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In-vitro antioxidant activity of the successive extracts of *Ricinus communis* leaves

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SUMMARY

Plants containing flavonoids have been reported to possess strong antioxidant properties. The successive extracts of *Ricinus communis* leaves were screened for in vitro antioxidant properties using standard procedures. The successive extracts i.e. Petroleum ether, benzene, chloroform, methanol, water and 50% methanolic extracts of *Ricinus communis* leaves exhibited IC_{50} values of 262.60 ± 5.07, 8.19 ± 1.23, 11.17 ± 1.89, 47.38 ± 3.80, 196.14 ± 4.52 and 41.40 ± 3.98 ìg/ml, respectively in DPPH and 30.25 ± 03.25, 32.00 ± 02.12, 27.38 ± 01.68, 26.75 ± 04.16, 83.62± 03.11 and 46.75 ± 08.73 ìg/ml, respectively in nitric oxide radical inhibition assays. These values were comparing with those obtained from ascorbic acid and quercetin, used as standards. These results suggest antioxidant potential of the successive extracts of *Ricinus communis* leaves.

Key words: *Ricinus communis,* Antioxidant; DPPH, nitric oxide; Peroxidation; Free radical scavenging.

Ricinus communis leaves belong to the family Euphorbiaceae. It is a small tree, which grows to 6 meters or more in height and found in India, South Africa, Brazil, Russia, etc. Leaves are used in the form of a poultice or fermentation on sores, boils and swelling. Leaves coated with oil and warms, are commonly applied over the abdomen to give relief in flatulence in children. An infusion of leaves is used for stomachache, and as a lotion for the eye. Fresh juice of the leaves is used as an emetic in poisoning by narcotics like opium[1]. They are useful as laxative, anti-inflammatory in G.I.T. disorders and skin disease[2-3]. Except for these studies, so far, no other chemical and biological investigations have been carried out on this plant.

Lipid peroxidation has gained more importance now a day because of its involvement in pathogenesis of many diseases like atherosclerosis, cancer, diabetes mellitus, myocardial infarction, and also in ageing. Free radicals or reactive oxygen species (ROS) are produced in vivo from various biochemical reactions and also from the respiratory chain as a result of occasional leakage. These free radicals are the main culprits in lipid peroxidation[4]. Plants containing flavonoids have been reported to possess strong antioxidant properties[5]. Hence, in the present study, the successive extract of *Ricinus communis* was screened for in vitro antioxidant properties using standard procedures.

MATERIALS AND METHODS

Plant material

The whole plant was collected from Jhansi, Jhansi district, Uttar Pradesh state, India in the month of April 2004 and authenticated by Dr. Gaurav Nigam, Department of Botany, Institute of Basic Sciences, Bundelkhand University, Jhansi, Uttar Pradesh, India.

Preparation of extracts and standards

The methanolic extract of the shade-dried powdered whole plant of *Ricinus communis* was obtained. The In-vitro experiments, a weighed quantity of the extract was dissolved in distilled dimethyl sulphoxide (DMSO) and used. Solution of ascorbic acid and quercetin used as standards for in vitro studies were prepared in distilled DSMO.

DPPH method

The antioxidant activity of the plant extract and the standards were assessed on the basis of the radical scavenging effect of the stable DPPH free radical[6]. A total of 200ìl of the methanolic extract (from 21 ìg/ml to 40 ìg/ml in DMSC solution) or standard was added to 4 ml of DPPH in methanol solution (100 ìM). After incubation at 37^0C for 30 minutes, the absorbance of each solution was determined at 490 nm the corresponding blank readings were also taken and remaining DPPH was calculated. IC_{50} value is the concentration of sample required to scavenge 50% DPPH free radical.

Nitric oxide radical inhibition assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which can be estimated with oxygen to produce nitrite ions, which can be estimated by the use of Griess IIIosvoy reaction[7]. Scavengers of nitric oxide compete with oxygen leading to reduce production of nitric oxide[8]. The reaction mixture (3ml) containing sodium nitroprusside (10mM, 2ml), phosphate buffer saline (0.5) and extract or standard solution (0.5 ml) was incubated at 25^0C for 150 minutes. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipette and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of

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