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RESEARCH ARTICLE

IN VITRO ANTI-INFLAMMATORY ACTIVITY OF DIFFERENT VARIETIES OF *MUSA SAPIENTUM* (BANANA) PEEL EXTRACT

Ramya, S., *Saraswathi, U. and Malathi, M.

Department of Biochemistry, PSG College of Arts and Science, Coimbatore-641014, Tamilnadu, India

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ABSTRACT

In the present study, the 50% hydro ethanolic extract of different varieties of *Musa sapientum* (Musa spp.) peel such as Nendran (Musa spp. - French Plantain - AAB), Pachanadan (Musa spp. - Pachanadan - AABS), Rasthali (Musa spp. - Rasthali – AAB and Sevvazhai (Musa spp. - Red banana - AAA) was evaluated for *in vitro* anti-inflammatory activity. Inhibition of protein denaturation, RBC membrane stabilization and proteinase inhibitory activity was assessed at a concentration of 0.1g/ml for all the varieties. The standards used in this study include aspirin and diclofenac sodium. Of the four varieties used in the present study, Rasthali was found to have maximum inhibiting effect on albumin denaturation (63.42%) and RBC membrane stabilization (53.94% and 46.18%). Proteinase activity was also maximally inhibited by Rasthali (71.78%) when compared to others. From the results it can be concluded that Rasthali peel extract exhibited significant anti inflammatory activity when compared to other varieties and it may be due to phytoconstituents in it.

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INTRODUCTION

Inflammation is a complex process which involves the reaction of living tissue to injury, infection and irritation. It is associated with pain, increase of vascular permeability, increase of protein denaturation and membrane alteration. It is a host defense mechanism and it is involved in the inflammatory reactions like release of histamine, kinin, prostaglandin, fluid extravasation, cell migration and tissue break down (Tortora and Reynolds, 1993). Lysosomal enzymes are released during inflammation which produce various pathological conditions such as cardiovascular diseases, inflammatory and auto immune disorders,neuro degenerative conditions, infection and cancer (Vijender Kumar *et al.,* 2011). The lysosomal membrane stabilization is important in minimizing the inflammatory response by inhibiting the lysosomal constituents. In inflammatory disorders phagocytes are activated which produce superoxide, hydroxyl and other non-radicals (Gilham *et al.,* 1997). The radicals damage the membrane through the process of lipid peroxidation. The human red blood cell membrane is similar to lysosomal membrane components (Mohamed Saleem *et al.,* 2011). To stabilize the human RBC membrane and to scavenge the free radicals, phytochemicals from medicinal plants play an important role. The WHO has estimated that 80% of population in developing countries relies on traditional medicine, mostly plant drugs for their primary health care needs (Prabakaran *et al.,* 2011). This rapid increase in the consumption of herbal remedies worldwide has been stimulated by several observations and one such observation is that these products are safe and effective (Saad *et al.,* 2005). Different varieties of Musa spp peels were used for the in vitro evaluation of anti-inflammatory activity. Musa spp is commonly called as banana. It is reported to prevent anemia by stimulating the production of hemoglobin in the blood (Akinyosoye, 1991). Banana peels are organic wastes that are highly rich in carbohydrates and other nutrients (Shobhana *et al.,* 2013). Bioactive compounds such as flavonoids, tannins, phlobatannins, alkaloids, glycosides and terpenoids are present in the banana peel. Bioactive compounds are reported to exert pharmacological effect, especially as an antioxidant, antidiabetic, anti-inflammatory and antibiotic.

***Corresponding author:* Saraswathi, U.**
Department of Biochemistry, PSG College of Arts and Science, Coimbatore-641014, Tamilnadu, India.

MATERIALS AND METHODS

Plant material

Musa spp fruits belonging to Nendran, Pachainadan, Rasthali and Sevvazhai were collected in fresh condition from Coimbatore, Tamilnadu. Fruits of the same size, shape, colour and without any physical defect were randomly selected. Banana peels were removed and dried under shade then ground into a uniform powder using a blender and stored in polythene bags at room temperature.

Preparation of extract

10g of dried powder was cold macerated with 50% hydro ethanol with occasional stirring for 3 days. After 3 days, the suspension was filtered through a fine muslin cloth and the filtrate was evaporated to dryness at low temperature (<40°C) under reduced pressure in a rotary evaporator. The powder was stored in a refrigerator and used for analysis.

Assessment of *In vitro* anti -inflammatory activity

Inhibition of albumin denaturation

The anti-inflammatory activity of Musa spp peels was studied according to Mizushima and Kobayashi, (1967) and Sakat *et al.,* (2010) followed with minor modifications. The reaction mixture consists of different varieties of banana peel extract of 1 ml and 1% aqueous solution of bovine albumin fraction. The pH of the reaction mixture was adjusted to 6.5 using small amount of 1N HCl. The peel extracts were incubated at 37°C for 20 minutes. After cooling the samples, the turbidity was measured spectrophotometrically at 660nm. The experiment was performed in triplicates. Percentage inhibition of protein denaturation was calculated as follows:

$$\text{The percentage inhibition} = \frac{\text{(Absorbance of control- Absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

Proteinase inhibition

The test was done according to the modified method of Sakat et al., (2010). The reaction mixture 2ml containing 0.06 mg trypsin, 1ml 20mM Tris HCl buffer (pH 7.4) and 1ml of different varieties of peel extract. The mixture was incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for additional 20 min. 2ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was recorded at 210nm. The experiment was performed in triplicates. The percentage inhibition of protienase inhibitory activity was calculated.

$$\text{The percentage inhibition} = \frac{\text{(Absorbance of control- Absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

Membrane stabilization

Preparation of Human Red Blood cells (HRBC) suspension
Preparation of Red Blood Cells suspension was done as per Sakat *et al.* (2010) and Sadique *et al.* (1989). The Blood was collected from healthy human volunteers and was centrifuged at 3000rpm for 10min.The pellet was washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline.

Heat induced haemolysis

Test was performed according to the modified method of Shinde *et al.* (1999). The reaction mixture 2ml consisted of 1 ml of different varieties of banana peel extract (0.1g/ml) and 1 ml of 10% RBCs suspension. For the control tube 1ml of saline was added instead of sample. Aspirin at a concentration of 0.1g/ml was used as a standard. All the tubes were incubated in a water bath at 56°C for 30min. After incubation the tubes were cooled and centrifuged at 2500rpm for 5 min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicates for all the test samples. The Percentage inhibition of haemolysis was calculated as follows:

$$\text{The percentage inhibition} = \frac{\text{(Absorbance of control- Absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

Hypotonicity-induced haemolysis

Hypotonicity induced haemolysis was studied as per Azeem *et al.* (2010). Different varieties of peel extract and control were separately mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension. Diclofenac sodium (0.1g/ml) was used as a standard drug. All the assay mixtures were incubated at 37°C for 30min and centrifuged at 3000rpm. The supernatant liquid was decanted and the amount of haemoglobin was estimated by a spectrophotometer at 560nm. The percentage hemolysis was estimated by assuming the haemolysis produced in the control as 100%.

Percentage protection = 100- (OD of sample / OD of control) x 100

Statistical analysis

The results are expressed as mean ± SD for three replicates.

RESULTS

The anti-inflammatory activity of the Musa spp peel extracts on protein denaturation, anti-proteinase activity and stabilization of red blood cell (RBC) membrane was shown in Table 1. The Musa spp peel extracts was effective in inhibiting heat induced albumin denaturation. Maximum protein inhibition of 63.42% was observed for hydroethanolic extract of Rasthali. For others like Sevvazhai, Pachanadan and Nendran extracts, the percentage protein denaturation was found to be 25.32%, 32.68%, 45.66% respectively at a concentration of 0.1 g/ml. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition 67.45% at the a concentration of 0.1g/ml. The different varieties of Musa spp peel extracts exhibited anti-proteinase activity as evident from table 1. Rasthali showed maximum inhibiton of proteinase activity (71.78%). Sevvazhai, Pachanadan and Nendran extracts showed an inhibition of 60.15%, 33.02% and 35.91% respectively. 72.56% of proteinase inhibition was observed with standard anti-inflammatory drug aspirin. The stabilization of RBC membrane by different varieties of Musa spp peel extracts is depicted in Table 1. The maximum inhibition of 53.94% was recored for Rasthali extract followed by Sevvazhai (51.03%), Pachanadan (13.08%) and Nendran (10.19%). Aspirin a standard drug showed the maximum inhibition of 56.45%.

Table 1. Effect of hydro ethanolic extract of different varieties of Musa spp peels on albumin denaturation, proteinase inhibition and membrane stabilization activity

% Inhibition			
Varieties	Albumin denaturation	Proteinase inhibition	Membrane stabilization
Rasthali	63.42±2.03	71.78±3.24	53.94±1.82
Sevvazhai	25.32±1.17	60.15±2.18	51.03±1.72
Pachanadan	32.68±1.30	33.02±1.06	13.08±0.31
Nendran	45.66±1.65	35.91±1.09	10.19±0.38
Aspirin	67.45±2.64	72.56±3.58	56.45±1.45

The values are expressed as Mean ± SD. (n=3)

Table – 2 showed the hypotonicity induced hemolysis by Musa spp peel extracts. Rasthali at a concentration of 0.1g/ml protect significantly the erythrocyte membrane against lysis induced by hypotonic solution and the percentage inhibition of haemolysis was found to be 46.18%. For other varieties like Sevvazhai, Pachanadan and Nendran the percentage inhibition of haemolysis was recorded as 43.10%, 19.01% and 26.85% respectively. Diclofenac sodium at a concentration of 0.1g/ml offered a significant protection of 51.03% against the damaging effect of hypotonic solution.

Table 2. Effect of hydro ethanolic extract of *Musa sapientum* peels on hypotonocity induced haemolysis

Varieties	% inhibition of haemolysis
Rasthali	46.18±1.39
Sevvazhai	43.10±1.77
Pachanadan	19.01±0.28
Nendran	26.85±0.94
Diclofenac sodium	51.03±1.02

The values are expressed as Mean ± SD. (n=3)

DISCUSSION

In the present study, results designate the anti inflammatory activity of different varieties of banana peel extract. However, Rasthali showed the greatest anti inflammatory activity by inhibiting protease, albumin denaturation and stabilizing the RBC membrane activity. Denaturation of protein is a well documented cause of inflammation in which proteins lose their tertiary structure and secondary structure by application of acid, base, organic salt, organic solvent or by heat. Most biological proteins lose their biological function when denatured (Megha *et al*., 2013). Rasthali peel extract possess maximum inhibition of protein denaturation than other varieties. Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinase which carries in their lysosomal granules many serine proteinases. It was previousely reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Sakat *et al.,* 2010). Our results confirmed that Rasthali peel extract have maximum ability to inhibit the proteinase. RBC membrane stabilization as an additional mechanism of the anti-inflammatory effect. The banana peel extracts possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The neutrophil lysosomal constituents include bacterial enzymes and protease, which upon extracellular release cause further tissue inflammation and damage (Chou, 1997). Protective effect on hypotonic saline induced erythrocyte lysis is known to be a incredibly good index of anti-inflammatory activity of any agent. Since the membrane of RBC is structurally similar to the lysosomal membrane it is important in limiting the inflammatory response by preventing the constituents which cause tissue inflammation and damage (Honnayakanahalli and Umesha, 2015). These findings clearly indicate that the Rasthali peel extract has a very good anti inflammatory activity.

Conclusion

The present investigation confirmed the anti-inflammatory activity of 50% hydro ethanolic extract of different varieties of banana peels and the maximum activity was observed with Rasthali. Further works on the in vivo anti-inflammatory activity of banana peel extract needs to be assessed.

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