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ANTIOXIDANT EFFECT OF *TEPHROSIA PURPUREA* L. ROOTS

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ABSTRACT

The plant *Tephrosia purpurea* L. is commonly known as Unhali. *T. purpurea* (Leguminosae) is a copiously branched perennial herb. Present work was undertaken to study antioxidant potential of the plant. Dried ethanol and aqueous extracts plant were screened for in-vitro antioxidant activity by DPPH assay and nitric oxide screening methods. Results showed that IC_{50} value of aqueous extract in DPPH assay and nitric oxide scavenging assay are 78 ug/ml and 89 ug/ml respectively. Phytochemical screening revealed that flavonoids, alkaloids, saponins, tannins and phenolic compound are present in aqueous extract and may be responsible for the activity. It can be concluded that the aqueous extract of *T. purpurea* L. root can be used as antioxidant and it can be recommended for the treatment of various disease.

INTRODUCTION: *Tephrosia purpurea* L. (Leguminosae) is a copiously branched suberect, herbaceous perennial which occurs throughout India, commonly known as Unhali [1]. *T. purpurea* is an important drug of indigenous systems of medicine and has been attributed a number of medicinal properties in ayurveda. The plant has been claimed to cure disease of kidney, liver, spleen, heart and blood. Plant is used as antipyretic, as a remedy for impotency and to treat asthma, bronchitis, diarrhea, rheumatism and dental caries [2, 3]. The root contains tephrosin, deguelin, isotephrosin and rotenone. Leaves contain 2% glycoside osyritin, β-sitosterol, rutin and lupeol [4]. Present work was undertaken to study antioxidant potential of the plant.

MATERIAL AND METHODS:

Plant material: The roots of *T. purpurea* were collected from Ahmednagar district (M.S.) and authenticated at Botanical Survey of India (Pune). Voucher specimen number SLL- 1.

Extraction: The roots were dried under shade and then powdered. The dried powdered material was subjected to extraction with ethanol in Soxhlet apparatus and then the marc left was extracted with water in reflux condenser [5]. Both the extracts were vacuum dried to yield 6.106 % (ethanol extract) and 8.104 % (aqueous extract).

Preliminary Phytochemical studies: Preliminary phytochemical studies were carried out as per procedures described by Khandelwal [6].

Evaluation of Antioxidant activity:

DPPH Assay: Free radical scavenging potential of extract was determined by DPPH assay [7]. 7.9 mg of DPPH was accurately weighed and dissolved in 100 ml methanol to obtain 200 µM solution of DPPH. Different concentrations of extracts (25-100 µg/ml) were prepared. To 2 ml methanol solution of DPPH, 2 ml of sample solution was added. The mixture was incubated in dark at room temp for 30 min. The degree of free radical scavenging activity in presence of different concentration of extracts and their absorbance were measured calorimetrically at 517 nm. The degree of free radical scavenging activity was expressed as;

$$\% \text{ inhibition} = \{(A_{control} - A_{sample})/ (A_{control})\} \times 100$$

$A_{control}$ = Absorbance of DPPH alone

A_{sample} = Absorbance of DPPH along with different concentrations of extracts.

IC_{50} was calculated from equation of line obtained by plotting a graph of concentration versus % inhibition.

Nitric oxide (NO) radial scavenging activity: All the extracts *T. purpurea* screened for nitric oxide (NO) radical scavenging activity [8]. 1 ml sodium nitropruside (10 mM) in 0.5 M phosphate buffer (pH 7.4) was mixed with 3.0 ml of the different concentrations (25 – 100 µg/ml) of the sample dissolved in methanol and incubated at 25^0C for 15 min. Above samples were reacted with

Greiss reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% N-(1-napthyl) ethylenediamine dihydrochloride in water). The absorbance of the chromophore formed during the diazotization of nitrate with sulphanilamide and subsequent coupling with N- (1-napthyl) ethylenediamine was read at 546 nm. The same reaction mixture without extract of plant but with equivalent amount of 0.5 M phosphate buffer served as control. Ascorbic acid was used as positive control. The antioxidant activity of the extracts was expressed as IC_{50}. As like DPPH method IC_{50} was calculated from equation of line obtained by plotting a graph of concentration (µ/ml) versus % inhibition.

RESULTS AND DISCUSSION: Reactive oxygen species (ROS) are involved in the pathogenesis of various diseases. Uncontrolled oxidation is caused by free radicals. Free radicals oxidize all major classes of biomoleculs. The products of these oxidation reactions diffuse from the original site of attack and spread the damage all over the body and produces serious damage to almost all the cells. Some important biomoleculs susceptible to free radical oxidation are Lipids, Proteins, Nucleic acids and Carbohydrates. Thus the need of antioxidant therapy arises.

In DPPH test the ability of a compound to act as donor for hydrogen atom or electron was measured spectrophotometrically. In nitric oxide scavenging activity, the sodium nitropruside solution spontaneously generates nitric oxide which reacts with oxygen to produce nitric ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduce production of nitric ions. Results showed that aqueous extract of *T. purpurea* shows better antioxidant activity than ethanol extracts. It is observed that, phenolic compounds are responsible for antioxidant activity [9, 10].

Hence it can be concluded that phenolic compounds from *T. purpurea* roots may be responsible for antioxidant activity. Ic_{50} value of aqueous extract is 79 µg/ml in DPPH assay and 89 µg/ml in nitric oxide scavenging method (Table 1). Ascorbic acid was used as a standard in both the methods; which showed Ic_{50} value of 13. 83 µg/ml in DPPH assay and 14.59 µg/ml in nitric oxide scavenging method. Hence it can be concluded that phenolic compounds from *T. purpurea* root are likely responsible at least in part for its antioxidant activity.

Name of extract	IC50 Values (µg/ml)	
	DPPH assay method	Nitric oxide (NO) radical scavenging method
Ethanol extract	86	98
Aqueous extract	79	89
Ascorbic acid (standard)	13. 83	14. 59

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