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Research Article

FREE RADICAL SCAVANGING ACTIVITY OF *SOPHORA INTERRUPTA* BY LIPID PEROXIATION AND NITRICOXIDE METHOD

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ABSTRACT

The study was aimed to evaluate the antioxidant property of *Sophorainterrupta* belonging to the family Fabaceae. It was evaluated by using the Nitric oxide scavenging method and Lipid peroxidation method. In the earlier mentioned method the IC_{50} value of the extract was found to be>1000 which was compared with the standard Rutin which showed an IC_{50} value of 65.44 and in the later method the IC_{50} value of 115.00 which was compared with the standard BHA which showed an IC_{50} value of 230.

Key words: Bilayered *Sophorainterrupta*, free radical scavenging, Lipid peroxidation.

1. INTRODUCTION

Oxygen is an essential element for life to perform biological functions such as catabolism in order to generate energy for growth. However, a parallel role of oxygen as a toxic agent for growth and tissues has also been discovered. They are reactive oxygen species (ROS) formed during metabolism can interact with biomolecules and leads to degenerative diseases like cancer, CVS problems. To protect against the destructive action of free radicals nature has created an antioxidant defensesystem. There are thousands of naturally occurring and synthetic antioxidants known[1-3].

*Sophorainterrupta*belongs to the family Fabaceae (Leguminaceae, Papilonaceae) which is commonly called as Edwariamadarasapatna. There are more than 200 species belongs to this family which have various pharmacological activities such as anti-cancer, anti-inflammatory, antispasmodic etc[4,]

2. EXPERIMENT

2.1. Plant material

The whole plant of *Sophorainterrupta* was collected from Tirupathi, Andhra Pradesh in Feb 2011 and shade dried.

2.2. Extraction

The dried plant was soxhelet extracted with 99% methanol. The extract was concentrated by evaporation to yield a concentrated extract.

2.3. Chemicals

All the chemicals were purchased from S.D.Fine chemicals and were analytical grade.

2.4. Phytochemical studies

From the preliminary phytochemical studies, it showed the presence of alkaloids, glycosides, flavonoids, phenols, carbohydrates, proteins.

2.5. Free radical scavenging activity by Nitric Oxide method[5]

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of modified GriessIlosvay reaction. In the present investigation, GriessIlosvay reagent is modified by using Naphthyl ethylene diaminedihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). Nitrite ions react with Griess reagent, which forms a purple azo dye. In presence of test components, likely to be scavengers, the amount of nitrites will decrease. The degree of decrease in the formation of purple azo dye will reflect the extent of scavenging. The absorbance of the chromophore formed was measured at 540 nm.

a) Reagents

Sodium nitroprusside solution

Weighed accurately 0.2998 g of sodium nitroprusside and dissolved in distilled water to make up the volume to 100 ml in a volumetric flask (10 mM).

Naphthyl ethylene diaminedihydrochloride (NEDD, 0.1%)

Weighed accurately 0.1 g of NEDD and dissolved in 60 ml of 50% glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask with distilled water.

Sulphanilic acid (0.33% w/v) reagent

Weighed accurately 0.33 g of sulphanilic acid and dissolved in 20% glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask.

b) Preparation of sample solutions

Sophorainterrupta stock solution was prepared in the concentration of 1000µg/ml, from the stock solution various dilutions viz, 15.6, 31.25, 62.5, 125, 250, 500, 1000µg/ml were prepared.

c) Preparation of standard solutions

Weighed accurately 10 mg of ascorbic acid and rutin and dissolved in 1 ml of DMSO separately. From these solutions, serial dilutions were made to obtain lower concentrations using DMSO.

d) Procedure

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, pH 7.4, 1 ml) and extract in DMSO at various concentrations or standard was incubated at 25o C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotisation. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink coloured chromophore was formed. The absorbance of these solutions was measured at 540 nm. The results are in Table 1 and figure 1.

2.6. Lipid peroxidation inhibitory activity[6]

Lipid peroxidation can be initiated by ROS such as hydroxyl radicals by extracting a hydrogen atom from lipids and forming a conjugated lipid radical. This reacts rapidly with oxygen to form a lipid radical until the chain reaction is terminated. Thelipidperoxidation adducts may induce the oxidation of bio molecules such as DNA, proteins and other lipids resulting in cellular damage.

a) Preparation of egg lecithin

Separate the egg yolk and wash it with acetone until yellow colour disappers. The creamy white powder thus obtained is used for the procedure by dissolving in phosphate buffer PH 7.4 (3mg/ml).

b) Procedure

The reaction mixture containing egg lecithin (1ml), ferric chloride (0.02ml), ascorbic acid (0.02ml) and extract or standard (0.1ml) in DMSO at various concentrations was kept for incubation for 1 hour at 37ºC. After incubation 2 ml of 15% TCA and 2ml of 0.37% TBA were added. Then the reaction mixture was boiled for 15 min, cooled, centrifuged and absorbance of the supernatant was measured at 532 nm. The values are tabulated in Table2 and Figure 2.

Table 1: Nitric Oxide Method

Concentration(µg/ml)	% of Inhibition
1000	32
500	24
250	22.3
125	19.6
62.5	17.5
31.25	10.5
15.6	5

Table 2: Lipid peroxidation Method

Concentration(µg/ml)	% Inhibition
1000	92.59
500	81.48
250	81.48
125	55.56
62.5	18.00
31.25	0.00
15.6	0.00

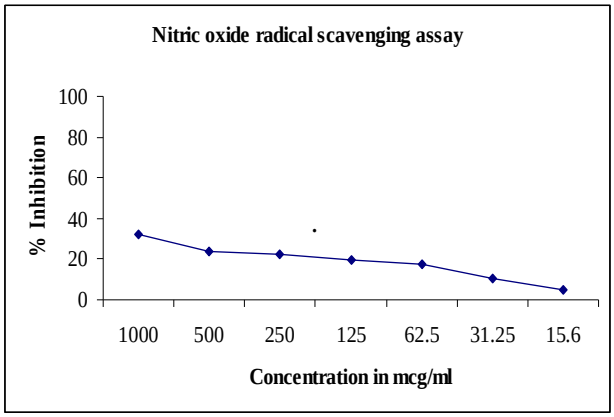

Fig. 1: Nitric Oxide Method

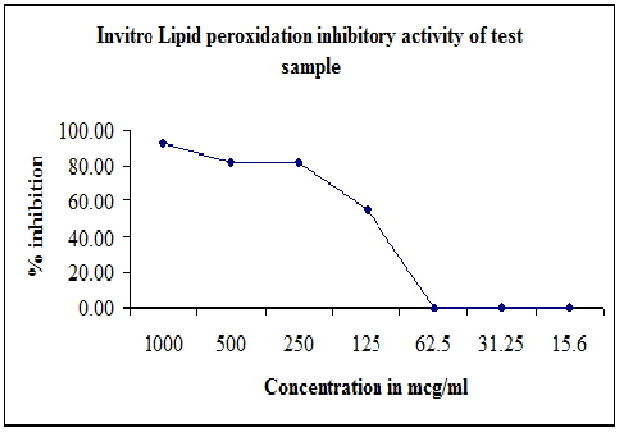

Fig. 2: Lipid Peroxidation Method

3. RESULTS AND DISCUSSION

The methanolic extract of whole plant of *Sophorainterrupta* showed antioxidant property. In Nitric oxide scavenging method the IC_{50} value of the extract was found to be >1000 which was compared with the standard Rutin which showed an IC_{50} value of 65.44.

The antioxidant property of the extract was evaluated by Lipid peroxidation method also. In this method the extract showed an IC_{50} value of 115.00 which was compared with the standard BHA which showed an IC_{50}value of 230.

From this it was clear that the extract showed a good scavenging property against lipid radicals and moderate scavenging property against nitric oxide radicals.

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