text{g/ml}$                             | 0.781                               | 0.773 | 0.777 |
| 4.    | 100 $\mu\text{g/ml}$                             | 0.811                               | 0.837 | 0.839 |
| 5.    | 50 $\mu\text{g/ml}$                              | 0.868                               | 0.896 | 0.881 |
| 6.    | 10 $\mu\text{g/ml}$                              | 0.925                               | 0.940 | 0.919 |
| 7.    | Acetyl salicylic acid                            | 0.225                               | 0.233 | 0.219 |

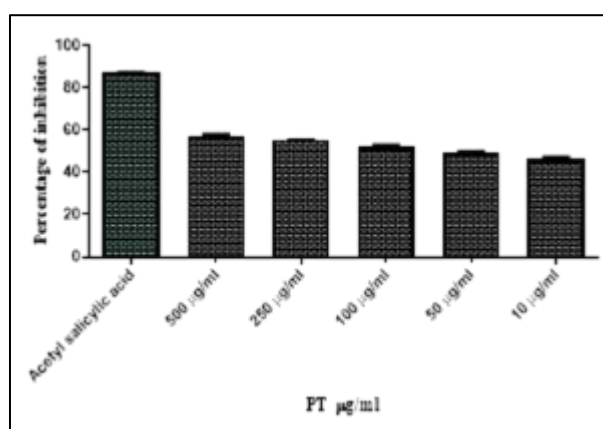


**Figure 7** Control vs PT

### 3.3.2. Inhibition percentage of albumin denaturation (%)

**Table 1** Inhibition % of albumin denaturation

| S. No | Tested sample concentration ( $\mu\text{g/ml}$ ) | Inhibition percentage albumin denaturation (%) (in triplicates) |        |       | Mean Value (%) |
|-------|--------------------------------------------------|-----------------------------------------------------------------|--------|-------|----------------|
| 1.    | Acetyl salicylic acid                            | 87.06                                                           | 86.601 | 87.40 | 87.02          |
| 2.    | 500 $\mu\text{g/ml}$                             | 58.25                                                           | 55.951 | 55.77 | 56.66          |
| 3.    | 250 $\mu\text{g/ml}$                             | 55.08                                                           | 55.549 | 55.31 | 55.31          |
| 4.    | 100 $\mu\text{g/ml}$                             | 53.36                                                           | 51.868 | 51.75 | 52.32          |
| 5.    | 50 $\mu\text{g/ml}$                              | 50.08                                                           | 48.476 | 49.33 | 49.30          |
| 6.    | 10 $\mu\text{g/ml}$                              | 46.80                                                           | 45.945 | 47.15 | 46.63          |



**Figure 8** Control vs PT for Inhibition % of albumin denaturation



**Figure 9** Albumin denaturation assay

#### 4. Conclusion

The ethanol extract of PT showed promising anti-inflammatory and anti-arthritis activity in vitro. It was effective in inhibiting protein denaturation, reducing proteinase activity, and protecting human red blood cells from lysis, suggesting its ability to stabilize cells under inflammatory conditions. These effects are likely due to the diverse phytochemicals present in the extract, including flavonoids, saponins, and alkaloids. Overall, the study highlights the potential of PT as a natural therapeutic agent for inflammation and arthritis. Further research is needed to isolate the active compounds and evaluate their effects in living systems.

#### Compliance with ethical standards

##### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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