Floral Bioactive of *Pongamia pinnata*: A Multifaceted Approach to Oxidative Stress, Inflammation, and Microbial Resistance

Abstract

Background

Pongamia pinnata (L.) (*P. pinnata)* Pierre is widely utilized in traditional medicine and is known for its diverse pharmacological properties. This study investigates the in vitro antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activities of *P. pinnata* flower extract dissolved in DMSO.

Methods

The antioxidant potential was assessed using DPPH, H_2O_2, FRAP, ABTS, and nitric oxide scavenging assays. The antimicrobial activity was evaluated against *Staphylococcus aureus*, *Streptococcus mutans,* and *Candida albicans* using the agar well diffusion method. Anti-inflammatory properties were analysed through protein denaturation and membrane stabilization assays. Cytotoxicity was determined by evaluating membrane stabilization inhibition percentages.

Results

The extract exhibited strong free radical scavenging activity, with DPPH inhibition ranging from 64.28% at 10 µg/mL to 90.12% at 50 µg/mL, and H_2O_2 scavenging increasing from 48.1% to 86.2%. Moderate antibacterial activity was observed against *S. aureus* (17 mm at 100 µg/mL) and *S. mutans* (14 mm at 100 µg/mL), while antifungal activity against *C. albicans* (13 mm at 100 µg/mL) was noted. The extract significantly inhibited protein denaturation (BSA: 80%, EA denaturation: 78% at 50 µg/mL) and demonstrated high membrane stabilization (84% at 50 µg/mL). Cytotoxic effects were observed with inhibition percentages of 68%–84% in membrane stabilization, indicating mild cytotoxicity compared to the standard drug.

Conclusion

P. pinnata flower extract exhibits significant antioxidant, anti-inflammatory, and antimicrobial

activities, with mild cytotoxic effects. These findings support its potential as a natural therapeutic agent, warranting further pharmacological exploration.

Keywords: *Pongamia pinnata*, Antioxidant, DPPH, antimicrobial, membrane stabilization, FRAP

1. Introduction

P. pinnata (L.) Pierre, commonly known as Indian beech or Karanja, is a versatile medicinal plant widely valued for its therapeutic applications in traditional medicine. Various parts of this plant, such as the leaves, seeds, bark, and roots, contain bioactive compounds like flavonoids, alkaloids, and terpenoids, contributing to its diverse pharmacological properties.[1] Inflammation is the body's physiological response to injury, infection, or tissue damage, characterized by heat, redness, swelling, pain, and disrupted bodily functions. This protective mechanism helps eliminate irritants, neutralize harmful organisms, and prepare tissues for repair. It is triggered by the release of chemical mediators from damaged tissues and immune cells.[2] While non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage inflammatory conditions, they are often associated with adverse effects, particularly gastrointestinal issues like gastric ulcers. Inflammation is a complex biological process involving vascular tissues, serving as a defence mechanism to eliminate harmful stimuli and initiate healing.[3] Key inflammatory mediators, including histamine, serotonin, prostaglandins, and plasma enzyme systems such as the complement, clotting, fibrinolytic, and kinin systems, play essential roles in enhancing vasodilation and vascular permeability. This response facilitates increased blood flow, plasma protein exudation, and leukocyte migration, primarily neutrophils, to the injury site.[4] Inflammation is broadly categorized into acute and chronic types. Acute inflammation represents the body’s immediate response to harmful stimuli and is characterized by vascular changes such as vasodilation and increased capillary permeability, driven by tissue-resident cells and inflammatory mediators.[5] In contrast, chronic inflammation is a prolonged response, involving simultaneous tissue damage and repair, often leading to progressive alterations at the site of inflammation. The exploration of natural products has significantly contributed to modern

medicine.[6] Recently, there has been renewed interest in traditional medicine due to extensive research on various plants and their active therapeutic compounds. Investigating the anti-inflammatory potential of natural products offers opportunities to discover bioactive compounds that may provide safer and more effective alternatives to synthetic drugs.[7] These natural compounds, such as polyphenols, flavonoids, terpenoids, and alkaloids, are known to target multiple inflammatory pathways while exhibiting reduced toxicity and fewer side effects. This is particularly relevant in chronic conditions where prolonged use of synthetic anti-inflammatory drugs can lead to adverse health outcomes. Several therapeutic agents, including aspirin and other NSAIDs, were inspired by plant-based substances.[8] Research into anti-inflammatory plants presents a promising pathway to identify novel bioactive compounds or chemical structures that could serve as the foundation for the development of more potent and targeted anti-inflammatory drugs.[9] Advancing this field requires cost-effective and reliable in vitro methods to evaluate the efficacy of natural anti-inflammatory compounds. The egg albumin denaturation method is one such approach, offering an accessible and effective tool for researchers to study the anti-inflammatory properties of natural substances.[10] Recent research has focused on its anticancer potential, particularly the cytotoxic effects of its extracts and bioactive compounds on cancer cells. Cancer continues to be a leading cause of morbidity and mortality worldwide, driving the search for safer, plant-based alternatives to synthetic chemotherapeutic agents. Natural products play a pivotal role in anticancer drug development due to their structural diversity and ability to modulate multiple biological pathways.[11] *P. pinnata* has shown promising cytotoxic activity, with studies demonstrating its ability to induce apoptosis, inhibit cell proliferation, and disrupt cancer cell metabolism.Extracts from the seeds, bark, and other parts of *P. pinnata* have exhibited cytotoxic effects against various cancer cell lines, including breast (MCF-7) and cervical (HeLa) cancer cells. Assays such as MTT and trypan blue dye exclusion have been used to assess its cytotoxic potential, revealing a dose-dependent reduction in cell viability. These effects are attributed to the plant's bioactive constituents, which act through mechanisms such as oxidative stress induction, mitochondrial dysfunction, and interference with cellular signalling pathway.[12] Research into *P. pinnata* as a source of anticancer agents is essential not only for drug discovery but also for understanding its role in complementary and alternative medicine. The study of plant-derived cytotoxic agents opens avenues to identify safer, more targeted therapies with minimal side effects compared to

conventional treatments.[13] This study explores the therapeutic potential of *P. pinnata* (L.) Pierre flower extract, widely known for its medicinal properties, by evaluating its antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activities. Given the need for safer, natural alternatives to synthetic drugs, the research investigates its efficacy through in vitro assays. Antioxidant potential was assessed using DPPH, H_2O_2, FRAP, ABTS, and nitric oxide scavenging assays, while antimicrobial activity was tested against *Staphylococcus aureus, Streptococcus mutans, Candida albicans,* and *Lactobacillus sp.* The anti-inflammatory effects were determined using BSA, egg albumin denaturation, and membrane stabilization assays. Additionally, cytotoxicity studies were conducted to ensure its safety profile, highlighting *P. pinnata* as a promising candidate for pharmacological applications.

2. Materials and methods

2.1. Plant materials

The plant material flower was collected from local area of Chennai India. The plant part such as flower were thoroughly washed and shade dried. 50g of flower powder material was extracted with different solvent fractions using a Soxhlet apparatus, and the obtained extracts were evaporated using a vaccum evaporator to get the crude dried extract (Fig. 1).

2.2. Preparation of plant extract

The flowers of Pongamia pinnata were collected, dried, and processed for extract preparation. The dried material was ground into a fine powder and subjected to extraction using dimethyl sulfoxide (DMSO) as the solvent. The extraction process involved maceration process to ensure the efficient dissolution of bioactive compounds into the solvent. The resulting extract was filtered to remove insoluble debris and concentrated under reduced pressure to obtain a semisolid DMSO extract, which was stored in a suitable container for further analysis.

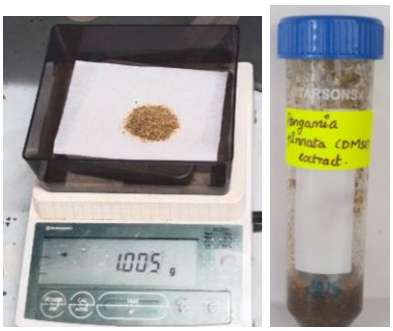


Fig. 1 This figure shows the extract of *P. Pinnata.*

2.3. Microorganisms

Staphylococcus aureus, Lactobacillus sp, Candida albicans, Streptococcus mutans microorganism were used in the present study. The microorganisms were procured from Saveetha dental college, microbiology lab.

2.4. Apparatus

Soxhlet apparatus, Rotatory evaporator, Freezedryer, Refrigerator, Hot air oven, Shaker (Gallen Kamp incubator orbital shaker), test tube, centrifuge tube, weighing balance, mechanical blender, Spectrophotometer, Crew cap vials, Ultra digital sonicator,

2.5. Chemicals

DMSO extract, normal saline, ethanol, diclofenac, amoxicillin and ascorbic acid. All chemicals used were of analytical grade.

2.6. Antioxidant Studies

2.6.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed to assess the free radical scavenging potential of *P. pinnata* flower extract. The experiment began with the preparation of reagents. A 0.1 mM DPPH solution was prepared by dissolving an appropriate quantity of DPPH in methanol. Various concentrations of the *P. pinnata* extract (10, 20, 30, 40, and 50 µg/mL) were obtained by diluting the stock solution in DMSO. For the reaction setup, clean test tubes were used, where 1 mL of the DPPH solution was combined with 1 mL of the extract at each concentration. A control was prepared by mixing 1 mL of the DPPH solution with 1 mL of

methanol in place of the extract. To serve as a positive control, a standard antioxidant, such as ascorbic acid, was prepared in the same concentrations as the extract (Figure 2). The reaction mixtures were incubated at room temperature in the dark for 30 minutes to allow sufficient interaction between the DPPH radicals and the extract. Following incubation, the absorbance of each solution was recorded at 517 nm using a UV-visible spectrophotometer. The percentage of DPPH radical scavenging activity was determined using the appropriate formula:

$$\text{DPPH scavenging activity (\%)} = \left(\frac{Acontrol - Asample}{Acontrol}\right) \text{x} 100$$

2.6.2. H_2O_2 (Hydrogen peroxide) Assay

The H_2O_2 assay was conducted to evaluate the antioxidant potential of *P. pinnata* flower extract. A 40 mM hydrogen peroxide solution was prepared in phosphate buffer (pH 7.4). Various concentrations of the *P. pinnata* extract (10, 20, 30, 40, and 50 µg/mL) were obtained by diluting the stock solution in DMSO. A standard antioxidant, such as ascorbic acid, was prepared at identical concentrations to serve as a positive control. For the reaction setup, clean test tubes were used, where 0.6 mL of the hydrogen peroxide solution was combined with 1 mL of the extract at each concentration. A control was prepared by mixing 0.6 mL of hydrogen peroxide solution with 1 mL of phosphate buffer instead of the extract (Figure 3). The reaction mixtures were incubated at room temperature for 10 minutes to facilitate the interaction between hydrogen peroxide and the antioxidant compounds in the extract. Following incubation, the absorbance of each reaction mixture was recorded. The percentage of hydrogen peroxide scavenging activity was calculated using the appropriate formula:

$$H_2O_2 \text{ scavenging activity (\%)} = \left(\frac{Acontrol - Asample}{Acontrol}\right) \text{x } 100$$

where,

A control =The absorbance of the control (H2O2 solution with phosphate buffer, no extract).

A sample = The absorbance of the reaction mixture containing the extract or standard.

2.6.3. FRAP Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was performed to evaluate the reducing ability of *P. pinnata* flower extract. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM ferric chloride solution in a 10:1:1 ratio. The extract solutions were prepared at varying concentrations (10, 20, 30, 40, and 50 µg/mL), with ascorbic acid serving as the standard antioxidant. The FRAP reagent was prepared by combining acetate buffer, TPTZ solution, and ferric chloride solution in the specified ratio and preheating it to 37°C before use. In clean test tubes, 1.5 mL of the FRAP reagent was mixed with 0.5 mL of the extract solution at each concentration. A blank solution was prepared by mixing 1.5 mL of the FRAP reagent with 0.5 mL of distilled water, while the standard solution was prepared following the same procedure as the extract solutions (Figure 4). The reaction mixtures were incubated at 37°C for 4 minutes to facilitate the reduction of ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions by the antioxidants present in the extract. The absorbance of the blue-coloured ferrous-TPTZ complex was then measured at 593 nm using a UV-visible spectrophotometer. The reducing power of the extract was expressed as a percentage relative to the standard, calculated using the appropriate formula:

$$\text{FRAP Activity} = \left(\frac{Asample}{Astandard}\right) \times 100$$

2.6.4. ABTS Assay

The ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay was conducted to assess the antioxidant potential of *P. pinnata* flower extract. The materials required for this assay included a 7 mM ABTS solution prepared in distilled water and a 2.45 mM potassium persulfate solution, which was used to generate the ABTS radical cation ($ABTS^{\bullet+}$). The extract solutions were prepared at varying concentrations (10, 20, 30, 40, and 50 µg/mL), with ascorbic acid serving as the standard antioxidant. The ABTS radical cation solution was formed by mixing equal volumes of the 7 mM ABTS solution and 2.45 mM potassium persulfate solution. This mixture was left to react in the dark at room temperature for 12–16 hours to allow complete generation of $ABTS^{\bullet+}$. Before use, the solution was diluted with ethanol or distilled water to achieve an absorbance of 0.7 ± 0.02 at 734 nm. For the reaction setup, clean test tubes were used, where 1 mL of the ABTS radical cation solution was mixed with 1 mL of the extract

at each concentration. A control solution was prepared by combining 1 mL of the ABTS radical cation solution with 1 mL of the solvent (such as ethanol) instead of the extract. Standard solutions were prepared using the same procedure as the extract solutions (Figure 5). The reaction mixtures were incubated at room temperature for 6 minutes to allow interaction between the antioxidants in the extract and the $ABTS^{\bullet+}$ radicals. Following incubation, the absorbance of each reaction mixture was measured at 734 nm using a UV-visible spectrophotometer. The ABTS radical scavenging activity was determined as a percentage using the appropriate formula:

$$\text{ABTS scavenging activity (\%)} = \left(\frac{Acontrol - Asample}{Acontrol}\right)x100$$

2.6.5. Nitric oxide Assay

The nitric oxide (NO) scavenging assay was carried out to determine the capacity of *P. pinnata* flower extract to neutralize nitric oxide radicals. The materials used in this assay included a 10 mM sodium nitroprusside solution, phosphate-buffered saline (PBS) at pH 7.4, and Griess reagent, which consisted of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride. Extract solutions were prepared at concentrations of 10, 20, 30, 40, and 50 µg/mL, with ascorbic acid serving as the standard reference antioxidant. For the preparation of the reaction mixture, 10 mM sodium nitroprusside was dissolved in PBS. In clean test tubes, 2 mL of this sodium nitroprusside solution was combined with 0.5 mL of the extract at different concentrations (10–50 µg/mL). A control was prepared by mixing 2 mL of the sodium nitroprusside solution with 0.5 mL of PBS instead of the extract (Figure 6). The reaction mixtures were incubated at 25°C for 150 minutes under light exposure to facilitate the generation of nitric oxide radicals. After incubation, 1 mL of each reaction mixture was mixed with 1 mL of Griess reagent and further incubated at room temperature for 10 minutes. During this period, a purple azo dye was formed due to the interaction between nitric oxide and the Griess reagent. The absorbance of the resulting solution was recorded at 540 nm using a UV-visible spectrophotometer. The percentage of nitric oxide scavenging activity was calculated using the formula:

$$\text{NO scavenging activity (\%)} = \left(\frac{Acontrol - Asample}{Acontrol}\right)x100$$

2.7. Antimicrobial activity against oral pathogens

Antimicrobial assay

The antimicrobial activity of *Pongamia pinnata* flower extract was assessed using the cup plate method, an agar diffusion technique. Muller Hinton Agar (MHA) was used to prepare sterile Petri plates, and microbial suspensions of oral pathogens (*Staphylococcus aureus*, *Lactobacillus* sp., *Candida albicans*, *Streptococcus mutans*) were prepared to match the 0.5 McFarland standard. After inoculating the plates with a microbial suspension using a sterile cotton swab, wells were punched into the agar, and 50 µL of *Pongamia pinnata* extract (at concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL) was added to separate wells. Amoxicillin was used as a positive control. The plates were incubated at 37°C for 24 hours for bacterial cultures and 28°C for 48 hours for fungal cultures, and the zone of inhibition was measured in millimeters (mm). The antimicrobial efficacy of the extract was compared to the standard antibiotic amoxicillin, and results were recorded accordingly.

2.8. Anti-inflammatory activity

2.8.1. BSA assay

To evaluate the denaturation inhibition of proteins, BSA was used as a model to simulate inflammation at pH 6.8. *P. pinnata* flower extract and Diclofenac samples were prepared in different concentrations (10 µg, 20 µg, 30 µg, 40 µg, and 50 µg) and diluted with DMSO in separate test tubes to achieve a final volume of 1 mL. Control test tubes containing only 50 µL of DMSO were also prepared. Each test tube, including samples and controls, received 5 mL of a 0.2% BSA solution prepared in tris-buffered saline at pH 6.8 (Figure 9). The samples and controls were incubated at 37°C for 20 minutes, followed by 5 minutes at 72°C. After incubation, the test tubes were allowed to cool for 10 minutes, and absorbance was measured at 660 nm. No denaturation inhibition was observed in the control test tubes. The denaturation inhibition of *P. pinnata* and Diclofenac was calculated and compared based on the results [16]. Inhibition of denaturation (%) was calculated using the following formula:

$$\text{Inhibition of denaturation (\%)} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

2.8.2. Egg Albumin (EA) Denaturation Assay

The egg albumin denaturation test is used to evaluate a substance's ability to prevent or inhibit the denaturation of egg albumin, which can serve as an indicator of anti-inflammatory properties. The premise of the experiment is based on the idea that compounds with anti-inflammatory effects may stabilize protein structures and prevent denaturation, a process often linked to tissue damage and inflammation. Therefore, substances that significantly reduce the denaturation of egg albumin in this test could potentially exhibit anti-inflammatory effects. Protein denaturation is considered a key factor in inflammation, and in addition to inhibiting denaturation, NSAIDs also block the COX enzyme. For the assay, 0.2 mL of egg albumin was mixed with 2.8 mL of phosphate-buffered saline (PBS) to prepare a 5 mL solution. Various concentrations of DMSO extracts, ranging from 10 µL to 50 µL, were prepared and stored (Figure 10). Diclofenac was used as a standard reference. The solutions were incubated at 37°C for 15 minutes, then allowed to cool to room temperature [17]. Absorbance was measured at 660 nm to assess the extent of protein denaturation.

2.8.3. Membrane stabilization assay

The membrane stabilization assay is used to evaluate the anti-cytotoxic properties of *P. pinnata* flower DMSO extract. To prepare a 10% v/v red blood cell (RBC) suspension, fresh blood (preferably from goat or cow) is centrifuged at 2500 rpm for 10 minutes to separate the plasma. The RBCs are then washed three times with isotonic phosphate-buffered saline (PBS, pH 7.4). Test solutions of the DMSO extract are prepared at varying concentrations (e.g., 50, 100, 200, and 400 µg/mL) in PBS, along with a standard drug solution (e.g., aspirin or diclofenac at 200 µg/mL) and PBS as the control. To prepare the reaction mixtures, 1 mL of RBC suspension is combined with 1 mL of the extract, standard drug, or PBS, followed by the addition of 2 mL of distilled water (hypotonic solution) (Figure 11). After incubating the mixtures at 37°C for 30 minutes, the tubes are centrifuged at 2500 rpm for 10 minutes to separate the supernatant. The amount of hemoglobin released into the supernatant is measured at 540 nm using a spectrophotometer to assess the extent of hemolysis:

$$\text{Membrane Stabilization (\%)} = \frac{\{\text{Absorbance of Control} - \text{Absorbance of Sample}\}}{\{\text{Absorbance of Control}\}} \times 100$$

A higher percentage indicates better anti-inflammatory activity of the extract compared to the standard drug. The assay is performed in triplicates to ensure accuracy and reproducibility [18].

3. Results and Discussion

The antioxidant activity of the Pongamia pinnata flower extract dissolved in DMSO was thoroughly evaluated through a series of in vitro assays: DPPH, hydrogen peroxide (H2O2), ferric reducing antioxidant power (FRAP), ABTS, and nitric oxide scavenging assays. The results consistently revealed a significant concentration-dependent antioxidant potential across all assays. These findings underscore the extract's efficacy as a natural source of antioxidants.

3.1. Antioxidant activity

3.1.1. DPPH Radical Scavenging Assay

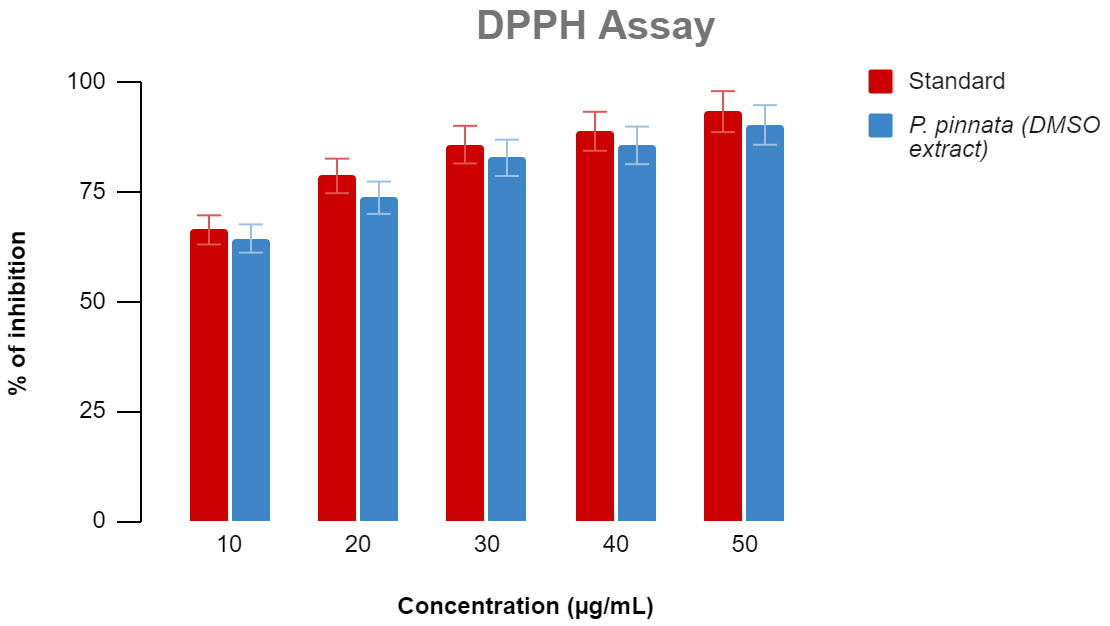


Fig. 2 The figure represents the DPPH Assay of *P.pinnata.* The bar graph represents the % inhibition of DPPH radicals by the standard and *P. pinnata* at different concentrations (10 to 50 µg/mL). Error bars indicate standard deviation (SD) from triplicate measurements.

Table 1 DPPH Assay of *P. pinnata*

DPPH conc. (µg/mL)	% of inhibition	
	Standard	***P. pinnata (DMSO extract)***
10	66.25	64.28
20	78.52	73.56
30	85.63	82.63
40	88.68	85.47
50	93.15	90.12

The table represents the percentage inhibition of DPPH radicals by the standard antioxidant and *P. pinnata* (DMSO extract) at different concentrations (10–50 µg/mL). The values indicate the mean percentage of inhibition observed at each concentration.

The DPPH assay is a widely used method to evaluate the ability of compounds to donate electrons or hydrogen atoms, neutralizing free radicals. The Pongamia pinnata extract displayed excellent scavenging activity, with values increasing proportionally to the concentration. At 10 µg/mL, the scavenging activity was 64.28%, and this increased steadily to 90.12% at 50 µg/mL (Table.1). This is comparable to the standard antioxidant, which achieved 93.15% at the same concentration. These results indicate that the extract contains bioactive compounds capable of effectively quenching DPPH radicals.

3.1.2. Hydrogen Peroxide (H2O2) Scavenging Assay

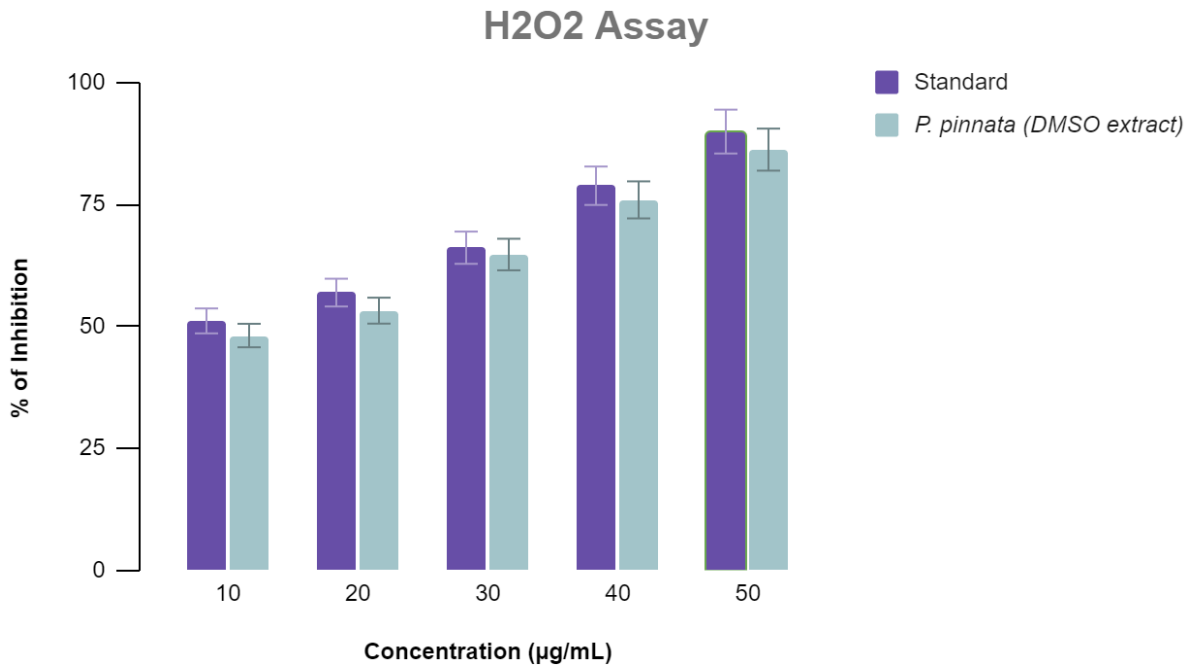


Fig. 3 Hydrogen Peroxide (H_2O_2) Scavenging Assay of *P. pinnata*. The bar graph illustrates the percentage inhibition of hydrogen peroxide (H_2O_2) by the standard antioxidant (purple) and *Pongamia pinnata* (DMSO extract) (light blue) at different concentrations (10–50 µg/mL). Error bars represent the standard deviation (SD) from triplicate measurements.

Table.2 Percentage inhibition of hydrogen peroxide by *P. pinnata*.

H2O2 conc. (µg/mL)	**% of inhibition**	
	Standard	***P. pinnata (DMSO extract)***
10	51.1	48.1
20	56.9	53.2
30	66.1	64.7
40	78.8	75.9
50	89.9	86.2

This table presents the percentage inhibition of H_2O_2 at different concentrations (10–50 µg/mL) for both the standard antioxidant and *P. pinnata* (DMSO extract). The inhibition increases with concentration, indicating dose-dependent antioxidant activity.

The hydrogen peroxide scavenging assay evaluates the capacity of an extract to neutralize H2O2, a reactive oxygen species that can diffuse across cell membranes and generate highly reactive hydroxyl radicals. The Pongamia pinnata extract showed promising activity, with scavenging percentages ranging from 48.1% at 10 µg/mL to 86.2% at 50 µg/mL. The extract's performance closely paralleled that of the standard antioxidant, which achieved 89.9% scavenging at the highest concentration (Table.2). This assay highlights the extract's ability to mitigate oxidative stress caused by hydrogen peroxide.

3.1.3. Ferric Reducing Antioxidant Power (FRAP) Assay

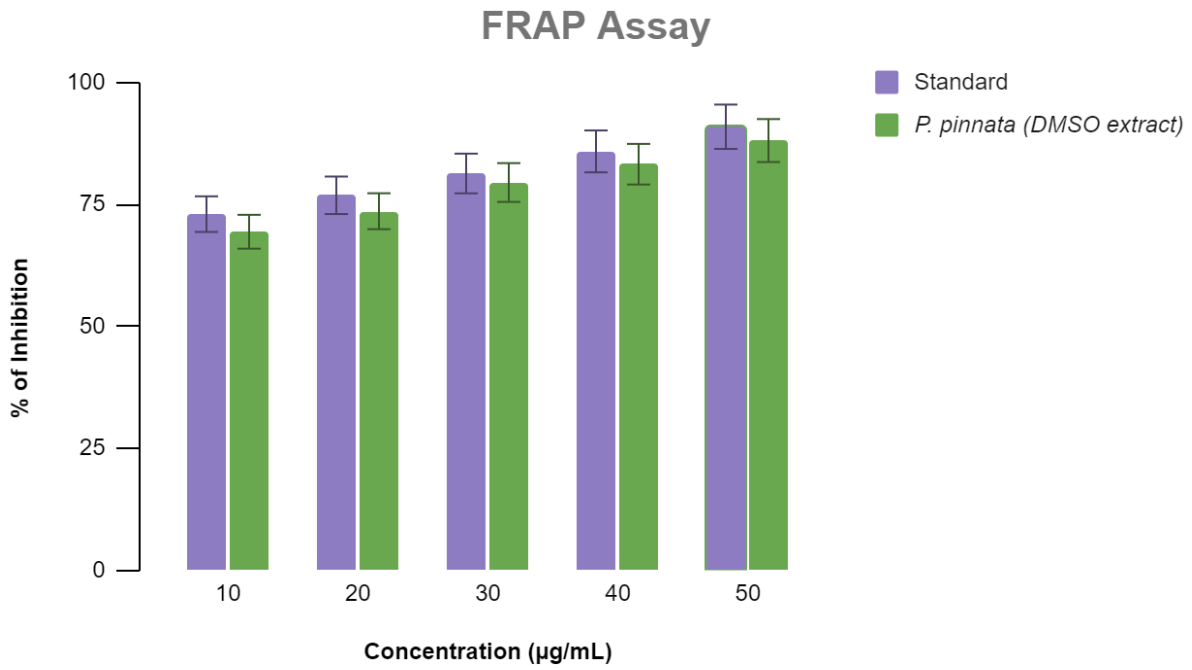


Fig. 4 Ferric reducing antioxidant power assay of *P. pinnata*. This figure illustrates the FRAP assay results, comparing the percentage inhibition of ferric ions at different concentrations (10–50 µg/mL) between the standard antioxidant and *Pongamia pinnata* (DMSO extract). The results indicate the antioxidant potential of the extract in a dose-dependent manner).

Table.3 Ferric Reducing Antioxidant Power (FRAP) Assay

Conc. (µg/mL)	**% of inhibition**

	Standard	*P. pinnata (DMSO extract)*
10	72.98	69.37
20	76.84	73.58
30	81.31	79.46
40	85.84	83.21
50	90.89	88.06

This table presents the percentage of inhibition observed in the FRAP assay at different concentrations (10–50 µg/mL) for both the standard antioxidant and *P. pinnata* (DMSO extract). The results indicate a dose-dependent increase in inhibition, demonstrating the extract's potential antioxidant activity comparable to the standard.

The FRAP assay evaluates the reducing power of a compound by measuring its ability to convert ferric ions. In this assay, *P. pinnata* extract demonstrated notable antioxidant activity, with values ranging from 69.37% at 10 µg/mL to 88.06% at 50 µg/mL. These results are similar to the standard antioxidant, which showed a peak activity of 90.89% (Table 3). The extract's ability to reduce ferric ions indicates its strong electron-donating potential, which is a characteristic of effective antioxidants.

3.1.4. ABTS Radical Scavenging Assay

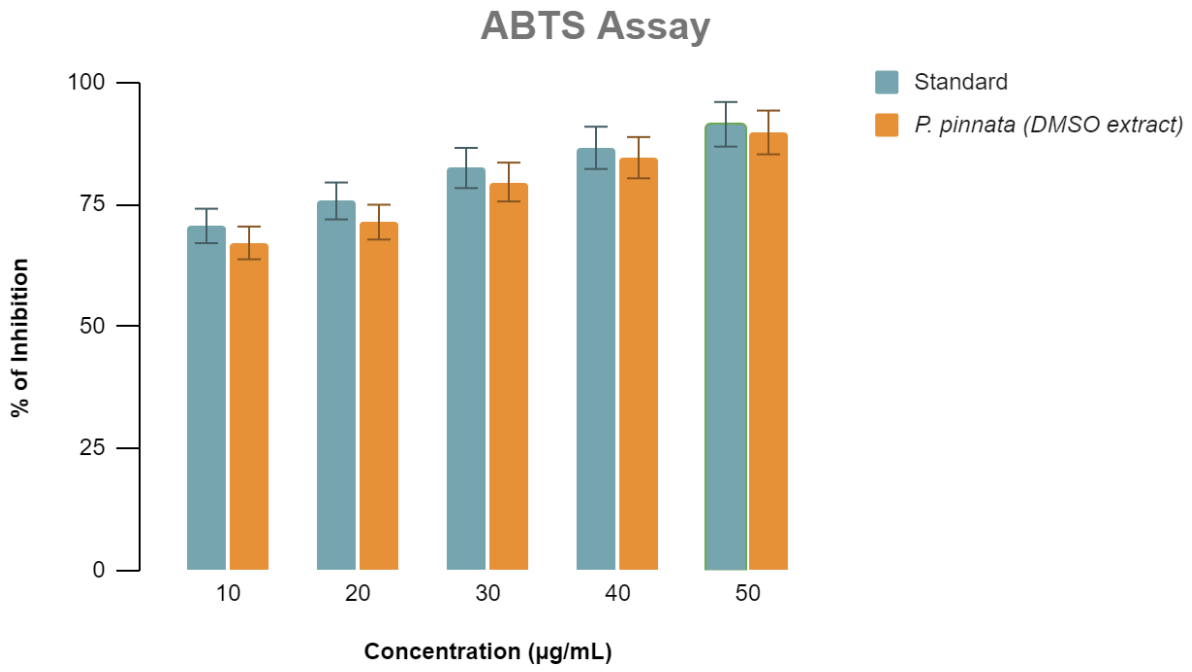


Figure.5 ABTS assay of *P. pinnata*

(The ABTS Assay graph illustrates the percentage of inhibition at varying concentrations (10–50 µg/mL) for both the standard antioxidant and *P. pinnata* (DMSO extract). The data indicate a concentration-dependent increase in inhibition, with the extract exhibiting antioxidant activity comparable to the standard).

Conc. (µg/mL)	% of inhibition	
	Standard	***P. pinnata (DMSO extract)***
10	70.56	67.08
20	75.68	71.34
30	82.43	79.56
40	86.57	84.55
50	91.39	89.72

Table.4 ABTS Assay Results of *P. pinnata.* This table presents the percentage of inhibition observed in the ABTS assay at different concentrations (10–50 µg/mL) for both the standard antioxidant and *P. pinnata* (DMSO extract). The results indicate a concentration-dependent increase in inhibition, demonstrating the extract's antioxidant activity comparable to the standard**.**

The ABTS assay measures the ability of antioxidants to neutralize the ABTS radical cation (ABTS•+). *Pongamia pinnata* extract showed significant scavenging activity, with percentages ranging from 67.08% at 10 µg/mL to 89.72% at 50 µg/mL. These values were slightly lower than the standard antioxidant, which reached 91.39% at the highest concentration (Table 4). These results highlight the extract's strong potential to interact with and neutralize charged radicals, reinforcing its antioxidant properties.

3.1.5. Nitric Oxide (NO) Scavenging Assay

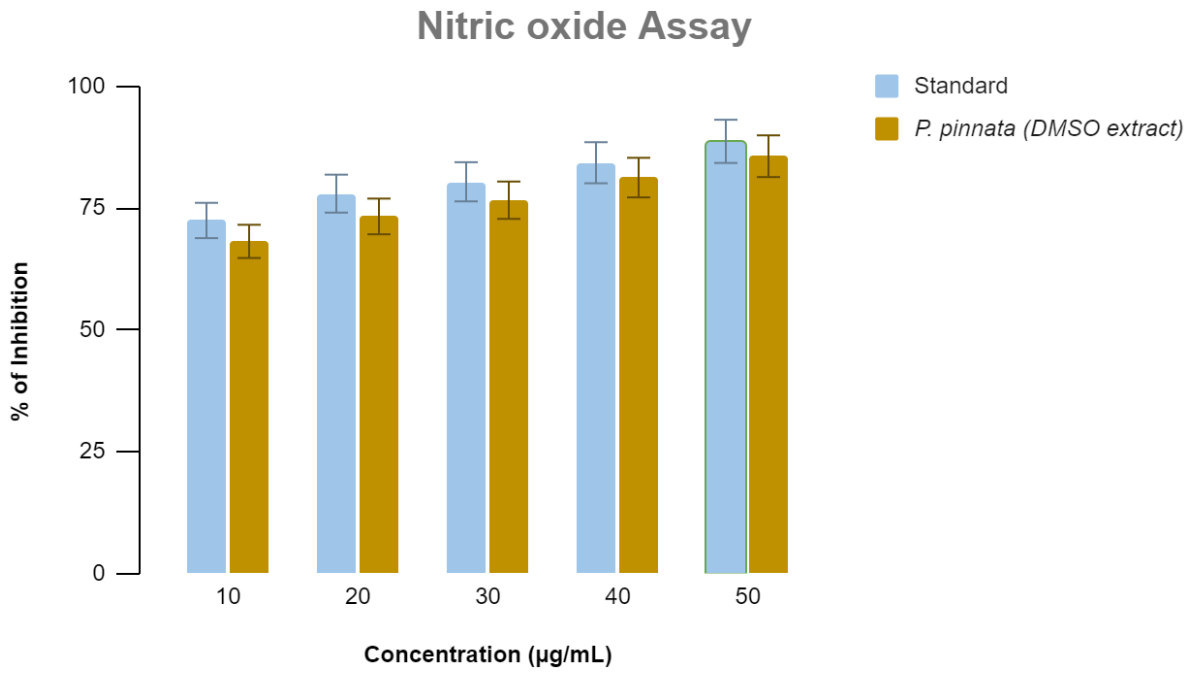


Figure.6 Nitric Oxide scavenging assay. This figure illustrates the percentage of inhibition observed in the NO scavenging assay at different concentrations (10–50 µg/mL) for both the standard antioxidant and *P. pinnata* (DMSO extract). The results show a dose-dependent increase in inhibition, highlighting the extract's potential nitric oxide scavenging activity comparable to the standard).

Table.5 Nitric Oxide (NO) Scavenging Assay Results

Conc. (µg/mL)	% of inhibition	
	Standard	*P. pinnata (DMSO extract)*
10	72.43	68.14
20	77.94	73.26
30	80.37	76.59
40	84.28	81.23
50	88.67	85.62

The table presents the percentage of inhibition observed in the NO scavenging assay at different concentrations (10–50 µg/mL) for both the standard antioxidant and *P. pinnata* (DMSO extract). The results indicate a concentration-dependent increase in inhibition, demonstrating the extract's potential NO scavenging activity, which is slightly lower than that of the standard antioxidant.

Nitric oxide is a reactive molecule involved in various physiological processes but can contribute to oxidative stress under pathological conditions. The nitric oxide scavenging activity of the Pongamia pinnata extract was concentration-dependent, starting at 68.14% at 10 µg/mL and reaching 85.62% at 50 µg/mL. These values were slightly lower than those of the standard antioxidant, which exhibited 88.67% activity at the highest concentration (Table.5). The ability of the extract to neutralize nitric oxide radicals suggests its potential in preventing nitrosative stress and related diseases.

Comparative Analysis and Implications

Across all assays, the Pongamia pinnata extract exhibited robust antioxidant activity, which was consistently close to that of the standard antioxidant. The slight variations in activity across assays are likely due to differences in the mechanisms of free radical neutralization or the specific compounds responsible for the activity. Nonetheless, the data confirm that the extract contains potent bioactive compounds capable of neutralizing a wide range of free radicals, thereby reducing oxidative stress. The antioxidant activity observed in the extract is attributed to the presence of phytochemicals, such as flavonoids, phenolic compounds, and tannins, which are known to exhibit strong free radical scavenging and reducing properties. These compounds are likely responsible for the extract's ability to interact with both charged (e.g., ABTS, nitric oxide) and uncharged radicals (e.g., DPPH, H2O2).

3.2. Anti-microbial assay

Figure.7 Anti-Microbial Assay of *Pongamia pinnata* (DMSO Extract)**.** The figure displays the antimicrobial activity of *P. pinnata* (DMSO extract) against selected microbial strains. The zones of inhibition observed at different concentrations (25 µg, 50 µg, and 100 µg) indicate the extract's effectiveness in inhibiting microbial growth. The variation in inhibition zones suggests a dose-dependent response, highlighting the extract's potential as an antimicrobial agent.

Table.6 Antimicrobial Activity of *P. pinnata* (DMSO Extract) against selected microorganisms

Organisms	**25 µg/mL**	**50 µg/mL**	**100 µg/mL**	**Standard**
S. aureus	11	12	17	40
Lactobacillus sp	9	9	9	40
C. albicans	11	12	13	9
S. mutans	9	11	14	50

This table presents the antimicrobial activity of *P. pinnata* (DMSO extract) at different concentrations (25 µg/mL, 50 µg/mL, and 100 µg/mL) against various microbial strains, including *S. aureus, Lactobacillus sp, C. albicans,* and *S. mutans.* The inhibition zone (measured in mm) increases with concentration, indicating a dose-dependent effect. The standard antibiotic used for comparison exhibited significantly higher inhibition zones.

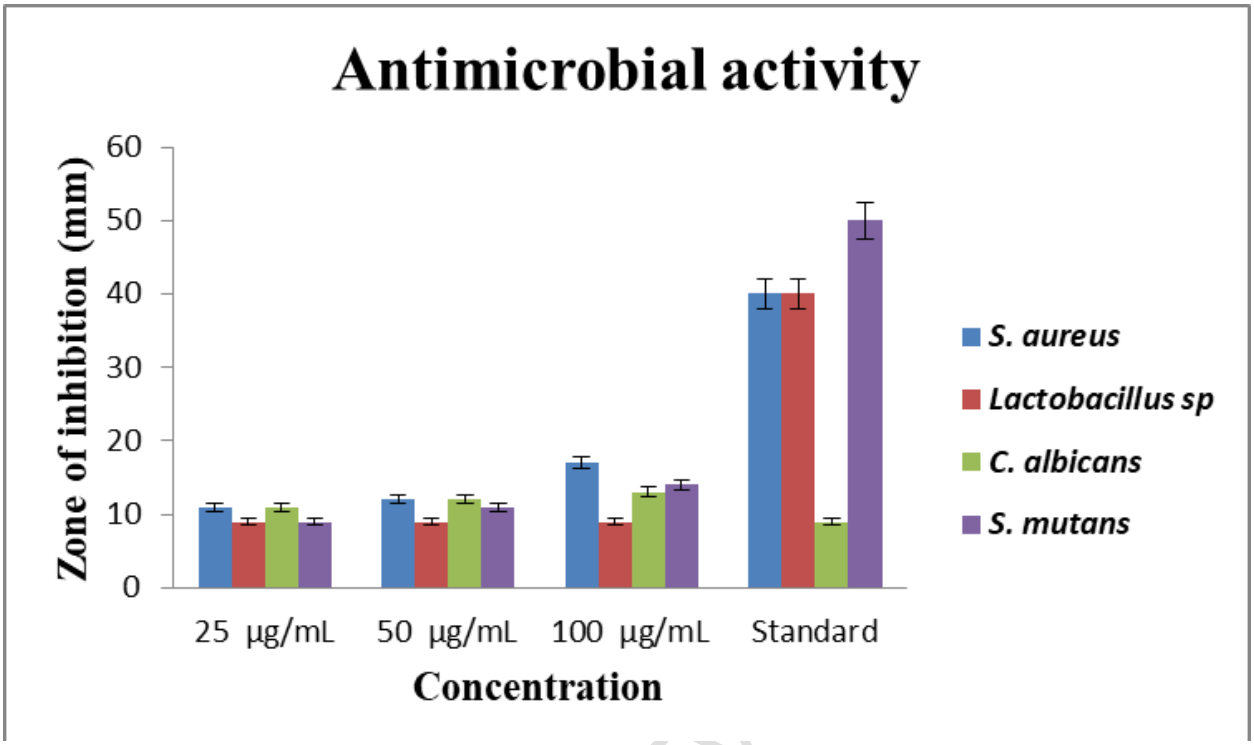


Figure. 8 Antimicrobial Activity of *Pongamia pinnata* Flower Extract

(This bar graph illustrates the antimicrobial activity of *Pongamia pinnata* flower DMSO extract against selected oral pathogens (*S. aureus, Lactobacillus sp, C. albicans,* and *S. mutans*). The inhibition zones (measured in mm) were recorded at different extract concentrations (25 µg/mL, 50 µg/mL, and 100 µg/mL), showing a dose-dependent increase in activity. The standard antibiotic demonstrated significantly higher inhibition zones compared to the extract). The antimicrobial activity of Pongamia pinnata flower DMSO extract against oral pathogens varied depending on the microorganism tested. The extract exhibited a dose-dependent increase in inhibition zones against Staphylococcus aureus, with values ranging from 11 mm at 25 µg/mL to 17 mm at 100 µg/mL. However, this activity was significantly lower than the standard antibiotic, which had a 40 mm inhibition zone. Against Lactobacillus sp., the extract showed minimal activity, with a constant inhibition zone of 9 mm across all concentrations, suggesting limited effectiveness against this bacterium. Interestingly, the extract demonstrated moderate antifungal activity against Candida albicans, with inhibition zones of 11 mm, 12 mm, and 13 mm at increasing concentrations. Notably, this activity surpassed that of the standard, which had a lower inhibition zone of 9 mm, indicating potential antifungal properties. For Streptococcus

mutans, the extract showed a gradual increase in inhibition, reaching 14 mm at 100 µg/mL, though it was still much weaker than the standard, which exhibited a 50 mm inhibition zone(Table.6) (Figure.8). Overall, Pongamia pinnata flower extract displayed promising antimicrobial potential, particularly against C. albicans, suggesting its usefulness as a natural antifungal agent. However, its antibacterial activity against S. aureus and S. mutans was moderate, and it had little to no effect on Lactobacillus sp. Further studies, including mechanism-based evaluations and formulation improvements, could enhance its efficacy and potential therapeutic application.

3.3. Anti-inflammatory and cytotoxic activity

3.3.1. BSA assay

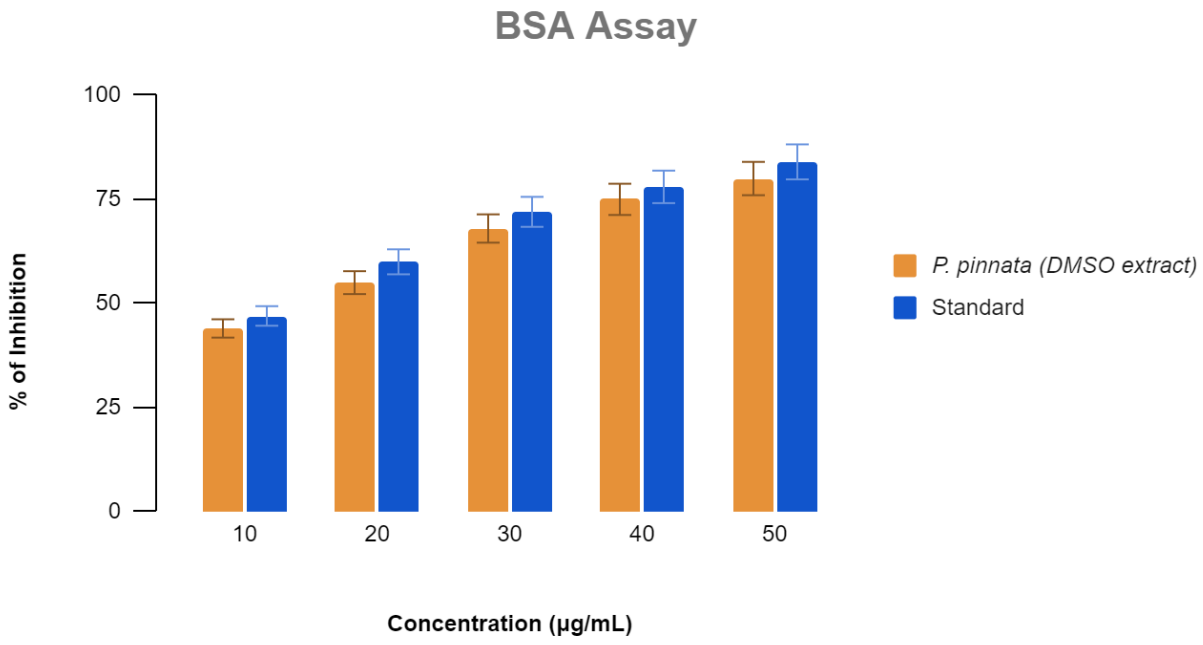


Figure. 9 BSA assay for protein denaturation inhibition. This graph represents the percentage inhibition of protein denaturation by *P. pinnata* DMSO extract compared to the standard across different concentrations (10–50 µg/mL). The results indicate a dose-dependent increase in inhibition, with the extract showing significant activity, though slightly lower than the standard. Error bars represent standard deviations from triplicate experiments.

Table 7 This table represents percentage inhibition of protein denaturation by *P. pinnata* DMSO extract at different concentrations (10–50 µg/mL) compared to the standard.

BSA		Concentration (µg/mL)				
		10	**20**	**30**	**40**	**50**
% of inhibition	*P. pinnata (DMSO extract)*	44	55	68	75	80
	Standard	47	60	72	78	84

The anti-inflammatory activity of *P. pinnata* flower extracts were evaluated using an in vitro BSA assay, with diclofenac serving as the standard. The results showed that Diclofenac achieved 100% inhibition at a concentration of 50 µg, while *P. pinnata* flower extracts demonstrated 84% inhibition at the same concentration, with a concentration-dependent effect (Table 7). This suggests that *P. pinnata* may exhibit comparable anti-inflammatory and cytotoxic effects to Diclofenac, potentially with fewer complications than the conventional drug.

3.3.2. Egg Albumin (EA) Denaturation Assay

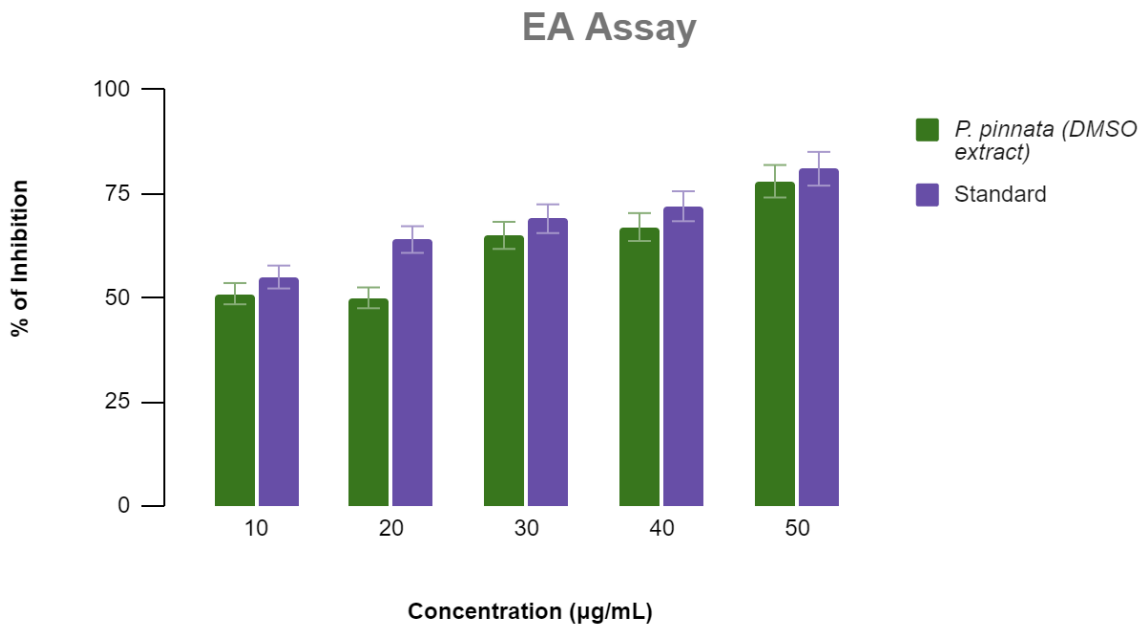


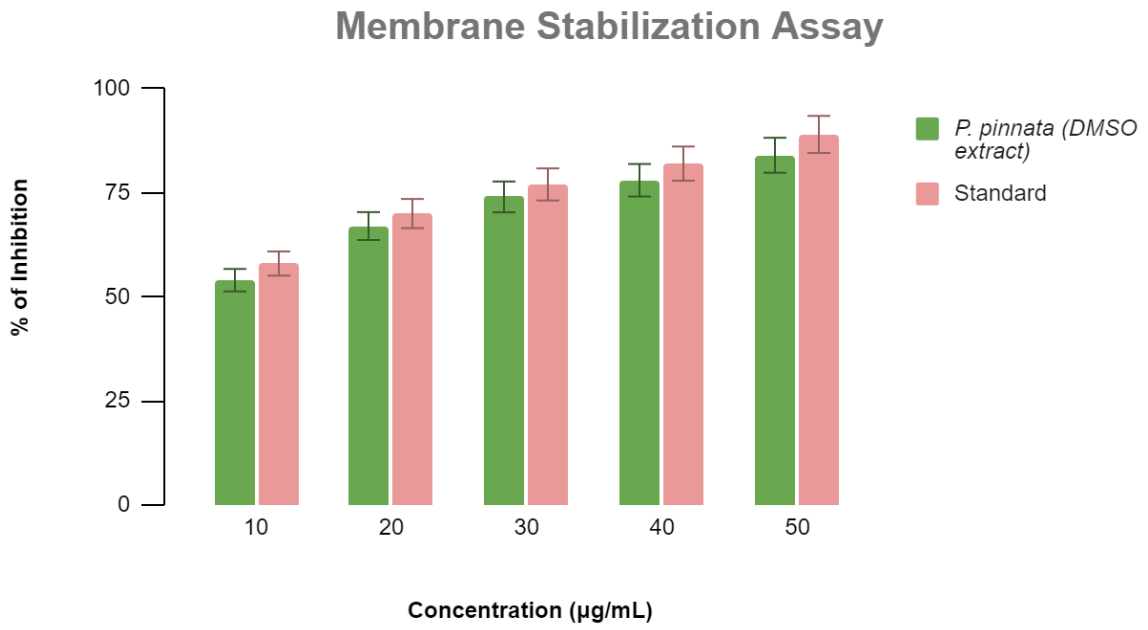
Fig. 10 EA Assay showing the percentage inhibition of erythrocyte membrane destabilization by *P. pinnata* DMSO extract at different concentrations (10–50 µg/mL) compared to the standard.

Table 8 Egg Albumin Assay results showing the percentage inhibition of protein denaturation by *P. pinnata* DMSO extract at different concentrations (10–50 µg/mL) in comparison with the standard.

Egg albumin		**Concentration (µg/mL)**				
		10	**20**	**30**	**40**	**50**
% of inhibition	*P. pinnata (DMSO extract)*	51	60	65	67	78
	Standard	55	64	69	72	81

Using the EA denaturation assay, the *P. pinnata* DMSO extract's anti-inflammatory properties were determined. The extract was tested at various concentrations and compared with diclofenac the standard. The results, which are displayed in Figure 2b, show that the P. pinnata extract reduced 51% of protein denaturation at a concentration of 10 µg/mL. By comparison, diclofenac also produced a 55% inhibition. The *P. pinnata* DMSO extract demonstrated a similar 78% inhibition to the diclofenac, which showed an 81% inhibition at a greater dose of 50 µg/mL(Table.8). Based on the EA denaturation experiment, these results indicate that the *P. pinnata* DMSO extract has strong anti-inflammatory properties and cytotoxic effect that are comparable to the effects of the standard diclofenac.

3.3.3. Membrane stabilization assay



by *P. pinnata* DMSO extract at different concentrations (10–50 µg/mL) compared to the standard.

Table.9 Membrane stabilization assay results representing the percentage inhibition of haemolysis by *P. pinnata* DMSO extract at varying concentrations (10–50 µg/mL) in comparison with the standard.

MSA		Concentration (µg/mL)				
		10	**20**	**30**	**40**	**50**
% of inhibition	*P. pinnata (DMSO extract)*	54	67	74	78	84
	Standard	58	70	77	82	89

The *P. pinnata* DMSO extract used in this investigation showed moderate to high membrane stabilizing levels in a concentration-dependent method. The extract showed higher membrane stabilizing action in comparison to the standard. Because membrane stabilization stops activated neutrophils from releasing membrane components like proteases, it is essential for reducing inflammatory reactions. This is essential because the release of these chemicals into the

extracellular space might induce further harm to tissues which cause inflammation and further cytotoxicity. Stabilizing cell membranes is the way by how steroidal anti-inflammatory drugs stop this process (Table 9). The capacity to prevent the lysis of the HRBC membrane is often used as a sign of cytotoxic and anti-inflammatory actions since it resembles the lysosomal membrane.

4. Discussion

This study investigated the antioxidant, anti-inflammatory, antimicrobial, and cytotoxic properties of *P. pinnata* flower extract dissolved in DMSO, revealing promising bioactivity and suggesting its potential for therapeutic use. The extract's antioxidant capacity was evaluated through several in vitro assays, including DPPH, H_2O_2, FRAP, ABTS, and nitric oxide scavenging tests. The extract demonstrated strong free radical scavenging abilities, with its efficacy increasing in a concentration-dependent manner. In the DPPH assay, the inhibition ranged from 64.28% at 10 µg/mL to 90.12% at 50 µg/mL, nearly matching the standard antioxidant (93.15% at 50 µg/mL). The H_2O_2 scavenging assay showed a similar trend, with activity increasing from 48.1% at 10 µg/mL to 86.2% at 50 µg/mL. The FRAP assay confirmed the extract's ability to reduce ferric ions, showing 88.06% inhibition at 50 µg/mL, compared to the standard (90.89%). These findings suggest the presence of bioactive compounds, such as flavonoids and phenolic compounds, which likely contribute to the extract's potent antioxidant properties. The antimicrobial potential of the *P. pinnata* flower extract was tested against several oral pathogens, including *Staphylococcus aureus*, *Streptococcus mutans*, *Candida albicans*, and *Lactobacillus* sp. The extract exhibited moderate antibacterial activity, with a 17 mm inhibition zone for *S. aureus* at 100 µg/mL, which was less than the standard antibiotic (40 mm). For *S. mutans*, the inhibition zone was 14 mm at 100 µg/mL, suggesting moderate antibacterial potential. The extract showed notable antifungal activity against *C. albicans*, with a 13 mm inhibition zone at 100 µg/mL, surpassing the standard (9 mm). However, its effect against *Lactobacillus* sp. was minimal (9 mm at all concentrations), indicating selective antimicrobial activity. These findings suggest that *P. pinnata* contains bioactive compounds with potential antibacterial and antifungal properties, making it a candidate for natural antimicrobial

formulations. The anti-inflammatory effects of the extract were assessed using BSA denaturation, egg albumin denaturation, and membrane stabilization assays. The extract significantly inhibited protein denaturation, with BSA denaturation reaching 80% at 50 µg/mL, which is close to the 84% inhibition seen with diclofenac. In the egg albumin denaturation test, the extract showed 78% inhibition at 50 µg/mL, compared to diclofenac's 81%. The membrane stabilization assay revealed 84% inhibition at 50 µg/mL, slightly lower than the 89% inhibition by diclofenac. These results suggest that *P. pinnata* flower extract exhibits anti-inflammatory effects similar to conventional NSAIDs, likely by stabilizing cell membranes and inhibiting the release of inflammatory mediators. Cytotoxicity was evaluated using membrane stabilization and protein denaturation assays, showing a dose-dependent effect. The extract exhibited inhibition rates of 68% to 84% in the membrane stabilization test, indicating moderate cytotoxicity when compared to the standard drug. In the egg albumin assay, the inhibition increased from 51% at 10 µg/mL to 78% at 50 µg/mL, suggesting controlled cytotoxicity. These findings imply that while *P. pinnata* exhibits some cytotoxic effects, they are lower than those of conventional drugs, indicating a potentially safer profile for therapeutic applications.

5. Conclusion

The results of this study highlight the significant antioxidant, antimicrobial, and anti-inflammatory properties of *Pongamia pinnata* flower extract, along with moderate cytotoxic effects. The antioxidant activity of the extract was found to be nearly on par with that of standard antioxidants, while its anti-inflammatory effects were comparable to those of diclofenac. The extract exhibited notable antimicrobial activity, particularly against *Candida albicans*, indicating its potential for antifungal applications. With its diverse pharmacological profile, *P. pinnata* flower extract shows promise as a natural therapeutic agent for treating inflammatory disorders and microbial infections. To fully assess its therapeutic potential, additional research, including in vivo studies and investigations into its mechanisms of action, is needed for further drug development.

Author contributions

Nantha Kumar Lingam: Data curation, writing original draft, Investigation, Resources, Validation; Hemapriya Thirugnanam: Validation, Formal analysis, Review & writing;

Vijayabharathi S: Data Curation, Resources, writing original draft, Methodology, Conceptualization; Saraswati Patel: Supervision, Review & writing.

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Ethical Approval

There is no ethical issue

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Conflicts of interest

The authors declare that they have no conflict of interest.

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