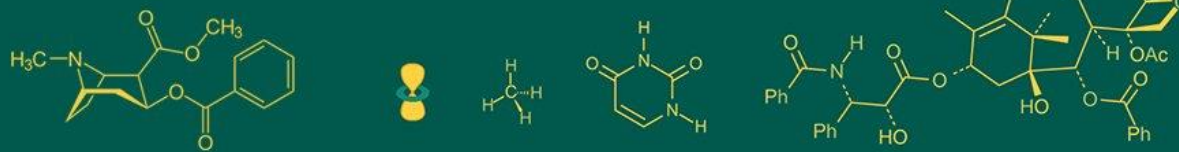


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In vitro protein denaturation inhibition property of *Pterospermum rubiginosum*

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Abstract

Non-steroidal anti-inflammatory drug (NSAID) are used widely to cure inflammatory conditions like sprains, arthritis and spondylitis. Some people experience side effects from these drugs, which led to the search of natural remedies. *Pterospermum rubiginosum* is known as a cure for sprains among the tribal healers of Kerala. The ethanol extract of bark and leaves were subjected to protein denaturation inhibition assay using diclofenac as standard drug. The inhibitory concentration (IC₅₀) values of standard, ethanol extract of bark (PRB) and ethanol extract of leaves (PRL) were 58.62, 82.87 and 180.34 µg/ml respectively. This indicates the efficacy of *Pterospermum rubiginosum* bark and leaf extract. As the concentration, increased there was an increase in efficacy of the extracts tested.

Keywords: *Pterospermum rubiginosum*, protein denaturation inhibition, natural remedies, anti-inflammatory

Introduction

Pterospermum rubiginosum belonging to the family Malvaceae is a medicinal plant used by the tribal healers of Western Ghats to cure fractures, sprains and wound. The *Kurichiya*, *Paniya*, *Kattunaika*, *Adiyan*, *Malampandaram*, and *Kani* tribes of Kerala use the bark of *Ellotti* along with various other ingredients to treat fractures, wounds, sprains, inflammation and pain (Prasad and Shyma, 2013; Mathew and George, 2013; and Latheef *et al.*, 2014) [8, 6]. It is said to have antioxidant, antinociceptive, anti-inflammatory and antimicrobial properties. An *in vitro* protein denaturation inhibition assay will help in getting scientific evidence in case of anti-inflammatory property of *Pterospermum rubiginosum*. The present study aims to evaluate the anti-inflammatory properties of the ethanol extract derived from both the bark and leaves of *Pterospermum rubiginosum* through an *in vitro* method called Protein denaturation inhibition assay. Additionally, it seeks to compare these effects with diclofenac sodium, a standard non-steroidal anti-inflammatory drug (NSAID), based on previous reports of its efficacy for similar purposes (Banerjee *et al.*, 2014) [3]. Diclofenac is one of the nonsteroidal anti-inflammatory drug (NSAID), functioning by impeding the body's synthesis of specific natural compounds responsible for inducing inflammation (Arrigoni-Martellie, 1977) [2]. It acts as the inhibitor of cyclooxygenase enzyme activity and has the potential to interact with the lipoxigenase enzyme pathway. With almost complete absorption, high protein-binding capacity, and excellent penetration into synovial fluid, diclofenac undergoes extensive metabolism. Comparative studies have demonstrated that diclofenac exhibits efficacy equivalent to, or greater than, aspirin and other NSAIDs in the treatment of rheumatic conditions like rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis and also possesses potent analgesic properties (Skoutakis *et al.*, 1988) [11].

Materials and Methods

Plant collection and extraction

The *Pterospermum rubiginosum* bark and leaf sample was collected from Wayanad region of Kerala. A voucher specimen of plant collected was deposited at Kerala Forest Research Institute, Peechi (Voucher No. 19367).

The bark and leaves of *Pterospermum rubiginosum* was cleaned and shade dried, till they could be pulverized with ease.

To obtain the extract, 5-10 g of powdered samples were extracted with ethanol in a soxhlet apparatus for 16-20 hours at 75 °C. The solvent was evaporated from the extract using a rotary evaporator and the extract was refrigerated for further use.

Protein denaturation inhibition assay

The study was carried out according to procedures described by Mizushima and Kobayashi (1968) [7] and Sakat *et al.* (2010) [9]. The reaction mixture (0.5 ml) consisted of 0.4 ml bovine serum albumin (3% aqueous solution) and varying test sample concentrations. The samples were incubated at 37 °C for 20 min, and 2.5 ml phosphate buffered saline (pH 6.3) was added to each tube and then heated at 80 °C for 10 min. Diclofenac was used as the standard. The absorbance was measured using a spectrophotometer at 660 nm. The rate of inhibition of protein denaturation was calculated as follows:

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

Results and Discussion

The present study found that the inhibition rate of ethanol extract of bark and leaves increased with the increase in concentration, which was similar to the trends shown by standard diclofenac which indicates the stabilisation of proteins. Up to a concentration of 50 µg/ml ethanol extract of bark exhibited higher inhibition compared to standard and ethanol extract of leaves. But, from 50 µg/ml the standard diclofenac had higher inhibition rate compared to bark and leaf extracts. The inhibitory concentration (IC₅₀) values of standard, ethanol extract of bark and ethanol extract of leaves were 58.62, 82.87 and 180.34 µg/ml respectively. From the IC₅₀ value it is clear that the standard was better among the three, followed by bark extract and leaf extract.

Prior research has demonstrated the therapeutic and anti-inflammatory qualities found in extracts from both the bark and leaves of this particular plant. According to Salam *et al.*, 2016 [10] *Pterospermum rubiginosum* leaf has analgesic, antinociceptive and anti-inflammatory properties. Similarly, Jacob and Sreejith (2019) [5] stated that methanolic extract of *Pterospermum rubiginosum* and *Pterospermum reticulatum* bark exhibited antiprotease activity at of 51.2% and 64.93% at 500 µg/ml respectively. Banerjee *et al.* (2014) [3] observed that the ethanol leaf extracts of *Mikania scandens* showed significant inhibition of protein denaturation at a concentration of 16000 µg/ml, exhibiting a 65% stronger inhibitory effect compared to the ethanol stem extract. This was attributed to the presence of alkaloids, tannins, flavonoids and steroids. Another study on *Ficus racemosa* also revealed that a highest inhibition rate of 27.71% and 27.65% was observed at a concentration of 1000 µg/ml in cold water and hot water extracts respectively. They noted that phytochemicals like phenols, flavonoids, tannins and phytosterols might be the reason for the anti-inflammatory property (Dharmadeva *et al.*, 2018) [4].

Phytochemical analysis of bark and leaves of *Pterospermum rubiginosum* have revealed the presence of biochemical compounds, such as flavonoids, tannins, and phenolic compounds, terpenoids and saponins in the plant, suggesting that these constituents, individually or in combination, contribute to its analgesic and anti-inflammatory effects (Anish *et al.*, 2021 and Salam, 2016) [1, 10]. The findings support the traditional use of this plant in treating painful and inflammatory conditions.

The egg albumin method offers an economical alternative for evaluating the anti-inflammatory potential of herbal medicine through denaturation technique, but further validation through additional studies is warranted. In conclusion, it can be affirmed that *Pterospermum rubiginosum* exhibits a verified anti-inflammatory effect according to the egg albumin denaturation method.

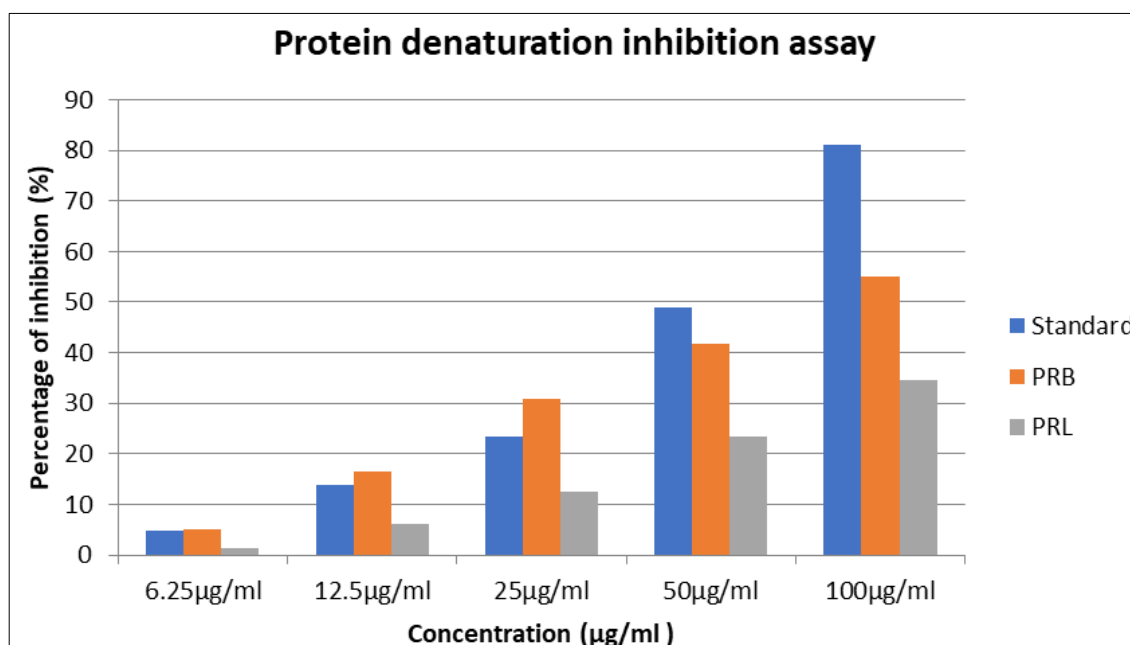


Fig 1: Rate of inhibition of protein denaturation in standard and plant extracts of *Pterospermum rubiginosum*

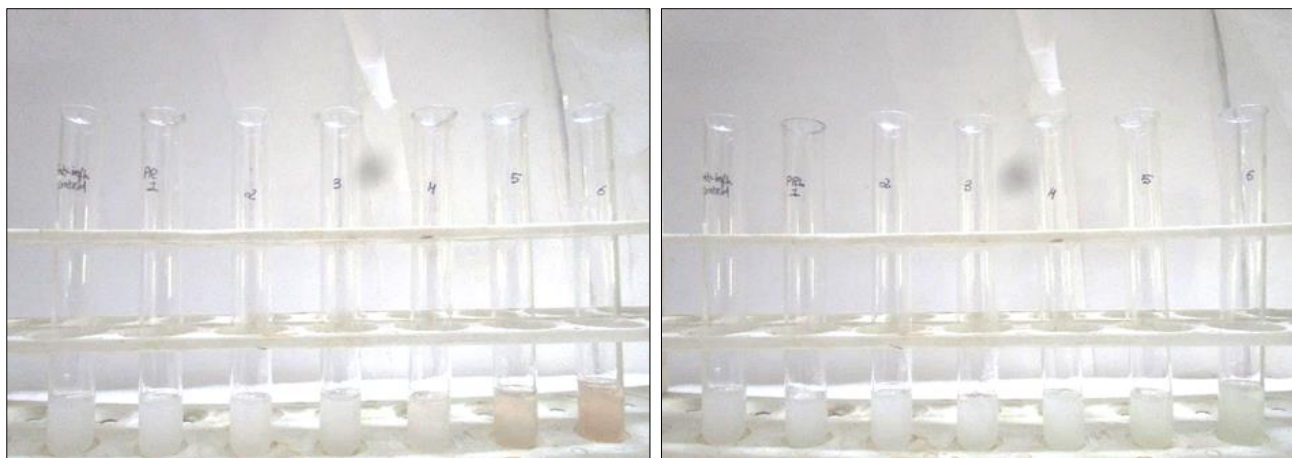


Fig 2: Protein denaturation inhibition assay in A) Bark extract B) Leaf extract

Conclusion

From the results it can be concluded that ethanol extract of *Pterospermum rubiginosum* bark and leaf extract has significant protein denaturation inhibition activity in *in vitro* analysis. Isolation of specific compounds involved in the inhibition of protein denaturation can be undertaken, which might be useful for the preparation of new formulations.

Acknowledgement

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