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## Antioxidant Activity of *Cuscuta reflexa* Stems

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Accepted 15 September 2000

Revised 16 August 2000

Received 7 April 2000

**The *in vitro* antioxidant activity of *Cuscuta reflexa* stem extract has been investigated by estimating degree of non-enzymatic hemoglobin glycosylation measured colorimetrically at 440 nm. The ethyl acetate fraction of ethanol extract showed higher activity than the other fractions. The antioxidant activity of extracts are very close and identical in magnitude and comparable to that of standard antioxidant compounds used.**

*Cuscuta reflexa* Roxb. known as Amarvel or Akashbel in Hindi, is a common golden yellow dodder like parasite belonging to the family Convolvulaceae. It is common throughout India growing on thorny or other shrubs as parasitic annuals. The plant useful in diseases of bile and blackbile as purge, and in affections of the brain such as fits, melancholy and insanity<sup>1</sup>. It is also useful externally against itch and internally in protracted fevers, retention of wind and in duration of the liver<sup>2</sup>. Recently a great deal of interest has been directed towards the bioactivity of flavonoids as dietary sources of antioxidant<sup>3</sup>. Since *Cuscuta* is one of the richest sources of flavonoids<sup>4,5</sup>, the present investigation attempts to explore the antioxidant properties of this plant.

Shade dried stems were exhaustively extracted with petroleum ether and ethanol using a soxhlet extractor. The extracts were concentrated to dryness *in vacuo*. The ethanolic extract was subjected to silica gel column chromatography and eluted with benzene (EB), chloroform (EC), and ethylacetate (EEA). Further fractionation of the ethyl acetate fraction using silica gel column and elution with benzene-ethylacetate afforded C<sub>6</sub>H<sub>6</sub>:EtOAc (1:2) EBE-1 and C<sub>6</sub>H<sub>6</sub>:EtOAc (1:8) EBE-2.

Since non-enzymatic glycosylation of hemoglobin is an oxidation reaction<sup>6</sup>, an antioxidant is expected to inhibit this reaction. The degree of glycosylation of hemoglobin *in vitro* can be measured colorimetrically<sup>7,8</sup>. Hemoglobin, 5 g/100 ml in 0.01 M phosphate buffer (pH 7.4) was incubated in presence of 2 g/100 ml concentration of glucose for 72 h in order to find out the best

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TABLE : 1. ANTIOXIDANT ACTIVITY OF DIFFERENT FRACTIONS OF *CUSCUTA REFLEXA* EXTRACT

Sample	Final concentration of tested compounds ( $\mu\text{g/ml}$ )		
	0.5	5	10
EB	Nil	Nil	Nil
EC	2	10	25
EBE-1	7	37	52
EBE-2	15	42	63
$\alpha$ -Tocopherol	12	38	65
Rutin	11	27	42

Per cent inhibition of hemoglobin glycosylation was measured at three concentrations of benzene eluent (EB), chloroform eluent (EC) and ethyl acetate fractions (EBE-I, EBE-2). The activity were compared with those  $\alpha$ -tocopherol and rutin.

condition for hemoglobin glycosylation. The assay was performed by adding 1 ml of glucose solution, 1 ml of hemoglobin solution and 1 ml of gentamycin (20 mg/ 100 ml) in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated in dark at room temperature. The degree of glycosylation of hemoglobin in the presence of different concentration of fractions and their absence were measured colorimetrically.

Result of antioxidant activity of *Cuscuta reflexa* extracts are summarised in Table 1. The result obtained indicates that ethyl acetate fraction has more antioxidant activity than the benzene and chloroform eluent. The detailed chemical nature of the active principles responsible for antioxidant activity is not known. However, the preliminary phytochemical screening has confirmed presence of flavonoids<sup>4,5</sup>.  $\alpha$ -Tocopherol and rutin were used as the standard antioxidant compounds.

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