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Studies on Analysis of Antioxidant and Enzyme Inhibitory Activities of Solanum indicum Linn.

Shelly Rana*[1], Ved Prakash[2] and Anand Sagar[3]
Department of Biosciences, H. P. University, Shimla-171005, India
*Corresponding author: Shelly Rana**

Abstract: *Since ages medicinal plants are bestowing us with good health. Medicinal properties of these plants are due to chemicals present in them. These may be tannins, saponins, alkaloids, flavanoids, glycosides etc. In the present study, antioxidant and enzyme inhibitory activity of Solanum indicum was determined. Free radical scavenging activity of this plant was evaluated (methanol, acetone, aqueous extracts) by DPPH (1,1-Diphenyl-2-picryl-hydrazyl) and reducing power methods. The plant came out as an effective DPPH scavenger and showed moderate reducing power potential. All the extracts of Solanum indicum were reported to possess good antioxidant and enzyme inhibitory activity around or greater than 50 % in the solvent used at the concentration of 20-100 mg/mL and 0.2-1.0 µg/mL respectively. Results of this plant support the presence of bioactive compounds responsible for its free radical scavenging and enzyme inhibitory activity. Therefore, further investigations should be done for determining its therapeutic potential.*

Keywords*: Solanum indicum, leaf extracts, DPPH, reducing power, α-Glucosidase*

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I. Introduction

Since 19th century, plants are known to produce various bioactive constituents with efficient and precise selectivity and number of chemical constituents have been isolated from various plant parts. The chemical constituents are base of many modern medicine[1]. Plants are rich source of phenols, flavanoids, alkaloids, tannins, sapnins, coumarins, lignans[2]. These compounds show good antioxidant activity. Antioxidants are the compounds which trap free radicals. Free radicals cause many diseases due to uncontrolled reaction within the human body by damaging DNA, proteins, lipids, therefore, plants play very important role in antioxidative defense and protect cells from injury.

Enzyme inhibition by plant-derived products has now become an important aspect of the modern pharmaceutical research. Due to significant importance in health care products and pharmaceutical industries, research on large scale has been carried out to screen the bioactivity of these inhibitors. Studies in this field have already led to the discovery of miraculous drugs useful in curing number of diseases and physiological disorders[3].

Isolation of alpha-glucosidase inhibitor has been done from plants with enhanced potential and less side-effects. Alpha-glucosidase is the important enzyme involved in the digestion of carbohydrates. It is absorbed by intestine and breaks down starch and disaccharide into glucose units. Alpha-glucosidase enzyme which is associated with type 2 diabetes and lipid per-oxidation in tissues is inhibited by plant based inhibitors and other food products which are rich in polyphenols[4].

Many research studies have reported that the major pharmacological properties of medicinal plants are associated and attributed to their antioxidant and enzyme inhibitory activities[5]. Our study gives good information on antioxidant and enzyme inhibitory properties of this plant. At present, there is an urgent need for exploration and development of cheaper and effective plant-based drugs with better bioactive potential and least side effect.

II. Material And Methods

Fresh leaves of *Solanum indicum* were collected from Kaloha village District Kangra, Himachal Pradesh, India. Plant was identified and authenticated in ethnobotany laboratory, Department of Bio-sciences, HP University Shimla.

Processing of Plant Material

Leaves of *S. indicum* were washed thoroughly under tap water and then with 2% Mercuric chloride. After that the leaves were cut into smaller pieces for quick drying. Cleaned leaves were shade dried for 15-20 days. The dried plant material was crushed into fine powder with the help of pestle mortar. Finally the fine powder was stored in an air tight container at room temperature.

Preparation of Plant Extracts

5 gm dried leaves of *S. indicum* were taken in separate Erlenmeyer flasks to which 50 mL of required solvents i.e. methanol, acetone and aqueous were added. The flasks were covered with aluminium foil and allowed to stand for 3-5 days for extraction. These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator. The extracts were collected and weighed. Finally, stock solution of concentration 50 mg/mL was prepared.

Antioxidant Activity Test

DPPH Radical Scavenging Activity Assay

The free radical scavenging activity of plant extracts was measured using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) as described by Blois[6]. Briefly, to 1 mL of different concentrations (20, 40, 60, 80 and 100 µg/mL) of plant or test extract, 1 mL of DPPH (0.1 mM in methanol) was added. Corresponding blank sample was prepared and ascorbic acid was used as reference standard. Mixture of 1 mL methanol and 1 mL DPPH solution (without plant extract) was used as control. All the tests were carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-VIS spectrophotometer. The percentage of inhibition was calculated using the following formula:

DPPH scavenging effect (%) = $\left(\frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}}\right) \times 100$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample

Reducing Power Assay

The reducing power was determined according to the method described by Oyaizu[7]. Different concentrations of plant extract (20, 40, 60, 80 and 100 µg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 mL) of Trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank solution. Higher absorbance of the reaction mixture indicated greater reductive potential. Experiment was performed in triplicates at each concentration to evaluate percent reducing power. The % reducing power (antioxidant activity) was calculated by using the formula:

% Reducing power = $\left(\frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}}\right) \times 100$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample.

Enzyme Inhibitory Activity Test

Examination of α-Glucosidase inhibition

The α-glucosidase inhibitory activity assay was carried out using modified methods of Apostolidis and Lee[8]. In a 96-well plate alpha-glucose inhibition assay was performed. Appropriate dilution of the plant extracts 0.2 to 1.0 mg/ mL (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ mL) and 200 µl of α-glucosidase solution was incubated at 25°C for 10 minutes. Thereafter, 50 µl of 5 mMol/L *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 mol/l phosphate buffer (pH 6.9) was added. 3 ml of 50 Mm sodium hydroxide was added to stop. The reacting mixture was then incubated at 25°C for 5 min before reading the absorbance. The yellow colour was quantified (due to p-nitrophenol formation) at 405 nm in the UV-Visible spectrophotometer.

% Inhibition = $[(A_{Reference} - A_{Sample}) / A_{Reference}] \times 100$

In this study, Acarbose solution (at the concentrations of 0.2, 0.4, .6, 0.8, 1 mg/mL) was used as positive control. The inhibition percentage of α-glucosidase was assessed by the following formula:

Iα-amylase % $= 100\times (\Delta A_{Control} - \Delta A_{Sample})/\Delta A_{Control}$

$\Delta A_{Control} = A_{Test} - A_{Blank}$

$\Delta A_{Sample} = A_{Test} - A_{Blank}$

III. Results And Discussion

Free radical scavenging activity of *Solanum indicum* was studied by DPPH inhibition by leaf extracts as shown in table 1.1. In DPPH assay, methanol leaf extract was found to be more effective as compared to acetone and aqueous leaf extract. The extracts were tested on concentration range (20-100µg/mL) and it was observed that the scavenging activity enhanced considerably with increase in concentration of plant extract. Highest DPPH scavenging activity was observed at 100µg/mL by leaf extract. A pattern of increasing antioxidant activity with increasing polarity has been reported in accordance with many reports published earlier.

Reducing power is an appropriate indicator of radical scavenging activity[9]. We investigated reducing capacity of *Solanum indicum* by measuring fe^{3+}-fe^{2+} conversion as given in table 2. In this assay, the test solution changed its colour green to blue depending upon reducing power of corresponding extracts. Ascorbic acid was taken as standard for comparing reducing power of plant extracts. The methanol extract showed more reductive ability than acetone and aqueous extract, which was capable for neutralizing the free radicals. The methanol extract showed more reductive ability because of conversion of fe^{3+}/ ferric cyanide complex to ferrous. *Solanum indicum* showed 60.15%, 54.10% and 46.40% reducing power for methanol , acetone and aqueous extracts respectively at 100µg/ml. Hamyet *et al.*[10] and Mahmood raza *et al.*[11] have reported that leaf extracts of *S. indicum* possess good reducing power which supported our findings.

The extracts were tested on concentration range (0.2-1.0µg/mL) and it was observed that the alpha-glucose inhibitory activity enhanced with increase in concentration of plant extract (62.22%, 53.65% and 46.45% for methanol, acetone and aqueous extracts respectively). Findings of Javed *et al.*[12] corresponds with our results compiled above.

Table 1.1 Free radical (DPPH) scavenging activity (%) of *Solanum indicum* leaf extracts at different concentrations

Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Ascorbic acid
20	22.05±0.40	16.10±1.00	15.40±0.42	30.00±1.00
40	32.05±1.00	26.22±2.22	26.20±1.45	49.52±0.40
60	41.00±0.05	40.10±1.30	32.55±0.70	61.30±1.25
80	55.32±1.82	51.66±1.00	44.05±0.55	72.44±0.08
100	64.75±2.40	60.60±2.65	51.75±0.45	82.20±0.28

Values are given as mean ± SD

Table 1.2 Antioxidant activity percentage (%) of *Solanum indicum* leaf extracts by reducing power method at different concentrations

Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Ascorbic acid
20	21.00±1.00	16.50±0.65	11.20±0.75	24.55±2.25
40	29.95±0.50	25.50±2.22	20.28±0.45	41.44±0.45
60	36.10±0.05	35.80±1.25	31.55±0.70	55.90±1.20
80	47.30±1.20	46.56±1.00	39.20±0.55	68.15±0.54
100	60.15±2.00	54.10±2.30	46.40±1.40	80.30±1.50

Values are given as mean ± SD

Table 1.3 α-Glucosidase inhibitory activity (%) of *Solanum indicum* leaf extracts at different concentrations

Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Acarbose
0.2	17.50±1.05	14.00±0.30	12.05±1.00	26.55±0.36
0.4	26.00±0.65	21.40±1.22	18.22±1.46	37.85±2.10
0.6	39.20±0.20	30.15±0.35	27.35±0.10	50.40±0.20
0.8	47.34±1.00	40.16±2.32	36.44±0.50	63.20±1.20
1.0	62.22±2.65	53.65±1.20	46.45±0.44	75.50±0.40

Values are given as mean ± SD

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