

## ***In vitro* Anti-Inflammatory and Antioxidant Activity of Seed Ethanolic Extract of Pongamia pinnata**

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**Pongamia pinnata** Merr., a member of the Fabaceae family, is extensively spread throughout tropical Asia. Historically, several parts of *P. pinnata* have been utilised in the indigenous medicinal systems of several cultures. Anti-inflammatory and antioxidant properties are basic requisites in treating any oral mucosal lesions. This study aims to investigate the antioxidant and anti-inflammatory properties of these leaf extracts. *pinnata* seeds were collected in Chennai, Tamil Nadu, India. For the extraction, shade-dried *P. pinnata* plant seeds were utilized. *P. pinnata* seed ethanolic extract was analysed qualitatively for the content of phenols, tannins, saponins, proteins, and acids, among other phytochemicals. Using the DPPH test and suppression of albumin denaturation, the anti-oxidant and anti-inflammatory activity of *P. pinnata* seed extract was determined. The phytochemical analysis of *P. pinnata* seed extract revealed the presence of phenols, tannins, saponins, proteins, and acids. The anti-oxidant and anti-inflammatory activity of *P. pinnata* seed extract was considerably higher at 500 g, as shown by the DPPH test and albumin denaturation inhibition. The results of this study show that *P. pinnata* seed extracts have both anti-inflammatory and antioxidant properties. Considering the results, *P. pinnata* shows high potential for management of oral mucosa lesions. Further clinical research needs to be done to analyse the effect of these properties.

**Keywords:** Anti inflammatory; Antioxidant; Ethanolic Extract; *P. pinnata*; Seed.

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The functional food and nutraceutical potential of phytochemical therapeutics is generating a great deal of interest today<sup>1</sup>. Bioactive compounds in these natural compounds contain anticarcinogenic action and provide several health-promoting benefits<sup>2</sup>. Numerous medicinal plants are known to generate bioactives with antioxidant and antibacterial properties. The phenolic acids, which hinder the growth of infections and

cause minimal damage to host cells, are also intriguing prospects for the development of novel antimicrobial medications. As a result, there is a rising interest in the development of several plant-derived medicines with different biological activities for the treatment of diverse infectious illnesses<sup>3</sup>.

*Pongamia pinnata* (L.) Pierre, a member of the Fabaceae family, is extensively dispersed

Historically, the parts of *P. pinnata* were utilised in the indigenous medicinal systems of several cultures. It has been observed that several primary phenolic compounds are found in diverse plant sections<sup>4,5</sup>. Karanjin, a furanoflavone extracted from this plant's seeds, has superior therapeutic properties. Extracts of the seed oil of *P. pinnata* were active against both Gram-positive and Gram-negative bacteria<sup>67</sup>.

Oral mucosal lesions, also known as OML, are any abnormal changes that occur on the surface of the oral mucosa. These changes can manifest as pigmented, ulcerative, red and white characteristics, or any swelling or developmental fault variations. Oral mucosal ulceration is an inflammatory lesion caused due to disintegration of the oral epithelium.<sup>8,9,6</sup> It has been established that medicinal plants that are abundant in a number of different chemical ingredients are quite beneficial in the treatment of OML. Several oral mucosal lesions are treated by anti-inflammatory and antioxidants like retinol, lycopene etc.<sup>10</sup>. Upon inflammation, toxins are released that cause cell damage and the body produces substances that activate the immune system. About half of analgesics are anti-inflammatory medicines, which treat pain by reducing inflammation as opposed to opioids<sup>11</sup>. Diclofenac is the typical anti-inflammatory medication, and its negative effects have been recorded<sup>12</sup>.

In turn, the antioxidant capacity is dependent on the total amount of polyphenolic compounds, essential oils, and other components<sup>13</sup>. Herbs are regarded as an excellent source of natural antioxidants, although very little research has been conducted on their potential use as antioxidants<sup>14,15</sup>. The majority of studies have been conducted on these leaves, with the exception of their anti-inflammatory properties. Therefore, the purpose of this study is to extract the essence of *P. pinnata*'s therapeutic properties using ethanol as a solvent. This study's objective is to investigate the antioxidant and anti-inflammatory properties of the seed extracts of *P. pinnata* in invitro methods.

## MATERIALS AND METHODS

### Collecting and extracting plants

*P. pinnata* seeds were gathered in Chennai, Tamil Nadu, India. For the extraction, shade-dried

*P. pinnata* plant seeds were used. The ethanol extract was made by soaking the seed powder in water and then evaporating it. The collection of dry powder for subsequent examination.

### Evaluation of the phytochemical properties of *P. pinnata* seed extract

In this study, the content of phenols, tannins, saponins, proteins, and acids in the seed ethanolic extract of *P. pinnata* was examined qualitatively using phytochemical techniques (Harbone & Baxter, 1993).<sup>16</sup>

#### Test for phenols

In a test tube, a tiny amount of ethanolic extract is combined with 1 mL of water, 1 to 2 drops of iron III chloride (FeCl<sub>3</sub>) are added, and a black colour change is noticed.

#### Test for tannins

3 drops of lead sub-acetate solution were mixed with 1 ml of filtrate. Creamy-coloured gelatin consistency implies tannins.

#### Test for saponins

Foam test: After forcefully shaking a 1 ml sample of the extract with water, persistent foam was detected.

#### Test for proteins

One millilitre of ninhydrin was dissolved in one millilitre of acetone, followed by the addition of a little amount of extract. The presence of proteins led to the development of a purple hue.

#### Test for acids

This is measured by boiling 3.0 g of the powder in 50 cc of 10% sulfuric acid for 5 minutes; the resulting ammonia is then freed, distilled into 0.1 N acid, and titrated.

### DPPH radical scavenging efficacy of ethanolic *P. pinnata* seed extracts

Using the approach of Hatano *et al.*, the DPPH radical scavenging was evaluated (1989). Briefly, 1.0 ml of DPPH solution was added to 1.0 ml of extract at doses ranging from 0.1 to 0.5 mg/ml. After 50 minutes at room temperature, the mixture's activity was measured at 517 nm. As a standard, identical amounts of ascorbic acid were utilised. Using the following formula, the capacity to scavenge the DPPH radical was computed and represented as a percentage (%).

$$\% \text{ inhibition} = \left[ \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \right] \times 100$$

In an Albumin denaturation inhibition assay, ethanolic seed extracts of *P.pinnata* demonstrate an anti-inflammatory effect in vitro.

The anti-inflammatory efficacy was investigated using inhibition of albumin denaturation analysis, as determined by Leela Prakash and Mohan Dass's approach (2010). The pH of the reaction mixture, which contained the test extracts as well as a 1% aqueous solution of bovine albumin fraction, was adjusted by adding a small amount of 1N hydrochloric acid. After heating the sample extracts for 20 minutes at 51 degrees Celsius after having been incubated at 37 degrees Celsius for 20 minutes, the turbidity was measured at 660 nanometers. The experiment was carried out three times. The proportion of protein denaturation inhibition was computed as follows:

$$\% \text{ inhibition} = \left[ \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right] \times 100$$

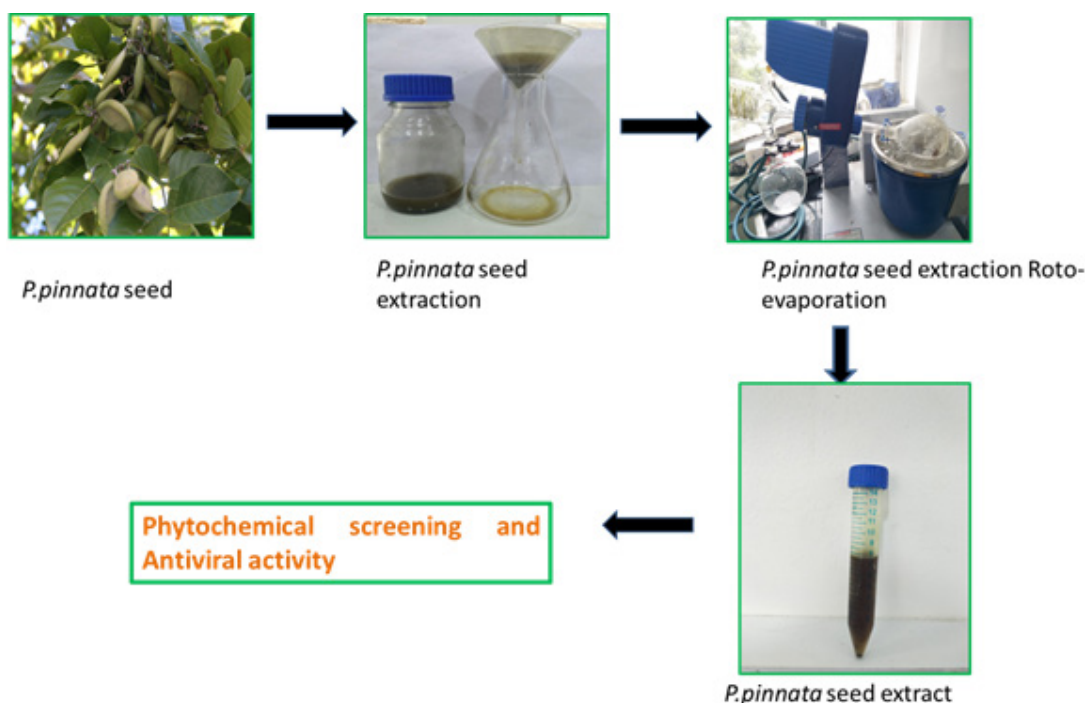
Statistical analysis using a one-way ANOVA in SPSS software v25, with a significance threshold of ( $p < 0.05$ ) considered.

## RESULTS

In the present study, results of the DPPH radical scavenging activity of *P.pinnata* seed ethanolic extract showed a significant ( $p < 0.05$ ) dose-dependent potential activity by inhibiting DPPH radical formation (figure 3). Inhibitory activity showed 10.74, 20.83, 36.71, 53.4 and 74.9% at 100, 200, 300, 400 and 500 $\mu$ g respectively. Protein denaturation inhibition activity of *P.pinnata* seed ethanolic extract showed a significant ( $p < 0.05$ ) dose-dependent potential anti-inflammatory activity (figure 4) such as 33,38,42,53 and 68% respectively whose activity was near to that of the standard drug diclofenac activity (14.8, 26.4, 37.1, 64.4 and 92%) and this study clearly indicates that *P.pinnata* seed extract exhibits a potential anti-inflammatory activity.

## DISCUSSION

In developing nations, pharmaceutical programmes continue to play a vital role as basic care therapeutic solutions. While phytochemical agents are predominantly used as a treatment



**Fig.1.** *P.pinnata* seed extraction preparation.

for minor diseases at home, several medical practitioners like Siddha, Ayurveda and Unani have gained attention and shown promising cures for chronic diseases<sup>17,18</sup>. Herbalists often do not treat acute psychological or physical illnesses; rather, the objective of herbal therapy is to promote long-term health improvements<sup>19</sup>.

The results were consistent with the previous study, which characterised *P.pinnata* leaf methanol extracts (400 mg/kg) as effective anti-

inflammatory agents, comparable to the present study<sup>20,21</sup>. The DPPH test is a standard technique for measuring the ability of antioxidant molecules to scavenge free radicals by neutralising the stable, coloured DPPH radical. The radicals generated by DPPH are neutralised by plant extracts containing antioxidants, offering an ambitious foundation for future *in vivo* investigations. According to studies, phytochemicals such as phenolics and flavonoids can donate hydrogen and neutralise DPPH radicals<sup>22,23,24</sup>.

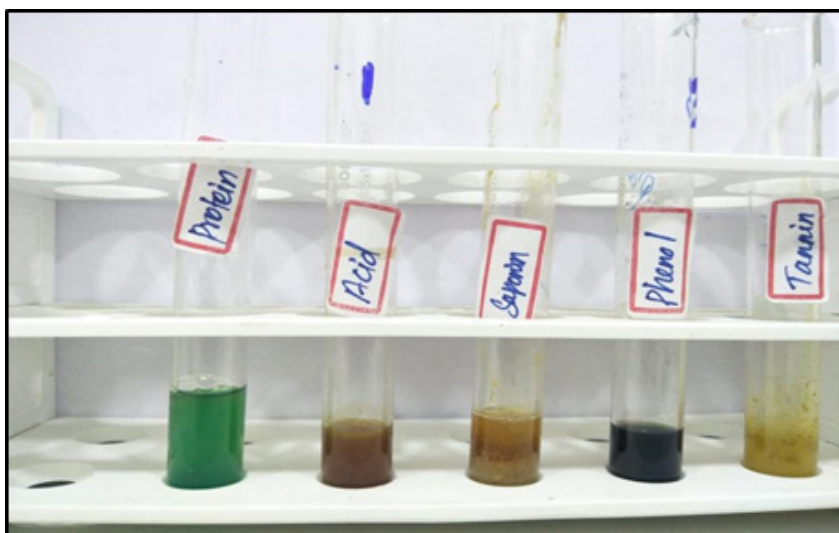


Fig. 2. Phytochemical screening of *P.pinnata* seed extract.

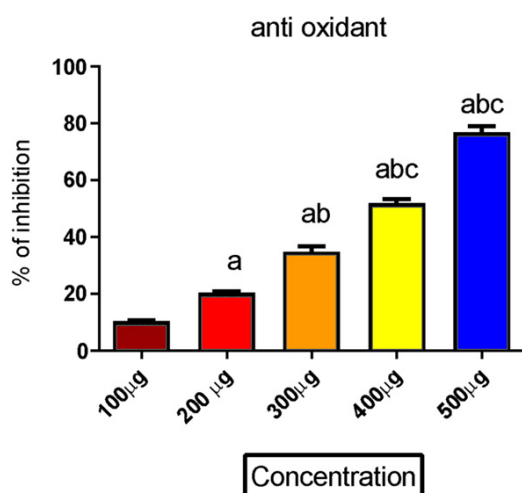


Fig. 3. In vitro antioxidant potential of *P.pinnata* seed ethanolic extract by DPPH radical scavenging activity

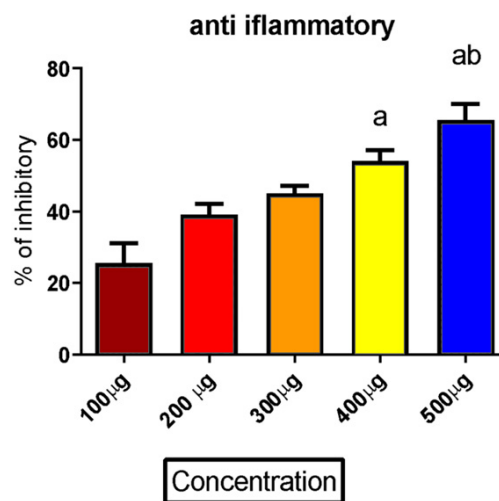


Fig. 4. In vitro anti-inflammatory potential of *P.pinnata* seed ethanolic extract by protein denaturation inhibition activity

Compared to the various concentrations, anti-inflammatory activities were clear in our investigation. Using the albumin denaturation assay, the strongest anti-inflammatory effect was discovered at 50µl. In an in vitro investigation, Srinivasan et al assessed the anti-inflammatory activity of a 70% ethanolic extract of *P.pinnata* leaves in rats and found significant anti-inflammatory activity without any ulcerogenic potential.<sup>25</sup> Singh et al aimed to assess anti-inflammatory potential of ethanolic seed extract of *P.pinnata* in rats and found the best anti-inflammatory activity against Bradykinin and prostaglandin E1 induced inflammation and minimal effects against histamine and serotonin induced inflammation<sup>26</sup>. Rekha et al also studied the characterization and anti-inflammatory properties of *P.pinnata* seed extract and found a significant increase in antioxidant and lipoxygenase inhibitory activity of ketone and oxide. The derivatives of *P.pinnata* showed higher anti-inflammatory activity in rat models<sup>7</sup>. Dinesh et al showed that a herbal combination of 3 herbal plants showed superior antioxidant and anti-inflammatory activities<sup>27,28</sup>.

Several oral lesions are treated by topical application of gels and topical agents with anti-inflammatory and antioxidant properties. Phytochemical agents like curcumin, aloe vera, etc show superior antioxidant and anti-inflammatory properties<sup>29</sup>. Curcumin is predominantly studied its therapeutic use of oral lesions and wound healing<sup>30,31</sup>. Plants like *P.pinnata* which are commonly and easily found and are being less explored in the field of medical research. Hence, an attempt was made to study its antioxidant and anti-inflammatory properties. The presence of metabolites like tannins, saponins, acids, phenols and proteins in *P.pinnata* is the reason for high anti-inflammatory and antioxidant properties. Further research is needed to implement the ethanolic seed extract of *P.pinnata* as a therapeutic agent for oral mucosal lesions.

### CONCLUSION

It was obvious from the investigation that the ethanolic seed extract of *P.pinnata* contained both antioxidant and anti-inflammatory properties. The anti-oxidant and anti-inflammatory properties of *P.pinnata* seed extract were considerably

higher at 500µg, as shown by the DPPH test and suppression of albumin denaturation, respectively. Additional research is required to confirm the therapeutic effects of these plant extracts. Further extensive research on the utilisation of *P.pinnata* seeds in the treatment of oral mucosal lesions using this plant's bioactives in in-vivo models is required in order to develop particular applications and synthesise novel and powerful medications of natural origin.

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### Conflict of Interest

No conflicts of interest with regard to the current work.

### Funding Sources

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