

ANALYSIS OF ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF PIPER NIGRUM LEAF EXTRACT BY IN VITRO ASSAY

¹S. Kavitha, ¹Dr. M. V. Rameshkannan, ²Dr. P. Mani

¹Department of Medicinal Plant Biotechnology, Sharmila Institute of Medicinal Products Research Academy, Thanjavur, Tamil Nadu, India

²Department of Biotechnology, Annai College of Arts and Science, Kumbakonam, Thanjavur, Tamil Nadu, India
Corresponding Author: S. Kavitha

Abstract: Diabetes shows damage of cells and organs due to improper sugar metabolism. It leads to oxidative damage which cause microvascular and macrovascular complications. It becomes requirement of drug development that act against hyperglycemia and oxidative damage, so the plants containing various bioactive compounds and minerals with less side effects were analyzed for drug development. Among various plants, the commonly used spices black pepper corn (*Piper nigrum*) is extensively used for various medicinal purposes traditionally. The compounds extracted from *P. nigrum* are used in many creams to treat various diseases at present selected for analysis. The leaf of *Piper nigrum* collected from karumandhuri and extracted with ethanol is used for further studies which showed antidiabetic and antioxidant activity in in vitro assay.

Keywords: Antioxidant activity, antidiabetic activity, Ethanol, *Piper nigrum*.

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I. Introduction

Diabetes is a complex disease with various symptoms. It occurs due to improper utilization of glucose obtained by digestion of food in the cells due to failure of secreting hormone by pancreas. Insulin is the hormone that regulates the glucose level in the blood[1,2]. Due to lack of hormone, the level of glucose raises in blood and lead to the condition called hyperglycemia. The patients with diabetics show other problems like kidney failure, cataract, heart problem, highly prone to infections, skin cancer, etc. due to rise in glucose level. During diabetics, the improper metabolization of glucose leads in to the formation of free radicals which can damage the cells that lead into early aging and cancer [3, 4]. In addition to this, the medication given to them also shows many side effects to overcome it. The medicinal plants are now used to treat them with fewer side effects.

There are many medicinal plants used to treat diabetics among which the piper corn also used to treat diabetics [5–7]. The leaves of piper also have various compounds that have antidiabetic activity in addition to that antioxidant activity which can help overcome the stress created by hyperglycaemic condition to the cells [8–10].

II. Materials And Methods

A. Collection of plant material

The plant sample was collected in a sterilized polythene bag from karumandhuri, then cleaned with double distilled water still the dirt was completely removed. The leaves were shade dried and powdered with sterile mixer and used for further purposes [11, 12].

B. Preparation of the extract

The powdered leaf sample was extracted with 30% of ethanol solvent using Soxhlet extractor. The extract was obtained in dried form using hot air oven [13, 14].

C. In vitro antioxidant activity

• DPPH radical scavenging assay:

Reagents required:

DPPH – 0.135 mM

Methanol -100 ml

Procedure

The plant extract were taken in different concentrations in an eppendorf tube such as 1, 10, 20, 50, 100, 250 and 500 µg to which 1.9 ml of DPPH was added. The sample was vortexed thoroughly and left in dark for 30

min. The absorbance was measured at 517 nm. The standard drug ascorbic acid was used as the reference to compare with the test sample [15, 16].

The ability of plant extract to scavenge DPPH radical was calculated from the following formula:

$$\% \text{DPPH inhibition} = \frac{[(\text{OD of control} - \text{OD of test}) / (\text{OD of control})] \times 100.}$$

- **ABTS radical scavenging assay**

Reagents required

ABTS stock solution:

ABTS -7 mM

Potassium per sulfate -140 mM

Deionized water -25 ml

The mixture was left in brown bottle for 15–16 h in dark at room temperature.

ABTS working solution:

The stock solution was diluted with ethanol to obtain absorbance of 0.700 ± 0.02 at 734 nm.

Procedure

The plant extracts were taken in different concentrations in an eppendorf tube such as 1, 10, 20, 50, 100, 250 and 500 μg to which 1.9 ml of ABTS working solution was added. The sample was vortexed thoroughly and left in room temperature for 20 min. The absorbance was measured at 734 nm using an UV–visible spectrophotometer. The standard drug ascorbic acid was used as the reference to compare with the test sample [17–21].

The radical scavenging activity was calculated by equation:

$$\text{ABTS radical scavenging effect (\%)} = \frac{[(A_0 - A_1) / A_0] \times 100.}$$

D. In vitro antidiabetic activity

- **α -amylase assay:**

Reagents required:

Phosphate buffer saline - 0.02 mol./L (pH 6.8)

α -amylase - 0.1 mg/ml of PBS

Starch solution: 1.0%

DNS reagent

Dinitrosalicylic acid - 1%

Sodium sulphite - 0.05%

Sodium hydroxide - 1%

III. Procedure

The plant extract of different concentration such as 50, 100, 250, 500 and 1000 μg were taken and dissolved with 0.25 ml of α -amylase solution and mixed thoroughly. The sample was incubated at 37°C for 5 min. Add 0.5 ml of starch solution and incubate for 3 min at 37°C. Then DNS reagent was added and boiled at 100°C for 5 min to stop the reaction. The reaction mixture was cooled to room temperature and the absorbance was read at 540 nm in spectrophotometer [22].

Percentage of inhibition was calculated using the equation:

$$\text{Inhibition (\%)} = \frac{[(\text{Abs1} - \text{Abs2}) / \text{Abs1}] \times 100}$$

IV. Result And Discussion

The antioxidant assay performed with DPPH and ABTS, the absorbance obtained was analyzed with graphpad prism to determine the IC_{50} which showed that the ethanol extract of *P. nigrum* leaf has moderate activity (26.78 μg and 42.68 μg) compared with the control ascorbic acid (2.54 μg and 4.39 μg), respectively. The activity of Piper leaf extract is higher when compared with the methanolic extract of fruit 444.1 μg [23].

The plant extract was also analyzed for anti-diabetic activity by inhibiting the α -amylase enzyme which showed that extract has showed inhibition maximum at 1000 μg (67.93%) comparing with the other concentration, but comparing with standard the activity is moderate at the same concentration (82.27%). The essential oil from black pepper has shown 60% of inhibition at 120 ml of the sample [24].

a) Antioxidant activity

• **DPPH assay:**

Compound	DPPH assay (IC ₅₀ μg)
PN	26.78
Ascorbic acid	2.54

• **ABTS assay:**

Compound	ABTS assay (IC ₅₀ μg)
PN	42.68
Ascorbic acid	4.39

b) Antidiabetic activity (α-amylase test)

Compounds	Concentration (μg)	% inhibition
PN	50	26.32
	100	29.41
	250	33.33
	500	50.29
	1000	67.93
ACARBOSE	50	33.85
	100	42.06
	250	61.12
	500	73.99
	1000	82.27

V. Conclusion

The leaf extract of *P. nigrum* has shown that antioxidant activity is maximum in non-enzymatic method than the fruit sample whereas the *in vitro* anti-diabetic against the α-amylase is maximum with leaf sample than the essential oil sample from piper. Thus, extract has to be further analyzed and evaluated for the presence of various compounds and to identify their medicinal properties.

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