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IN VITRO ANTI INFLAMMATORY ACTIVITY OF *PTEROSPERMUM RUBIGINOSUM* BARK EXTRACT BY USING ALBUMIN DENATURATION METHOD

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ABSTRACT
Natural components present in any natural source have the potential to inhibit the pathways responsible for causing inflammation. It has gained huge demand as they tend to possess lesser side effects. *Pterospermum rubiginosum*, belonging to the family, Sterculiaceae, is a plant used in the treatment of bone fracture and its related complications. The use of different parts of *P. rubiginosum* has been in practice by the traditional people, particularly the tribal communities living in deep interior forests. However, only a little research has been performed on this drug to document any of its therapeutic capabilities. The present research was done with this in view and in an attempt to study anti-inflammatory potential by invitro protein denaturation activity. Different solvent extracts of the bark obtained by soxhelation method were tested for the activity. The concentration gradient of each extract was prepared using bovine serum albumin and phosphate buffered saline. These extracts were adjusted to pH 6.3 and incubated at 37^{o}C for 20 minutes. Diclofenac sodium was used as standard and the percentage inhibition of protein denaturation was calculated. The inhibition rate of bovine serum albumin denaturation for all the solvent extracts increased gradually with an increase in concentration. A commendable high rate of inhibition was observed in the ethanol extract compared to others which indicated the anti-inflammatory potential of the bark part of the plant that increases with the concentration of the extract.

KEYWORDS: Anti-inflammatory, bovine serum albumin denaturation, ethanolic extracts.

INTRODUCTION
The progression in the field of science and the latest technology has brought about tremendous changes in the medical treatment scenario. Inflammation is a defense mechanism of the body that is a result of external damage due to conditions like fracture of bone, infection, cancer, allergens, and many more. It is identified by appearances on the body in the form of redness, heat in the area around it, and swelling accompanied by certain physiological changes in the body. The enzymes are responsible for this inflammation and the denaturation of protein present in the enzymes reduces them. Denaturation of protein is brought about by the application of heat, reaction with acids, etc. whereby the secondary and tertiary structure of proteins are disoriented making them different from their actual form.[1,2] Therefore, the active site of the enzyme is lost and substrates are unable to attach resulting in the loss of its activity.[3] In modern medicine, NSAIDs are majorly responsible for the denaturation of protein, however, due to their adverse effects such as the formation of gastric ulcers, indigestion[4,5], medicinal plants have been gaining attention as an alternative source with good therapeutic activity. They are also found to be economical with lesser side effects.[6] The acceptance of medicines from plant sources and the identification of biologically active compounds from them followed by their isolation has led to the development of novel drugs from plant sources.[7,8] Plants are said to possess various therapeutic actions and the search for constituents responsible for it has been carried out for many years. The present research deals with the investigation of the anti-inflammatory potential of different solvents extract from the bark part of *P. rubiginosum* that thrives in the Western ghat region in India, against bovine serum albumin for its efficiency.

MATERIAL AND METHODS
Plant material
The plant part, that is the barks of *P. rubiginosum* were collected from the forest region in Wayanad, India. It was authenticated from the Department of Plant Genetic Resources, Indian Institute of Horticultural Research, Bengaluru, Karnataka.

Extraction

The bark part of the plant was washed thoroughly in tap water followed by distilled water to remove any further impurities that are likely to be present. They are air-dried at room temperature to obtain a uniform dried weight. The dried parts were then size reduced to a moderately coarse powder. About 250 grams of the powder was taken and subjected to successive Soxhlet extraction by solvents such as petroleum ether, chloroform, ethyl acetate, acetone, and ethanol. In all the cases the extract was filtered using a muslin cloth and then concentrated to get a semi-solid or syrupy consistency. These extracts were then subjected to denaturation of protein studies to determine which among them processed a good denaturation activity.

Standard and sample preparations

Different concentrations of samples such as 62.5µg/mL-500µg/mL from a stock solution of 10mg/mL were prepared by serial dilution. The standard diclofenac sodium was also prepared similarly. The test control consisted of bovine serum albumin fraction V (HI MEDIA, MUMBAI) and 0.05 mL of distilled water whereas the test solution consisted of 0.45 mL of bovine serum albumin and different concentrations of the samples. The product control consisted of 0.45 ml of distilled water and different concentrations of the samples.

Inhibition of protein denaturation

All the reaction mixtures were subjected to pH 6.3 using 1N HCl. They were incubated in a water bath at 37^{o}C for 20 minutes, and later, the temperature was increased to 57^{o}C for 3 minutes. Then, the reaction mixture was allowed to cool at room temperature for about 20 minutes. After cooling, 2.5 ml of phosphate buffer was added to the solutions. The absorbance was measured using a UV-VISIBLE spectrophotometer at 416nm. The percentage of inhibition of protein was determined with respect to the control by employing the following formula.

% inhibition= 100 - [((optical density of test solution – optical density of product control) ÷ optical density of test control)) ×100]

IC_{50} values of the standard and samples were determined using ED50 PLUS V 1.0 software.

Statistical Analysis

Data were expressed as mean ± SEM and were analyzed statistically by one way ANOVA procedures followed by using Dunnett's test. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

The anti-inflammatory activity of the bark extracts of *P. rubiginosum* was evaluated against the denaturation of the bovine serum albumin method. The extracts of petroleum ether, chloroform, acetone, ethyl acetate, and ethanol were evaluated for comparison as to which extract can exhibit the maximum inhibitory activity of protein denaturation at concentrations range of 62.5 µg/mL, 125µg/mL, 250µg/mL, and 500µg/mL. It was observed that the ethanol extract at all concentrations gave higher inhibitions when compared to other extracts. The highest rate of inhibition of ethanol extract was found at a concentration of 500 µg/mL. The different conditions of inflammation, such as rheumatic arthritis, diabetes, and fracture of bones cause the denaturation of proteins and thereby inflammation. The method of inhibition of protein denaturation results in the inhibition of the inflammatory process.[9] The current research employs an NSAID which is used as a standard drug. These have their mechanism of action by hindering the cyclooxygenase enzyme activity, also its use is accompanied by certain side effects like ulceration, haemorrhage.[10] The anti-inflammatory activity of ethanolic bark extract of *P. rubiginosum* has been reported in this study for the first time. The only reports are of the traditional use of the plant by the tribal communities leaving in the forests.[11] However, there are only a few scientific studies on this plant and the present study supports the traditional use of the plant as an agent responsible for the regeneration of fractured bones. The presence of biologically active compounds like flavonoids, phenolic compounds, etc. in the plant may be responsible for the anti-inflammatory effects. In this study, it was observed that the rate of inhibition of protein denaturation increases gradually with the increase in the concentration of all the extracts that were tested and also the standard used. It was also found that the highest concentration of 500 µg/mL of the bark ethanol extract gave a significant inhibition of 88.94% ($p < 0.05$) compared to the standard at 89.36% (Table 1 and Table 2) at the same concentration. When denaturation of protein occurs, the autoantigens are produced that aid in the reduction of inflammation caused due to rheumatic arthritis. This method of anti-inflammatory potential by bovine serum albumin is a cheap and reliable method of study in understanding the anti-inflammatory potential of medicinal herbs, moreover, further studies are recommended to identify the components responsible for the activity. These studies revealed that *P. rubiginosum* has a good potential as an anti-inflammatory against bovine serum albumin denaturation technique and therefore the bark extract of ethanol possesses a good in vitro anti-inflammatory effect.

IC_{50} values of diclofenac sodium against bovine serum protein denaturation is 61.55µg/mL, petroleum ether extract IC_{50} value is 165.39µg/mL, chloroform extract value is 106.22µg/mL, acetone is 236.39µg/mL, ethyl acetate is 341.99µg/mL and that of ethanol is 69.88µg/mL.

Table 1: % rate of inhibition of Diclofenac sodium (Standard).

Concentrations (µg/mL)	OD of test solution	OD of product control	Percentage of inhibition
62.5	0.22	0.19	50.77
125	0.03	0.01	74.27
250	0.05	0.04	81.13
500	0.08	0.08	89.36

OD of test control = 0.0583, values are means of three replicates ±SD

Table 2: % rate of inhibition of different solvent extracts.

Concentrations (µg/mL)	OD of test solution	OD of product control	Percentage of inhibition
Acetone extract			
62.5	0.052	0.039	36.18
125	0.060	0.050	46.73
250	0.089	0.079	50.25
500	0.098	0.092	66.33
Chloroform extract			
62.5	0.022	0.010	38.69
125	0.036	0.028	59.80
250	0.048	0.041	64.82
500	0.072	0.069	80.40
Ethyl acetate extract			
62.5	0.028	0.011	12.56
125	0.039	0.024	24.62
250	0.059	0.048	47.74
500	0.072	0.065	64.82
Petroleum ether extract			
62.5	0.032	0.020	40.70
125	0.057	0.047	49.75
250	0.068	0.059	55.28
500	0.097	0.092	74.37
Ethanol extract			
62.5	0.034	0.023	43.72
125	0.052	0.043	54.77
250	0.071	0.065	73.87
500	0.096	0.092	88.94

OD of test control = 0.0199, values are means of three replicates ±SD

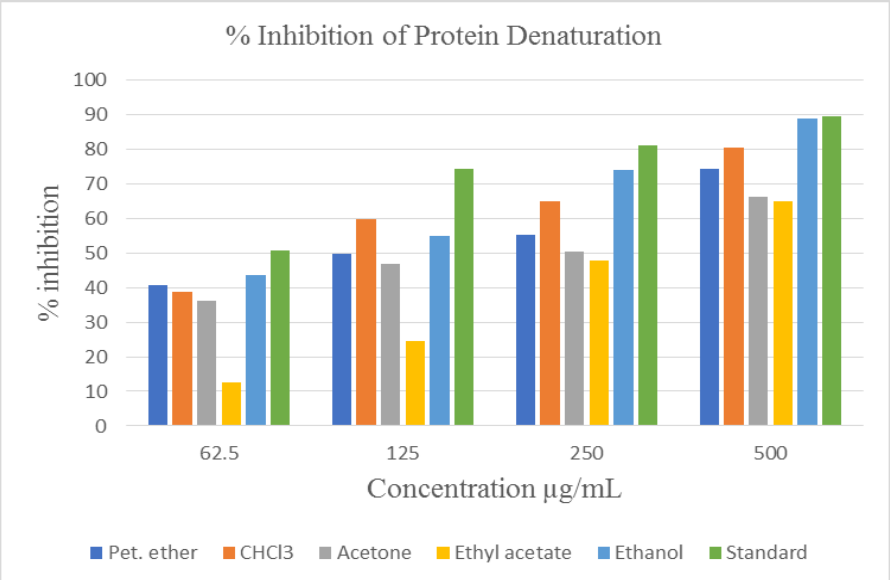

Fig. 1: % Inhibition of Protein Denaturation by Standard and samples.

CONCLUSION

P. rubiginosum bark contains those constituents responsible for the anti-inflammatory potential against the bovine serum albumin technique. The active constituents may be present at a higher proportion in ethanol extract when compared to petroleum ether, chloroform, acetone, and ethyl acetate extracts. It is also observed that the ethanol extract has almost similar potential as that of the standard drug, diclofenac sodium.

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