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Anti-haemolytic and Antioxidant Activity of *Piper Longum* Seeds

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Abstract

Our work is to identify the medicinal property of Long pepper seeds (*Piper longum*). Being one of the traditionally used Ayurvedic ingredients in recipes, known to regulate diabetes, cholesterol, obesity and rheumatism, this medicine plant has many ayurvedic properties. But it has not been studied well in scientific research. Antioxidant and anti-haemolytic activity are important parameters for anti-diabetic applications. Hence, the anti-oxidant property and anti-hemolytic property of Piper longum seeds were analyzed. The photochemical were extracted with water and methanol. DPPH (2,2-diphenyl-1-picryl-hydrazyl) assay was used for antioxidant analysis. Anti-haemolytic activity was studied by hydrogen peroxide mediated haemolysis. Result of the study show that, both aqueous and methanolic extract of long pepper seeds have significant antioxidant and anti-haemolytic activity. However, methanolic extracted demonstrated greater scale of antioxidant and anti-haemolytic activity. This concludes that, the methanolic extract of *Piper longum* seeds has significant potential for application in anti-diabetic studies, due to its antioxidant and anti-haemolytic activity.

Keywords: Antioxidant activity; Anti-hemolytic activity; Radical scavenging; *Piper longum* seeds; Long pepper

Introduction

Traditional ayurvedic plants have many medicinal values. Many plants have biologically active substances. Antioxidant provides protection against different types of chronic diseases including coronary heart disease, Alzheimer's disease which also includes oxidative damage to the cellular components [1]. Reactive oxygen species (ROS) are the causes of these coronary disease and also cause aging of cells thereby damaging the DNA [2,3]. ROS attack the membranes of erythrocytes which leads to the oxidation of the lipids and proteins present in the membrane [4]. Also it causes lysis of red blood cells (hemolysis) by changing the structure of haemoglobin [5]. The damage caused by this ROS to the erythrocytes can be inhibited by some of the antioxidants especially Vitamin C [6,7].

Some medicinal plants are really more conserved as it contains photochemical with significant antioxidant capacities and hence helpful in various health problems [8,9]. The fruits of long pepper contain alkaloids, resins and some essential oils. Main chemical found is piperine (alkaloid) which gives the spiciness of the pepper. Long pepper possess anti diabetic and anti-bacterial activity. It is used to improve appetite and digestion, and to treat diarrhoea, cholera intestinal gas and also lung problems like cough, asthma and bronchitis [10]. The aim of the present study is to investigate the anti-hemolytic and antioxidant activity of aqueous and methanol extract of Piper longum seed.

Materials and Methods

Sample preparation

Fresh *Piper longum* seeds were purchased from local markets of kottakkal, Kerala.

Preparation of extract

The collected seeds were dried in the shade for 1 week and were powdered and sieved. Aqueous extract was prepared by mixing 10g of powder in 100ml of distilled water. Then it was incubated in a boiling water bath for 3 hours with continuous shaking and was kept for 24 hours in shaker. Then the extract was filtered using Whattmann No.1 filter paper and extract was lyophilized using freeze dryer and extract was collected. 10g of finely powdered seed were mixed in 100ml of methanol and kept in shaker for 24 hrs. Then the solvent was filtered using

Whattmann No.1 filter paper and then methanol is evacuated using Rotary Vacuum evaporator.

DPPH assay for antioxidant activity

Free radical scavenging activity was performed using DPPH (2,2-diphenyl, 1-picrylhydrazyl) assay. Stock solutions (10mg/ml) of plant extracts were prepared in methanol. Serial dilutions were carried out to obtain concentrations of 50, 100, 150, 200 and 250µg/ml. Extracts of each concentration (0.5ml) were mixed with DPPH (3ml) 0.004% W/V solution in methanol. The mixture was shaken well and allowed to stand at room temperature in the dark for 30minutes, the absorbance were read at 517nm using spectrophotometer. Butylated hydroxyl Toluene at various concentrations 100µg/ml was used as a standard. Blank was prepared using 3ml of DPPH. The entire test were run in triplicates and averaged. The percentage of inhibition was calculated using,

$$\% \text{ inhibition} = 100-[(Ab_s - Ab_b)/Ab_c]*100$$

Where;

Abs = absorbance of sample; Ab_b = absorbance of blank; Ab_c = absorbance of control.

Anti-hemolytic activity

Human blood (10ml) was collected from a healthy adult in EDTA containing vials and was diluted with PBS and centrifuged at 1000rpm for 10min to separate the RBC. The separated RBCs were then diluted with phosphate buffer saline to give 4% suspension [1]. The extract was dissolved in PBS (1gm/ml) and was added to 2ml of RBC suspension and the volume is made up to 5ml with PBS. The mixture is incubated for 5min in room temperature and then 0.5ml of H2O2 solution in saline buffer was added to induce oxidative degradation of membrane lipids. The concentration of H2O2 in the reaction mixture was adjusted to bring about 90% of hemolysis of blood cells after 240min [4]. The reaction mixture was centrifuged at 1500rpm for 10min after incubation. 2ml of water is added to 2ml of RBC suspension which serves as positive control [2]. And the rate of hemolysis was determined by measuring the absorbance at 540nm. Percentage of anti-haemolytic activity was calculated by the following formula.

$$\% \text{ inhibition} = [(Abs^{control} - Abs^{extract})/ Abs^{control}]*100$$

Results and Discussion

DPPH radical scavenging activity

The radial scavenging activity of methanol extract and aqueous extract was shown in Table 1 which shows the percentage of inhibition of various concentrations of aqueous and methanolic extracts. The highest percentage of antioxidant activity was shown by methanolic extract (98.11%) at 250µg/ml concentration. Although both the extracts are significantly potent in antioxidant activity, methanolic extracts demonstrated increased radical scavenging activity.

Table 1: Antioxidant activity of *Piper longum* seed extracts.

Concentrations µg/ml	Methanol Extract (%)	Aqueous Extract (%)
50	75.52	78.83
100	96.47	94.53
150	96.59	95.22
200	97.52	91.43
250	98.11	93.64

Anti-hemolytic activity

Table 2: Anti haemolytic activity of Piper longum seed extracts.

Extracts	Antihemolytic Activity (%)
Methanol	60.3
Aqueous	54.5

Methanolic extract demonstrated greater anti-hemolytic activity of 60.3% when compared to aqueous extract of 54.5% at 1mg/ml concentration. This anti-hemolytic activity results are directly in relation to the observed antioxidant activity. A result of anti-hemolytic activity is tabulated in Table 2. Erythrocytes are the major target for the free radicals because of the presence of both high membrane concentration of poly saturated fatty acids and the transport of oxygen associated with redox active hemoglobin molecules, which are the promoters of activated oxygen species [8]. It was found that the extent of lysis of RBC (hemolysis) was greater when RBC were treated with hydrogen peroxide (toxicant). This is due to the oxidizing nature of H2O2 with respect to the cell membrane destruction and liberation of hemoglobin from cells. The destruction of the cell membrane is the major factor for hemolysis. i.e., various antioxidant mechanisms together form the reason for the anti-hemolytic activity.

Thus the results suggest that, since both methanolic and aqueous extracts demonstrate significant antioxidant activity, they exhibited anti-haemolytic activity, by preventing the oxidative damage caused by hydrogen peroxide.

Conclusion

The seeds of Piper longum exhibited good antioxidant activity and showed inhibitory activity against hemolysis of RBCs induced by hydrogen peroxide. This suggests that the plant seeds contain promising bioactive photochemical that can be studied for its anti-diabetic applications. This study opens opportunity for discovery of valuable photochemical with potential for anti-diabetic applications.

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