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ANTIOAXIDANT ACTIVITY OF METHANOL EXTRACT OF *CUSCUTA HYALINA* ROTH

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Abstract:
The aim of the present study was designed to evaluate the in vitro antioxidant activity of the methanol extract of whole plant of Cusuta hyalina Roth. The activity was evaluated by using Diphenyl picryl hydrazyl (DPPH), superoxide and hydroxyl radical scavenging methods. The radical scavenging activity of the extract was found to be concentration dependant and maximum scavenging activity was at a dose of 300 µg and comparable with ascorbic acid. Antioxidant potential could be due to the presence of flavonoids, alkaloids, triterpenoids, glycosides, steroids and carbohydrates.

Key words: *Cusuta hyalina, antioxidant activity, methanol, DPPF, Superoxide, hydroxyl radical*

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INTRODUCTION:

Free radicals are generated in several biochemical reactions in the body. A free radical is an unstable atom or molecule that has an unpaired electron. This unstable radical has the tendency to become stable through electron pairing with biological macromolecules such as proteins, lipids, and DNA in healthy human cells, thus causing protein and DNA damage. The presence of free radicals within the body has a significant role in the development and progression of many diseases like heart disease, congestive heart failure, hypertension, liver disorders and diabetic complications [1]. The oxidative stress results when the production of damaging free radicals exceeds the capacity of the body's antioxidant defenses to detoxify them. Antioxidant supplements are vital to combat oxidative damage. Many plant extracts and plant products are rich source of free radical scavenging molecule having significant antioxidant activity. Recently, much attention has been directed towards the development of ethnomedicines with strong antioxidant properties but low cytotoxicities. *Cuscuta* species also known as dodders as they feed on commonly used medicinal herbs are given special attention by traditional healers[2]. Many species of *Cuscuta* are known to contain several antibacterial, antiviral, and antiproliferative agents[3,4]. Many tribes and traditional communities have long used the different forms of *Cuscuta* for treatment and prevention of many diseases. The present study is designed to evaluate the *in vitro* free radical scavenging activity of methanol extract of whole plant of *Cuscutta hyalina* **by** DPPH, superoxide and hydroxyl radical scavenging assays. The methanol extract was also subjected to phytochemical screening to determine the presence of alkaloids, carbohydrates, glycosides, phytosterols, proteins and aminoacids, saponins, tannins and terpenoids.

MATERIALS AND METHODS:

Plant material:

The whole plants of Cusuta hyalina (Convolvulaceae) collected from Tamilnadu and were authenticated by V Chelladurai, Research officer (Retired)-Botany, Central Council for Research in Ayurveda and Siddha, Government of India.

Preparation of the extract:

Freshly collected plant materials were shade dried, powdered and subjected to soxhlet extraction using methanol as a solvent for 48 hours. The extracts were concentrated under reduced pressure and controlled temperature to dryness in rotary flaks evaporator. The percentage yield was found to be 27.52% w/w.

Drugs and chemicals:

Ascorbic acid (Sigma Aldrich Chemie, Germany), Riboflavin (S.D chemicals, India), all others reagents and chemicals used in this study were of analytical grade purchased from local source.

Phytochemical analysis

Qualitative Phytochemical screening of extracts was carried out for the presence of alkaloids, flavonoids, glycosides, phytosterols, tannins and triterpenoids. Carbohydrates, proteins and aminoacids.

***In-vitro* antioxidant activity**

The methanol extract of *Cusuta hyalina* was screened for antioxidant activity against DPPH, superoxide radical and hydroxyl radicals. The percentage inhibition of the extract was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of plant/ascorbic acid}}{\text{Absorbance of control}} \text{ X } 100$$

The percentage inhibition and 50% inhibition concentrations (IC_{50}) was calculated. The optical density was plotted taking concentration on X-axis and percentage inhibition on Y-axis of the extract / ascorbic acid. The graph was extrapolated to find the IC_{50} of extract/ascorbic acid.

DPPH-Radical-Scavenging activity[5]:

The free radical scavenging activity of the extract was determined by using stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH). An aliquot of 3 ml of 0.004% DPPH solution in methanol and 0.1 ml of plant extract at various concentrations (40-360 µg/ml) were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition activity was calculated.

Superoxide radical scavenging activity:

Superoxide radical scavenging activity of the extract was measured according to Riboflavin photo reduction method[6]. 0.1 ml of different concentrations (40-360 µg/ml) of plant extract and 0.1 ml of 6 µM ethylene diamine tetraacetic acid (EDTA) containing NaCN, 0.1 ml of 50 µM nitroblue tetrazolium, 0.05 ml of 2 µM riboflavin were transferred to a test tube, and final volume was made up to 3 ml using phosphate buffer. Then the assay tubes were uniformly illuminated with an incandescent light (40 Watt) for 15 minutes and measured optical densities at 560 nm. In the place of

plant extract/ascorbic acid a control was prepared using 0.1 ml of respective vehicle. The percentage inhibition of superoxide production was evaluated.

Screening for Hydroxyl Radical Scavenging Activity:

Hydroxyl radical scavenging activity was measured by Deoxyribose degradation method to screen the different concentrations (40-360 µg/ml) of the extract[7]. Fenton reaction mixture consisting of 200 µl of 10 mM ferrous sulphate ($FeSO_4$. $7H_2O$), 200 µl of 10 mM EDTA and 200 µl of 10 mM 2-deoxyribose and was mixed with 1.2 ml of 0.1 M phosphate buffer (pH 7.4) and 200 µl of plant extract. 200 µl of 10 mM H_2O_2 was added before the incubation at 37ºC for 4 h. 1 ml of Fenton reaction mixture was treated with 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 0.8% thiobarbituric acid and 1.5 ml of 20 % acetic acid. The total volume was then made to 5 ml by adding distilled water and kept in an oil bath at 100ºC for 1 hour. The mixture had been cooled; 5 ml of 15:1 v/v butanol-pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substances was measured at 532 nm. A control was prepared using 0.1 ml of vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of hydroxyl radicals of the extract was determined by comparing the absorbance values of the control and the experimental tubes for hydroxyl radical.

RESULTS AND DISCUSSION:

DPPH is one of the most common and relatively quick methods used for testing radical scavenging activity of several plant extract and it is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity[8]. DPPH is a stable free radical which is reduced in the presence of hydrogen donating antioxidants. The scavenging ability of methanol extracts of *Cuscuta hyalina* for free radicals of 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) showed remarkable scavenging activities (**Figure 1).** *Cusuta hyalina* extract and ascorbic acid standard showed antioxidant activity in a dose-dependent manner in the range of 40-360 µg/ml and produced maximum scavenging activity at a dose of 360µg. The parameter IC_{50} is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color). The IC_{50} values for *Cusuta hyalina* and ascorbic acid were 244.88 and 201.75 µg/ml respectively.

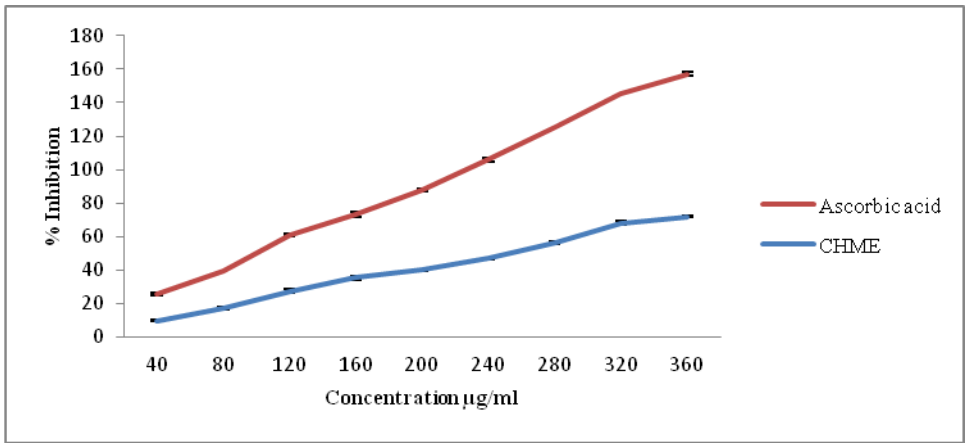

Fig. 1: DPPH radical scavenging activity

Superoxide radicals are highly reactive molecules that react with various substances produced through metabolic processes. Superoxide is a highly reactive molecule that reacts with various substances and causes oxidative damage in lipids, proteins, and DNA resulting in tissue damages and human Diseases[9]. Methanol extract of *Cusuta hyalina* and standard ascorbic acid showed encouraging response in quenching radical antioxidant activity in a dose dependent in the range of 40-360 µg/ml and produced maximum scavenging activity at a dose of 360µg **(Figure 2).** The IC_{50} values for *Cusuta hyalina* and ascorbic acid were 236.54 and 176.95µg/ml respectively.

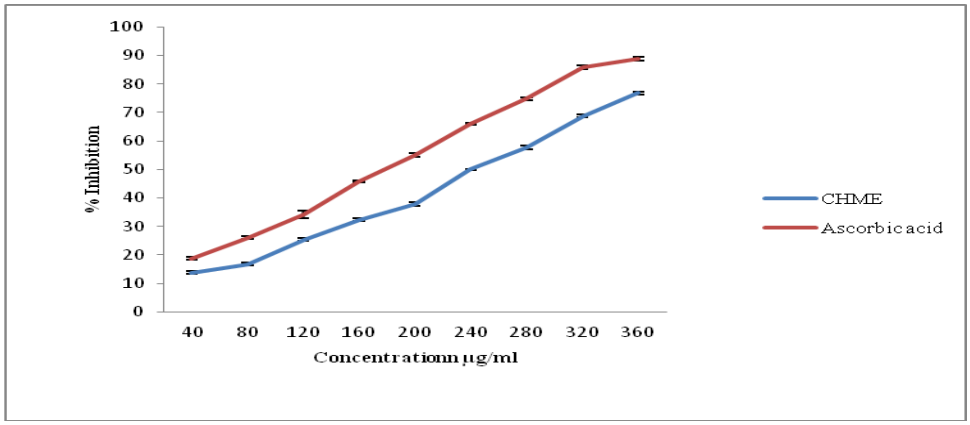

Fig. 2: Super oxide radical scavenging activity

Hydroxyl radical is the most reactive oxygen centered species and causes severe damage to adjacent biomolecule. The hydroxyl radical can induce oxidative damage to DNA, lipids and proteins[9]. The hydroxyl radical scavenging ability of the extracts was determined by the ability of the extracts to compete with deoxyribose for hydroxyl radical. The methanol extract of *Cuscuta hyalina* compete with deoxyribose and diminish chromogen formation in a dose dependant manner in the range of 40-360 µg/ml. The IC_{50} values for *Cusuta hyalina* extract and ascorbic acid were 213.69 and 211.61 µg/ml respectively (**Figure 3).**

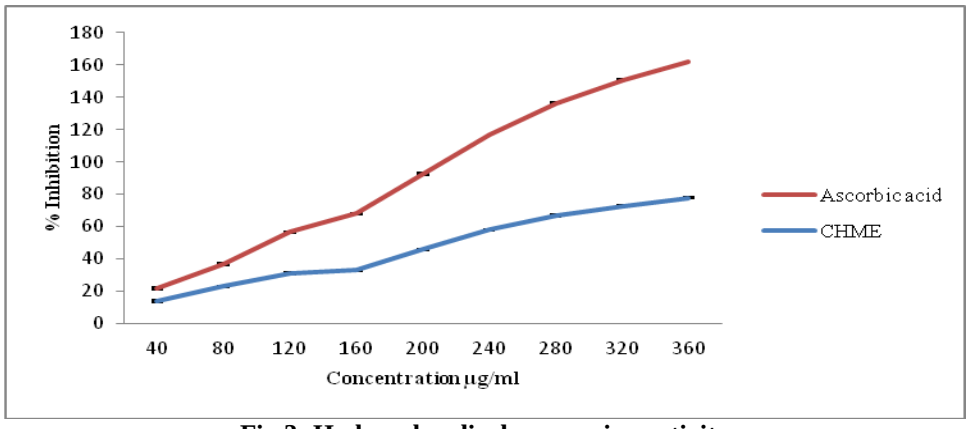

Fig.3: Hydroxyl radical scavenging activity

Qualitative phytochemical studies revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phytosterols and triterpenoids **(Table No1).** The presence of above said constituents in selected plant extract alone or in combination might be responsible for the observed antioxidant potential.

Table 1: Phytochemical analysis of methanol extract of *Cuscuta hyalina*

Sl No	Phytoconstituents	Methanol extract
1	Alkaloids	+
2	Carbohydrates	+
3	Flavonoids	+
4	Glycosides	+
5	Phytosterols	+
6	Proteins & amino acids	-
7	Saponins	-
8	Tannins	-
9	Triterpenoids	+

+' Present, '-' Absent

CONCLUSION:

The methanol extract of *Cusuta hyalina* can be considered as a source of natural antioxidants. Our findings justified the traditional use in the treatment of many diseases.

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