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In vitro evaluation of *Tephrosia purpurea* Pers for antioxidant activity

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Abstract

This research was taken up to investigate the antioxidant activity of leaves of *Tephrosia purpurea*. The *in vitro* antioxidant activity of ethanolic extract of leaves of *Tephrosia purpurea* was investigated by DPPH free radical scavenging, and nitric oxide scavenging methods. The ethanol extract showed good antioxidant activity in these above methods. This activity may be due to the presence of flavanoids.

Keywords: Antioxidant activity, *Tephrosia purpurea*, Free radicals, DPPH, Nitric oxide.

1. Introduction

Free radicals and oxygen derivatives are constantly generated *in vivo* both by accident of chemistry and specific metabolic purposes. The reactivity of free radicals vary with many causing inflammation or even severe damage to biological molecules, especially to DNA, Lipids and Proteins. These have been implicated in human diseases such as lung diseases, heart failure, nephrotoxicity, inflammation and diabetes. Antioxidant defense system scavenges and minimizes free radicals formation. The action of free radicals are counteracted by antioxidants, either endogenous or exogenous [1]. Modification in our diet with increased intake of antioxidants may also prove to be effective in decreasing the incidences of these diseases [1].

Tephrosia purpurea Pers. is pantropical coastal shrub that grows up to 1 m in high [2]. It occurs through out the Indian subcontinent. Previous phytochemical investigation on this plant have shown the presence of coumarins, [3] flavonoids and rotenoids, [4, 5] flavanones [6, 7]. isoflavanones [8] and quercetin [9]. The present study was carried out to screen antioxidant activity of the leaves of *Tephrosia purpurea*.

2. Materials and Methods

The plant of *Tephrosia purpurea* was collected from the forest around Dr. H.S. Gour Vishwavidyalaya, Sagar in the month of September and identified at Department of Botany, Dr. H.S. Gour Vishwavidyalaya, Sagar (M. P.).

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Plant leaves were selected for assessment of the activity. The dried leaves (dried under the shade) were extracted with 95% ethanol for 48 hours after defatting with petroleum ether (for 72 h). The extract was filtered and concentrated in vacuum under reduced pressure and dried in desicator.

2.1 Evaluation of Free Radical Scavenging Activity

The antioxidant activity of alcoholic extract of leaves of *Tephrosia purpurea* was studied. The extract was studied with different concentration from 0.015 mg/ml to 1 mg/ml. *In vitro* methods (DPPH Scavenging and nitric oxide Scavenging) were used to screen the plant for the antioxidant activity. Ascorbic acid was used as positive control in the same concentrations for DPPH Scavenging and nitric oxide Scavenging [10].

2.2 Scavenging of Nitric Oxide [11]

The ethanolic extract was dissolved in PBS in different concentration and sodium nitroprussdide was added (5 uM) in each tube and tubes were incubated at 25°C for 5 h. Control experiments without test compound were carried out with identical conditions. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess reagent. The absorbance was taken at 546 nm. Experiment was repeated for three times.

2.3 DPPH Radical Scavenging Method [12]

A stock solution of 0.1 ml of DPPH was prepared in ethanol. This solution was mixed with equal volume of solutions (different concentrations) of test compound in ethanol. The reaction was allowed to complete in the dark for about 20 min. The absorbance was taken at 517 nm. Experiment was repeated three times. The difference in absorbances between the test and the control was calculated and expressed as percentage scavenging of DPPH radical (Table 1).

Table 1
Free Radical Scavenging Activity of the Leaf of Tephrosia purpurea

	% Inhibition			
Concentration (mg/ml)	**DPPH scavenging**		**Nitric oxide scavenging**	
	Ascorbic acid	**Drug extract**	**Ascorbic acid**	**Drug extract**
1.0	97.23	73.1	93.45	70.2
0.50	93.58	61.3	89.75	58.04
0.25	90.48	42.08	80.64	40.1
0.125	45.22	22.08	41.20	19.5
0.05	20.24	13.7	18.06	13.2
0.025	14.21	08.03	13.28	07.2
0.015	12.15	2.04	11.5	1.07

3. Results and discussion

From the study it is evident that the alcoholic extract of leaves of *Tephrosia purpurea* Pers has promising antioxidant activity against Nitric oxide and DPPH induced free radicals (Table 1). Reactive oxygen species (ROS) are formed continuously in cells as consequence of both oxidative biochemical reactions and external factors. However, they become harmful when they are produced in excess under certain abnormal conditions such as inflammation, ischemia and in the presence of iron ions.

Under these conditions, the endogenous antioxidants may be unable to counter ROS formation. [13, 14] Reactive oxygen species formed may cause cellular damage and this damage may involve in etiology of diverse human diseases. Exogenous antioxidant supplement is helpful to overcome this severe problem of free radicals, which can scavenge these free radicals.

The free radical scavenging activity of natural compounds can evaluates through their ability to quench the synthetic nitric oxide and DPPH free radicals, in which absorbance of reaction mixture is taken in visible range to know whether the compound is having antioxidant property.

4. Conclusion

From the study it was concluded that the alcoholic extract of leaves of *Tephrosia purpurea* is antioxidant in nature and can be used in various form as antioxidant. It can also be concluded that this antioxidant activity of the drug could be attributed to flavanoids, which are present in *Tephrosia purpurea* Pers.

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