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IN-VITRO ANTIOXIDANT ACTIVITY OF THE SUCCESSIVE EXTRACTS OF *RICINUS COMMUNIS* STEMS

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ABSTRACT

There is increasing evidence to support the involvement of free radical reactions in several human diseases. n recent years, it has become increasingly apparent that in man, free radicals play a role in a variety of normal regulatory systems, the de-regulation of which may play an important role in inflammation. Active oxygen species and other free radicals have long been known to be mutagenic. Further, these agents have more recently emerged as mediators of the other phenotypic and genotypic changes that lead from mutations to neoplasia. Therefore, free radicals may contribute widely to cancer development in humans. The antioxidant activities of the plant extract and pure compounds were assessed on the basis of radical scavenging effect of the stable DPPH free radical. Generally plants containing flavonoids having strong antioxidant properties. The six extracts *Ricinus communis* stem and two standards tested for antioxidant activity using DPPH method, the benzene and 50% methanol successive extracts showed the maximum antioxidant activity with IC_{50} values of 36.19 $\pm$ 2.332 μg/ml and 34.40 $\pm$ 5.98 μg/ml, respectively. The methanol and chloroform extract also showed antioxidant activity with IC_{50} values of 64.18 $\pm$ 3.20 and 66.17 $\pm$ 6.30 μg/ml. The distilled water crude extracts showed IC_{50} values of 106.14 $\pm$ 4.33 μg/ml, respectively.

INTRODUCTION: *Ricinus communis* stems belong to family Euphorbiaceae. The plant is native of India and cultivated throughout the country in gardens and fields and also grows wild in waste places**.** The castor plant is not toxic to most insects, even small amounts of the toxic protein ricin, and the alkaloids tricinine occurs in vegetative parts of the Plants. They required immediate attention if attacked by insects and diseases [1]. *A Ricinus communis stem contains* Ricinine (1- methyl- 3- cyano- 4- methoxy- 2- pyridine), amino acid. It is also contains carbohydrate, saponins, flavonoids, tannins etc. castor plants having larvicidal activity and castor oil used as traditional medicine as purgative, antiarthritis [2] . Casters are used as Antidote for poisonous bites and nitric oxide synthase [3].Free radical or reactive oxygen species are produced *in vivo* from various biochemical reactions and also from the respiratory chain as a result of occasional leakage[4]. Lipid peroxidation has gained more importance nowadays because of its involvement in pathogenesis of many diseases like atherosclerosis, cancer, diabetes mellitus, rheumatoid arthritis, schizophrenia, cataracts, retinopathy, asthma, and also in aging [4-5]. Flavonoids also inhibit the cytotoxicity of LDL and increase intake of flavonoids might reduce the risk of cardiovascular disease. On the basis of above trend we tested the anti oxidant effect of successive extract of *Ricinus communis* stem. The biologically generated superoxide anion dismutase into molecular oxygen and hydrogen peroxide in the presence of proton and this reaction is highly favored in the presence of superoxide dismutase (SOD) [6].

$$2\dot{O}_2^- \xrightarrow{2\,H^+} H_2O_2 + O_2$$

Hydrogen peroxide induces cellular damage in the presence of ferrous ions by a Fenton reaction resulting in the formation of OH free radicals as follows;

$$Fe^{+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + O_2$$

Evidence has accumulated that the free radical process known as lipid peroxidation plays a crucial and causative role in the pathogenesis of atherosclerosis [7], cancer, myocardial infarction [8] and also in aging [9].

MATERIALS AND METHODS:

Plant Material: The stems of Ricinus communis were collected from the Town - Koraon, District Allahabad, Uttar Pradesh in the Month of April 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 fresh stems were dried under shade, powdered and the successive extracts of the powdered stems of *Ricinus communis* was obtained. For In-vitro study of Ricinus communis stems, a weighed quantity of the extract was dissolved in distilled dimethyl sulphoxide (DMSO) or in methanol. Ascorbic acid and Quercetin was used as standards and were prepared by 10 mg of each of these weighed separately and dissolved in 0.95 ml of DMSO to get 10.5 mg/ml concentration. This solution was serially diluted with DMSO to get lower dilutions.

DPPH Method-: The antioxidant activities of the plant extract and pure compounds were assessed on the basis of radical scavenging effect of the stable DPPH free radical[10]. To 2.0 ml of DPPH solution, 1.0 ml of each of the test sample or the standard solution was added separately in microtitre plates. The final concentration of the test and standard solutions used are 1, 0.5, 0.25,

0.125, 0.0625, 0.03125, 0.016, 0.008, 0.004, 0.002 and 0.001 mg/ml. The plates were incubated at 37°C for 30 minutes and absorbance of each solution was measured at 490 nm against the corresponding test and standard blanks. The remaining DPPH was calculated. IC_{50} is the concentration of the sample required to scavenge, 50% DPPH free radicals [11].

Nitric Oxide Radical Inhibition Method: Sodium nitroprusside, 0.2998 gm, was weighed accurately and dissolved in distilled water to make up the volume to 100 ml in a volumetric flask [12] (10 mM). Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interact with oxygen to produce nitrite ions, which were measured by using Griess Ilosvog Reaction [13-16]. The reaction mixture (6ml) containing sodium nitroprusside (10 mM, 4ml), phosphate buffer saline (1ml) and extracts (1ml) was incubated at 25°C for 150 minutes.

After incubation, 0.5ml of the reaction mixture containing nitrite was removed, 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was mixed well and allowed to stand for 5 minutes for completing diazotization, and then 1ml of 1- Naphthylamine (5%) was added, mixed and allowed to stand for 30 minutes. A pink colored chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solution. IC_{50} value is the concentration of sample required to inhibit 50% of nitric oxide radical.

RESULTS AND DISCUSSION: The six extracts *Ricinus communis* stem and two standards tested for antioxidant activity using DPPH method, the benzene and 50% methanol successive extracts showed the maximum antioxidant activity with IC_{50} values of 36.19±2.332 μg/ml and 34.40±5.98 μg/ml, respectively. The methanol and chloroform extract also showed antioxidant activity with IC_{50} values of 64.18±3.20 and 66.17±6.30 μg/ml. The distilled water crude extracts showed IC_{50} values of 106.14±4.33 μg/ml, respectively. However, petroleum ether extract showed lowest antioxidant activity with an IC_{50} value of 149.60±3.432 μg/ml. The known antioxidants ascorbic acid and Quercetin exhibited IC_{50} values of 78.17±4.05 and 53.60±1.79 μg/ml, respectively as shown in **table 1**.

TABLE 1: ANTIOXIDANT ACTIVITY OF *RICINUS COMMUNIS* STEM EXTRACTS USING DPPH METHOD

TEST COMPOUNDS	IC_{50} Values ± SE* (μg/ml)
Petroleum ether extract	149.60 ± 3.432
Benzene extract	36.19 ± 2.332
Chloroform extract	66.17 ± 6.30
Methanol extract	64.18 ± 3.20
50% Methanol crude extract	34.40 ± 5.98
Aqueous crude extract	106.14 ± 4.33
Ascorbic acid	78.17 ± 4.05
Quercetin	53.60 ± 1.79

* Average of 10 determinations

The six extracts of *Ricinus communis* stem and two standards tested for antioxidant activity using nitric oxide radical inhibition method, the Chloroform and benzene extracts showed the maximum antioxidant activity with IC_{50} values of 27.28±4.88 and 23.30±2.62 μg/ml, respectively.

The petroleum ether, methanol and 50% crude methanol extracts also showed antioxidant activity with IC_{50} values of 43.87±3.65, 46.15±7.16 and 52.15±7.76 μg/ml, respectively. However, water extract showed lowest antioxidant activity with an IC_{50} value of 87.95 ± 4.76 μg/ml. The known antioxidants ascorbic acid

and quercetin exhibited IC_{50} values of 20.50±01.16 and 19.5±1.85 µg/ml, respectively as shown in **table 2**.

TABLE 2: ANTIOXIDANT ACTIVITY OF *RICINUS COMMUNIS* STEM EXTRACTS USING NITRIC OXIDE RADICAL INHIBITION ASSAY

TEST COMPOUNDS	IC_{50} Values ± SE* (µg/ml)
Petroleum ether extract	43.87 ± 3.65
Benzene extract	23.30 ± 2.62
Chloroform extract	27.28 ± 4.88
Methanol extract	46.15 ± 7.16
50% Methanol crude extract	52.15 ± 7.76
water	87.95 ± 4.76
Ascorbic acid	20.50 ± 01.16
Quercetin	19.5 ± 1.85

* Average of 10 determinations

Flavonoids are polyphenolic compounds that are ubiquitous in nature, found in fruits, vegetables, and certain beverages and have diverse beneficial biochemical and antioxidant properties. Flavonoids provide protection against cancer and carcinogenesis through inhibition of oxidative damage [17]. Hence the antioxidant activity of *Ricinus communis* stem is due to presence of flavonoids in their extracts.

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