

## IN VITRO ANTI-INFLAMMATORY, ANTICANCER AND ANTIMICROBIAL ACTIVITY OF *ANNONA RETICULATA* PEEL AND PULP EXTRACTS – A COMPARATIVE STUDY

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### Abstract

Recently, research has been focused on the pharmacological properties of natural compounds as potential therapeutic agents. *Annona reticulata* a member of family Annonaceae is a source for both industrial and medicinal products. It has a therapeutic effect, including anthelmintic, antipyretic, anti-inflammatory, cytotoxic and wound-healing properties. Phytochemicals such as flavonoids, phenols, alkaloids, steroids, tannins, and glycosides are widely distributed in it. The present study compared the antibacterial, anti-inflammatory, and anticancer activities of *Annona reticulata* (red custard apple) fruit pulp and peel extracts of fruits. The anti-inflammatory activity of pulp and peel extract of *Annona reticulata* was determined by inhibition of albumin denaturation and membrane lysis assay. The anticancer activity was tested against the MCF-7 using the MTT assays. The antibacterial properties against *Pseudomonas aeruginosa* and *Proteus mirabilis* was studied by disc diffusion method. The findings showed that the fruit pulp extract had more potential for anti-inflammatory, antibacterial, and anticancer action than the peel extract chosen for testing. Based on our findings, we suggested that *Annona reticulata* pulp serve as a valuable source of ingredients for both the culinary and pharmaceutical industries.

**Key words:** Antibacterial, Anticancer, Anti-inflammatory, *Annona reticulata*, Breast cancer.

**Abbreviations:** ROS-reactive oxygen species; CVD-cardiovascular diseases; DMEM-dulbecco's modified eagle medium; FBS-fetal bovine serum; MTT-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMSO-dimethyl sulfoxide; PBS-phosphate buffered saline; RBC-red blood cell; NSAID-nonsteroidal anti-inflammatory drug.

### Introduction

Cancer, inflammation, and the emergence of antibiotic-resistant diseases, among other human ailments, drive the ongoing search for novel bioactive compounds sourced from natural origins (Rahman *et al.*, 2021). Natural chemicals from medicinal plants have been used historically to treat a variety of chronic ailments. Secondary metabolite such as phenolic acids, flavonoids, quinones, lignans, alkaloids, and coumarins exhibit potent free-radical scavenging properties and have play a significant role in cancer therapy (Shi *et al.*, 2021). Natural substances show significant promise in combating cancer with minimal side effects, sparking substantial interest in their utilization from plants. Approximately 80% of individuals in developing nations reportedly resort to natural or herbal remedies for treating diverse ailments (Nobili *et al.*, 2009). The diverse pharmacological effects of plants stem from their secondary metabolite (Hussein & El-Anssary, 2019). These bioactive compounds, as noted by Zilani *et al.*, (2017), mitigate the risk of diseases induced by ROS (reactive oxygen species) through various mechanisms, such as inhibiting oxidative enzymes, antioxidants, and quenching ROS Plant extracts have been extensively documented to exhibit a variety of biological properties, such as anti-allergic, antibacterial, anticancer,

anti-inflammatory, antiviral, antioxidant, and antifungal effects. (Alshehri *et al.*, 2022).

An imbalance between the body's defence systems, which are meant to eradicate ROS, and their production is what leads to oxidative stress. This imbalance ultimately leads to chronic inflammation. Reports indicate that chronic inflammatory diseases are exacerbated by oxidative stress, with ROS identified as a primary contributor to inflammatory conditions such as cancer, non-insulin-dependent diabetes mellitus, and CVD (cardiovascular diseases) (Hussain *et al.*, 2016). Bacterial infections caused by organisms like *Pseudomonas aeruginosa* and *Proteus mirabilis* contribute to various diseases. For instance, *Pseudomonas aeruginosa* is responsible for lung infections and sepsis in individuals with cystic fibrosis or compromised immune systems (Campodónico *et al.*, 2008), while *Proteus mirabilis* commonly causes urinary tract infections (Torzewska *et al.*, 2019).

*Annona reticulata*, belonging to the Annonaceae family, comprises over 100 species within the *Annona* genus (Ngbolua *et al.*, 2018). Known as Bullock's heart, it has a rich traditional history of treating conditions such as fever, ulcers, heart issues, epilepsy, worm infestations, dysentery, bacterial infections, bleeding, and painful urination (Suroowan *et al.*, 2019). Indigenous populations from regions like the Philippines and India historically used this plant as a remedy

for inflammation, stress, and helminthic infections (Wen *et al.*, 2019). Notable bioactive constituents of *Annona reticulata* include steroids, tannins, flavonoids, saponins, alkaloids, and cardiac glycosides (Sangeetha *et al.*, 2014). The plant's leaves have been employed in treating helminthic infections, acting as insecticides, combating tumors, relieving toothaches, dysentery, fever, and as a topical suppurant (Parthiban *et al.*, 2019). The bark of *Annona reticulata* is utilized for deworming and diarrhea treatment, while other plant parts (root, leaves, and stem) contain isoquinoline alkaloids with potential acetylcholinesterase inhibition, antiviral, and insecticidal properties (Amudha & Varadharaj, 2017). Notably, the leaf extract of *Annona reticulata* demonstrated significant antimicrobial activities against both gram strains, indicating its potential therapeutic efficacy (Rani *et al.*, 2013).

*Proteus mirabilis* is a Gram-negative bacterium often found in the human urinary tract, known for causing urinary tract infections (UTIs) and producing a distinct ammonia odor due to its urease activity (Kafe *et al.*, 2023). *Pseudomonas aeruginosa* is a Gram-negative, opportunistic pathogen notorious for its resistance to antibiotics and its role in severe infections, particularly in immunocompromised individuals. Both bacteria are significant in healthcare settings, particularly in patients with catheters or chronic wounds (Paprocka *et al.*, 2022).

The study aims to explore the potential anti-inflammatory, antibacterial, and anticancer properties of *Annona reticulata* fruit pulp and peel extracts using an *In vitro* model.

## Material and Methods

**Gathering of botanical specimens:** *Annona reticulata* fruits were gathered in Nagercoil, Kanyakumari district, Tamilnadu. The Department of Botany of Annamalai University in Annamalai Nagar identified and authenticated the fruit.

**Extract preparation:** The plant materials are washed with running tap water. It was cut into small pieces of unripe fruit, and ripe fruit pulp was scraped thoroughly. The pulp was stored refrigerator. Then, the fruit peel was taken, shade dried for about 15 days and powdered using a blender. The fruit pulp pastes and peel was soaked in 50 ml of methanol and used homogenized. The sample was centrifuged, and the supernatant obtained was used for anti-inflammatory and anticancer studies at different concentrations. 5 g of powdered fruit pulp and peel were immersed for 48 hours with occasional shaking in 50 ml of various solvents, including acetone, methanol, ethanol, and aqueous. Using Whatman No. 1 filter paper, the plant extract was filtered. For the subsequent experiment, the filtrate was collected and stored in a clean beaker.

## Cytotoxicity assay (anticancer activity)

**Cell culture maintenance:** The MCF-7 cell line was obtained from the National Centre for Cell Lines, located in Pune, India. Dulbecco's modified Eagle medium (DMEM) was used to cultivate these cells. Fetal Bovine Serum (FBS) (10%) and antibiotics (1%), namely penicillin-streptomycin, were added as supplements. The cultures were kept in a humidified environment with 5% CO<sub>2</sub> at 37°C.

**MTT assay:** The pulp and peel extracts of *Annona reticulata* were examined for their effect on cell viability using the MTT assay. In a 96-well plate, cells (5×10<sup>4</sup> cells/well) were planted and allowed to develop for 24 hours. After a subsequent treatment of the cells with different dose of *Annona reticulata* pulp and peel extract (25, 50, 100, 200, and 400 µl/ml), in each well 2 µl of a MTT (5 mg/ml) were added. After incubating for 4 hours, formazan crystals were dissolved in 100 µl of dimethyl sulfoxide (DMSO), and a microplate reader was used to measure the absorbance of the crystals at 490 nm. The untreated cells made comprised the control group.

## Anti-inflammatory activity

**Protein denaturation:** According to Mizushima & Kobayashi (1968) and Sakat *et al.*, (2010), the protein denaturation test was used to assess the anti-inflammatory properties of *Annona reticulata* pulp and peel extracts. In summary, 2.9 mL of phosphate buffered saline (PBS) at pH 6.4 was mixed with 2 mL of various extract strengths (20-160 µl/ml). After adding 100 µL of egg albumin, the mixture was incubated for a further 15 minutes at 37°C. To denaturize the proteins, the reaction mixture was incubated for ten minutes at 70°C in a water bath. Using pure water as the reference sample, the mixture's absorbance at 660 nm was measured after cooling. Aspirin, a well-known anti-inflammatory medication, served as the positive control. The following approach was used to determine the percentage of protein denaturation inhibition based on the average findings of three tests conducted in duplicate.

$$\% \text{ Of inhibition} = (\text{OD } C_0 - \text{OD } S_0) \times 100 / \text{OD } C_0$$

C<sub>0</sub>-Control OD and S<sub>0</sub>- Sample OD

## Membrane lysis assay

**Preparation of red blood cells (RBCs) suspension:** Blood was obtained from a volunteer who was in good health and had refrained from taking NSAIDs. It was then placed into centrifuge tubes along with an anticoagulant. The tubes were spun in a centrifuge for 10 minutes at 3000 rpm, followed by three washes with saline solution of equal volume. To reach a concentration of 10% (v/v), the blood was mixed with ordinary saline solution (Sakat *et al.*, 2010, Sadique *et al.*, 1989).

**Heat-induced hemolysis:** The final reaction mixture comprised 1 mL of a 10% red blood cell (RBC) suspension and varying concentrations (ranging from 20 to 160 µl/ml) of extracts from *Annona reticulata* pulp and peel, along with aspirin serving as a positive control. Saline was used to replace the extracts in the control group. Following a 30-minute incubation at 56°C and subsequent cooling with running water, the supernatants were subjected to optical density measurement at 560 nm after centrifugation at 2500 rpm for 5 minutes, as described by Sakat *et al.*, (2010) and Shinde *et al.*, (1999). Each experiment was conducted in triplicate, and the resulting hemolysis inhibition rate was calculated.

$$\% \text{ Of inhibition} = (\text{OD } C_0 - \text{OD } S_0) \times 100 / \text{OD } C_0$$

C<sub>0</sub>-Control OD and S<sub>0</sub>- Sample OD

**Hypotonicity-induced hemolysis:** In the experiment, 0.5 mL of *Annona reticulata* pulp and peel extracts at concentrations ranging from 20 to 160 µl/ml were added to a reaction mixture that also included 1 mL of phosphate buffer, 2 mL of hyposaline, and 0.5 mL of RBC suspension. Aspirin was used as the positive control. After being incubated for 30 minutes at 37°C, each reaction mixture was centrifuged at 3000 rpm. Following centrifugation, the liquid supernatant was collected, and spectrophotometer readings were taken to determine the optical density at 560 nm (Azeem *et al.*, 2010). With the given expression, the percentage of hemolysis inhibition was computed.

$$\% \text{ Of inhibition} = (\text{OD } C_0 - \text{OD } S_0) \times 100 / \text{OD } C_0$$

$C_0$ -Control OD and  $S_0$ - Sample OD

**Antibacterial assay:** The disc diffusion method was used by Bauer *et al.*, (1966) to assess the antibacterial qualities of extracts from *Annona reticulata*'s pulp and peel. To produce and sterilise Muller Hinton agar media, an autoclave was used, set to 121°C for 15 minutes and 15 pounds of pressure. The medium was sterilised, then filled sterile Petri plates and let to harden.

Test organisms were taken out of the stock culture and added to 0.1 millilitre of broth. To properly distribute the organisms around the Muller Hinton agar surface, sterile cotton swabs were dipped into the broth culture. For five minutes, cultures were allowed to air dry.

The extracts were evaluated at 50, 100, 150, and 200 µl/ml concentrations. Using sterile forceps, sterile discs impregnated with the plant extracts were put onto the agar's surface and gently pushed down to achieve full contact. Furthermore, a control disc containing streptomycin was made and put on the agar surface. Next, the plates were incubated at 37°C for the whole night. Each plate's "zone of inhibition" diameter was measured in millimeters (mm) after incubation.

## Result

**Anticancer activity of *Annona reticulata* pulp and peel extracts:** We examined the potential of extracts from both the pulp and peel of *Annona reticulata* to suppress the

growth of MCF-7. By using the MTT test and morphological examinations, the pulp and peel extracts of *Annona reticulata* were determined to have anticancer properties. The MTT test and morphological research findings, which are shown in (Figs. 1 and 2), demonstrated the extract's particular anticancer efficacy against MCF-7 cells. Compared to untreated cancer cells, the pulp and peel extracts of *Annona reticulata* showed dose-dependent cytotoxicity against MCF-7 cells (Figs. 3 and 4). The pulp and peel extracts of *Annona reticulata* were reported to have IC<sub>50</sub> values of 202 ± 1.01 and 298 ± 1.90 µl/ml, respectively. The morphological analysis demonstrates a substantial difference between the extract-treated MCF-7 cell lines and the untreated ones in terms of their shape (reduced in size and abnormally shrunken). Compared to the peel extract of *Annona reticulata*, the pulp extract possesses higher anticancer activity.

**Inhibition of protein denaturation:** The anti-inflammatory action of *Annona reticulata* pulp and peel extracts was shown to be mediated by their capacity to suppress protein denaturation. It was successful in preventing albumin denaturation brought on by heat. At 160 µl/ml, the pulp and peel extracts of *Annona reticulata* showed maximum inhibition at 72.25 and 63.84%, respectively (Fig. 5). Standard anti-inflammatory medication aspirin demonstrated a maximal inhibition of 78.39% at a concentration of 160 µl/ml. The IC<sub>50</sub> of protein denaturation for pulp and peel extracts of *Annona reticulata* were found to be 39.31 ± 1.54 and 45.89 ± 2.67 µl/ml, respectively.

## Membrane stabilization

**Heat induced haemolysis:** At different concentrations, *Annona reticulata* pulp and peel extracts were able to stop heat-induced hemolysis. The results showed that *Annona reticulata* pulp and peel extracts at a concentration of 160 µl/ml protect 69.84 and 64.35% of the erythrocyte membrane against heat-induced lysis (Fig. 6). Aspirin 160 µl/ml provided 69.7% protection against the harmful effects of heat solution. The IC<sub>50</sub> of heat induced haemolysis for pulp and peel extracts of *Annona reticulata* were found to be 47.85 ± 2.23 and 80.00 ± 0.001 µl/ml, respectively.

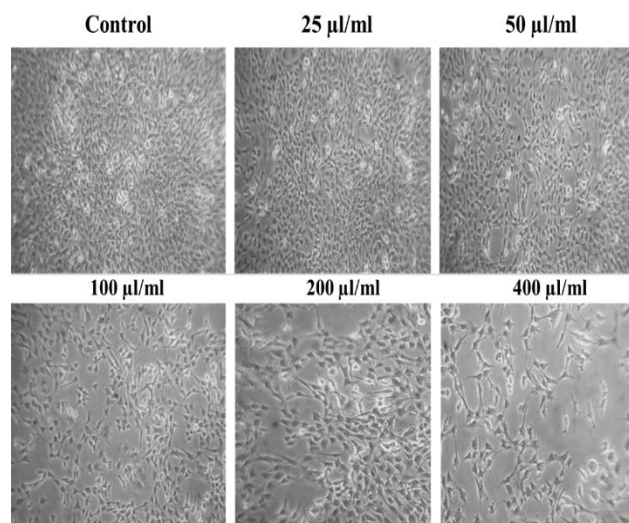


Fig. 1. Morphological alterations in MCF-7 cell lines treated with various concentrations of *Annona reticulata* pulp extract.

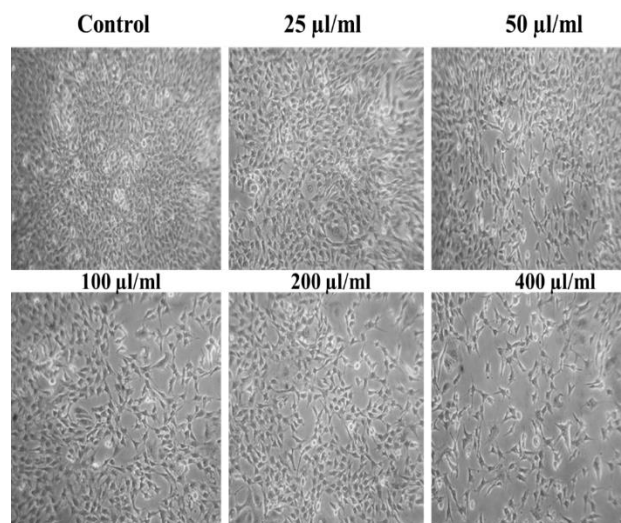
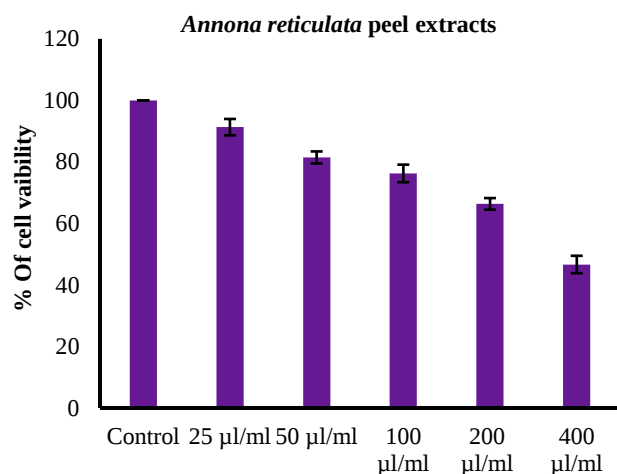
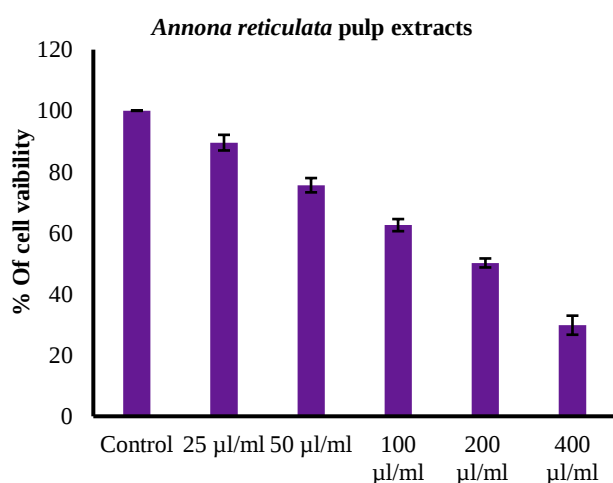


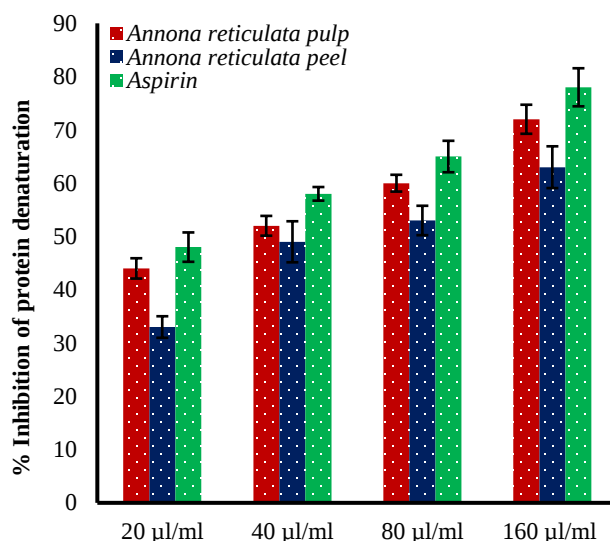
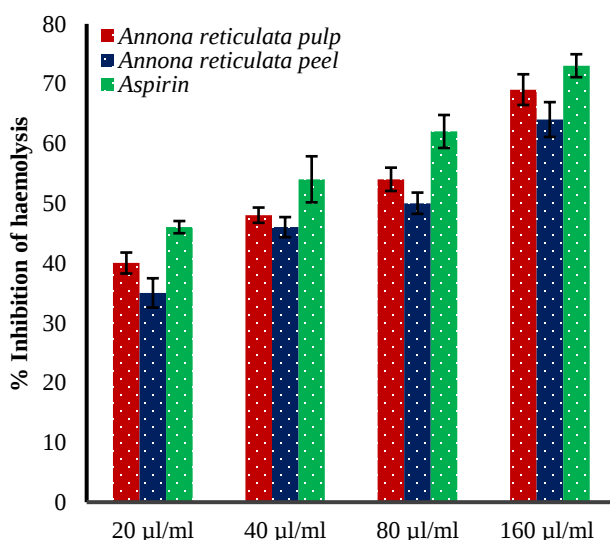
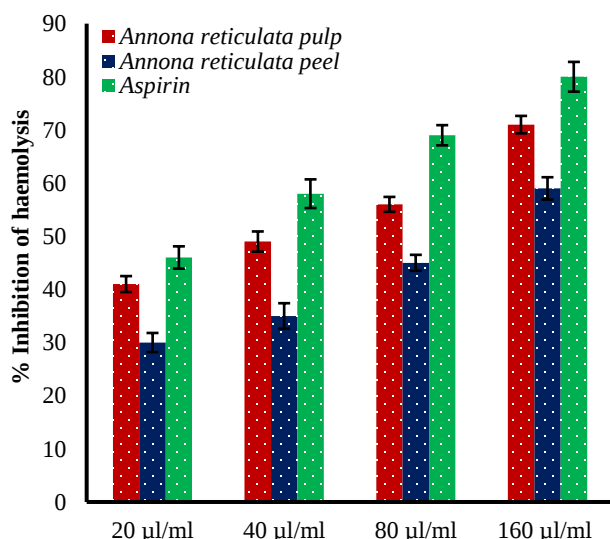
Fig. 2. Morphological alterations in MCF-7 cells treated with various concentrations of *Annona reticulata* peel extract.

Fig. 3. Anticancer activity of *Annona reticulata* pulp extracts.Fig. 4. Anticancer activity of *Annona reticulata* peel extract.  
Anti-inflammatory activity of *Annona reticulata* pulp and peel extracts

**Hypotonicity induced haemolysis:** The results demonstrated that *Annona reticulata* pulp and peel extract at a concentration of 160 µl/ml in 71.62 and 59.05%, respectively, prevents erythrocyte membrane lysis brought on by a hypotonic solution (Fig. 7). The 81.86% protection provided by aspirin 160 µl/ml against the harmful effects of hypotonic solution. The  $IC_{50}$  of hypotonicity induced haemolysis for pulp and peel extracts of *Annona reticulata* were found to be  $63.19 \pm 1.65$  and  $108.57 \pm 1.39$  µl/ml, respectively.

**Antibacterial activity of *Annona reticulata* pulp extract against *Proteus mirabilis* and *Pseudomonas aeruginosa*:**

The study findings indicated that both solvent extracts derived from *Annona reticulata* pulp and peel exhibited antibacterial properties, as depicted in Figures 8 and 9, and detailed in Tables 1 and 2. The methanol extract was better at killing bacteria than the acetone solvent extract. The methanol extraction of *Annona reticulata* pulp showed the largest zone of inhibition for *Proteus mirabilis* (21 mm) and *Pseudomonas aeruginosa* (23 mm), while the methanol extraction of *Annona reticulata* peel showed zones of inhibition against *Proteus mirabilis* (20 mm) and *Pseudomonas aeruginosa* (19 mm). *Proteus mirabilis* and *Pseudomonas aeruginosa* were not as affected by acetone solvent extracts as they were by methanolic extracts. When it came to killing bacteria, peel extracts were better than pulp extracts.

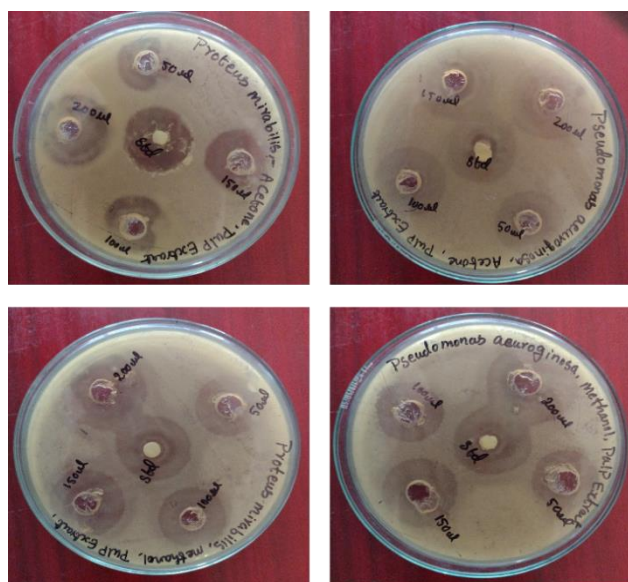
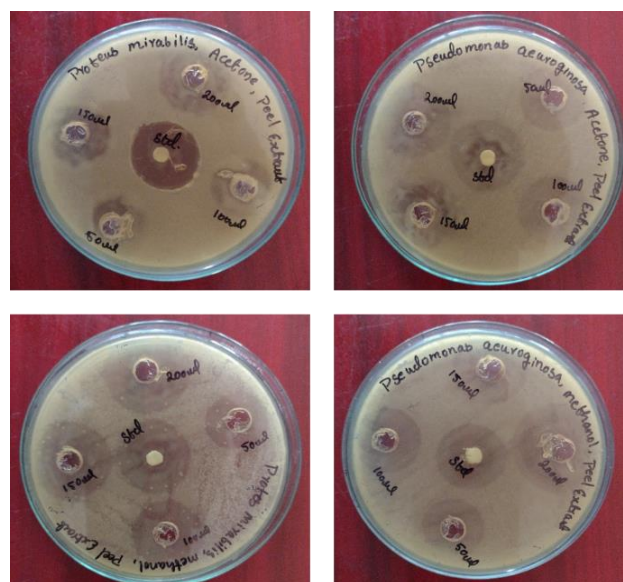
Fig. 5. Heat induced protein denaturation of *Annona reticulata* pulp and peel extract.Fig. 6. Heat induced haemolysis of *Annona reticulata* pulp and peel extractFig. 7. Hypotonicity induced haemolysis of *Annona reticulata* pulp and peel extract

**Table 1. Antibacterial activity of *Annona reticulata* pulp extract against *Proteus mirabilis* and *Pseudomonas aeruginosa*.**

|              | Bacteria                      | Solvents used | Area of inhibition zone (mm) |                |                |               |                  |
|--------------|-------------------------------|---------------|------------------------------|----------------|----------------|---------------|------------------|
|              |                               |               | 200 $\mu$ l/ml               | 150 $\mu$ l/ml | 100 $\mu$ l/ml | 50 $\mu$ l/ml | Positive control |
| Pulp extract | <i>Proteus mirabilis</i>      | Acetone       | 17                           | 15             | 13             | 12            | 22               |
|              |                               | Methanol      | 21                           | 18             | 16             | 14            | 24               |
|              | <i>Pseudomonas aeruginosa</i> | Acetone       | 18                           | 16             | 14             | 10            | 23               |
|              |                               | Methanol      | 23                           | 19             | 17             | 15            | 25               |

**Table 2. Antibacterial activity of *Annona reticulata* peel extract against *Proteus mirabilis* and *Pseudomonas aeruginosa*.**

|              | Bacteria                      | Solvents used | Area of inhibition zone (mm) |                |                |               |                  |
|--------------|-------------------------------|---------------|------------------------------|----------------|----------------|---------------|------------------|
|              |                               |               | 200 $\mu$ l/ml               | 150 $\mu$ l/ml | 100 $\mu$ l/ml | 50 $\mu$ l/ml | Positive control |
| Peel extract | <i>Proteus mirabilis</i>      | Acetone       | 16                           | 14             | 12             | 10            | 22               |
|              |                               | Methanol      | 20                           | 17             | 15             | 12            | 23               |
|              | <i>Pseudomonas aeruginosa</i> | Acetone       | 15                           | 13             | 12             | 09            | 21               |
|              |                               | Methanol      | 19                           | 17             | 14             | 12            | 22               |

**Fig. 8. Antibacterial potential of pulp extract against *Proteus mirabilis* and *Pseudomonas aeruginosa*.****Fig. 9. Antibacterial potential of peel extract against *Proteus mirabilis* and *Pseudomonas aeruginosa*.**

Positive control (Streptomycin). The result showed that pulp extract of *Annona Reticulata* showed appreciable antibacterial effect against *Proteus mirabilis* and *Pseudomonas aeruginosa*. The maximum of zone of inhibition for methanolic extract of pulp shows 21 and 23 mm in 200 $\mu$ l for *Proteus mirabilis* and *Pseudomonas aeruginosa*, respectively.

Positive control (Streptomycin). The result showed that peel extract of *Annona Reticulata* showed appreciable antibacterial effect against *Proteus mirabilis* and *Pseudomonas aeruginosa*. The maximum of zone of inhibition for methanolic extract of peel shows 20 and 19 mm in 200 $\mu$ l for *Proteus mirabilis* and *Pseudomonas aeruginosa*, respectively.

## Discussion

*Annona reticulata*, commonly known as red custard apple, holds significant biological importance due to its diverse array of medicinal and nutritional properties. This tropical fruit belongs to the *Annonaceae* family and is native to Central America. The plant has been traditionally used in various cultures for its therapeutic benefits. The fruit is a rich source of vitamins, particularly vitamin C, which contributes

to immune system health and acts as a potent antioxidant. Additionally, red custard apple contains essential minerals such as magnesium, potassium, and copper, contributing to overall well-being (Handique *et al.*, 2022).

Beyond its nutritional value, *Annona reticulata* has been recognized for its medicinal attributes. Various parts of the plant, including the leaves, seeds, and roots, have been utilized in traditional medicine for treating ailments like fever, dysentery, and digestive issues. Extracts from the red custard apple have also demonstrated potential anti-cancer properties in scientific studies, highlighting its importance in the realm of medical research (Omkaresh *et al.*, 2023).

Additionally, the pharmacological effects of the plant's bioactive components, such as acetogenins and alkaloids, including their antibacterial and anti-inflammatory properties, have been studied. The broad spectrum of bioactive compounds found in *Annona reticulata* makes it a valuable resource for pharmaceutical and nutraceutical purposes. Overall, the red custard apple's biological significance lies in its dual role as a nutrient-rich food source and a repository of compounds with potential health benefits, contributing to both traditional medicine and modern scientific advancements (Pathak *et al.*, 2021). The aim of this study is to explore the *In vitro* anti-inflammatory,

anticancer, and antimicrobial properties of extracts derived from both the peel and pulp of *Annona reticulata*.

Anti-inflammatory substances reduce inflammation in the body, alleviating pain and swelling. Common examples include nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, and natural compounds like turmeric and omega-3 fatty acids. These agents work by inhibiting inflammatory pathways and can be beneficial for conditions like arthritis (Maroon *et al.*, 2010).

Denaturation occurs when external stressors or compounds, like potent acids or bases, concentrated inorganic salts, organic solvents, or heat, disrupt the tertiary and secondary structures of proteins (Pathania *et al.*, 2019). Denatured proteins generally no longer serve their intended biological purpose. Inflammation can be caused by denaturing proteins, which is a well-known fact (Ramos *et al.*, 2017).

The anti-inflammatory characteristics of human red blood cell membrane reinforcement have been examined *In vitro*, according to Chippada *et al.*, (2011). The lysosomal and erythrocyte membranes are similar, which is why this occurs. According to De *et al.*, (2017), the extract may also be able to stabilise lysosomal membranes based on its ability to stabilise erythrocyte membranes. Since activated neutrophils release lysosomal components like bacterial enzymes and proteases when they leave the cell, increasing tissue damage and inflammation, stabilising lysosomal is crucial for reducing the inflammatory response (Alu *et al.*, 2020). Many different disorders are brought on by the release of lysosomal enzymes during inflammation. It is believed that either acute or chronic inflammation is related to the activity of these enzymes outside of cells. Nonsteroidal medications function by either increasing the stability of the lysosomal membrane or inhibiting the activity of these lysosomal enzymes (Mezzelani *et al.*, 2018). The peel and pulp extracts of *Annona reticulata* demonstrated anti-inflammatory activity in the current study by preventing hemolysis and the protein albumin. According to Malike *et al.*, (2021), the citrus nobilis peel methanolic extract demonstrated anti-inflammatory properties in membrane stabilisation and albumin denaturation tests.

The prevalence of breast cancer has been steadily rising as a result of recent lifestyle changes, such as eating meals low in or devoid of vegetables and fruits, doing little physical activity or exercise, drinking excessive amounts of alcohol, and being exposed to dangerous chemicals (Salman, 2023). Although while regular checkups and early identification have decreased the mortality rate, breast cancer still takes a significant number of lives worldwide each year. Hence, it is crucial to find innovative treatments or drug candidates that selectively target cancer cells while having no negative effects on healthy cells (Chari, 2008). Certain plant extracts, like those from turmeric (curcumin), green tea (catechins), and garlic (allicin), exhibit promising anticancer properties. These natural compounds have demonstrated anti-inflammatory, antioxidant, and apoptotic effects, suggesting their potential as adjuncts to conventional cancer therapies (Tan & Norhaizan, 2018). Our study revalued that *Annona reticulata* peel and pulp extracts possess anticancer activity against MCF-7 cell line. According to Ediriweera *et al.*, (2017), *Mangifera zeylanica* fruit peel and pulp extract demonstrated anticancer efficacy against the MCF-7 cell line.

The increase in pathogenic microorganisms and their resistance to various antibiotics, along with the economic and social issues they pose, has spurred increased research into the efficacy of herbal medicines (Mancuso *et al.*, 2021). So, looking at these plants could/might unveil novel compounds that can stop pathogenic microorganisms from spreading. Antimicrobial drugs could be made from substances that stop pathogenic microorganisms from growing or kill them without hurting the cells of the host (Gill *et al.*, 2015). Researching new antimicrobial agents that exhibit promising natural activities is very important if we want to find alternatives to common antibiotics (Moloney, 2016). Plant extracts exhibit potent antibacterial properties, harnessing bioactive compounds such as alkaloids, flavonoids, and essential oils. These natural agents disrupt bacterial cell membranes, inhibit enzymatic activities, and offer promising alternatives for combating bacterial infections. Methanolic extract of *Annona reticulata* pulp and peel had better antibacterial properties compare to acetone against *Proteus mirabilis* and *Pseudomonas aeruginosa* bacteria. This suggests that methanol is more effective in extracting bioactive compounds with antibacterial potential from the plant material. The solvent used in extraction can significantly impact plant extract biological activity. Similarly, Fawole *et al.*, 2012 documented that methanolic extract of pomegranate fruit peel and pulp had antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*.

Pulp extract is often more potent than peel extract because it contains higher concentrations of bioactive compounds, such as flavonoids, phenolics, and vitamins, which are directly linked to anti-inflammatory, anticancer, and antimicrobial activities. Additionally, the pulp's nutrient-rich environment promotes the synthesis and accumulation of these beneficial compounds.

The study highlights the significant pharmacological potential of *Annona Reticulata* extracts, particularly from the pulp, as promising candidates for anti-inflammatory, anticancer, and antimicrobial applications. However, further research is required to isolate the active compounds and validate these findings through *In vivo* models for broader application.

## Conclusion

In our current investigation, findings suggest that both pulp and peel extracts from *Annona Reticulata* possess notable pharmacological activity (anti-inflammatory, anticancer, and antimicrobial). Notably, the pulp extract demonstrates particularly high levels of activity in these regards compared to the peel extract. These extracts hold promise as potent candidates for anti-inflammatory, anticancer, and antibacterial medications. To isolate and identify the active chemicals causing these reported effects, further research is necessary. Additionally, confirmation of these results through *In vivo* models is essential for comprehensive understanding and application.

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