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Antioxidant Activity of the Successive Extracts of *Grewia asiatica* Leaves

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Plants containing flavonoids have been reported to possess biological properties. The successive extracts of *Grewia asiatica* leaves were screened for *in vitro* antioxidant properties using standard procedures. The successive extracts such as petroleum ether, benzene, ethyl acetate, methanol, water and 50 % crude methanol extracts exhibited IC_{50} values of 249.60 ± 7.37, 16.19 ± 2.132, 26.17 ± 1.49, 27.38 ± 1.80, 176.14 ± 5.53 and 56.40 ± 3.98 µg/mL, respectively in DPPH and 22.12 ± 02.65, 27.00 ± 01.62, 47.38 ± 05.88, 56.85 ± 06.16, 152.75 ± 5.76 and 72.75 ± 13.76 µg/mL, respectively in nitric oxide radical inhibition assays. These values are comparable with standards such as an ascorbic acid and quercetin. The *Grewia asiatica* leaves are showing antioxidant activity.

Key Words: *Grewia asiatica*, Antioxidant, DPPH, Nitric oxide, Peroxidation, Free radical scavenging.

INTRODUCTION

Grewia asiatica leaves belong to the family Tiliaceae. It is a scraggly shrub and small tree, which grows to 4 m or more in height and found in India, especially in Punjab, Uttar Pradesh and Mumbai, Pakistan, Southeast Asia and USA, *etc.* The leaves are applied on skin eruptions and they are known to have antibiotic action. The fresh leaves are valued as fodder. The ether extract of leaves possesses antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*[1]. 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 nowadays 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

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occasional leakage. These free radicals are the main culprits in lipid peroxidation[2]. Plants containing flavonoids have been reported to possess strong oxidant properties[3]. Hence, in the present study, the successive extracts of *Grewia asiatica* leaves was screened for *in vitro* antioxidant properties using standard procedures.

EXPERIMENTAL

The whole plant of *Grewia asiatica* was collected from Chaumuhan, Mathura district, Uttar Pradesh state, India in the month of April 2003. The plant was authenticated by Dr. Gaurav Nigam, Department of Botany, Institute of Basic Sciences, Bundelkhand University, Jhansi, Uttar Pradesh, India.

Preparation of extracts and standards: The successive extracts of the shade-dried powdered leaves of *Grewia asiatica* was obtained. The *in vitro* experiments, a weighed quantity of the extract was dissolved in distilled dimethyl sulphoxide (DMSO) or methanol and used. Solution of ascorbic acid and quercetin used as standards for *in vitro* studies were prepared in distilled DMSO.

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[4]. A total of 200 µL of the methanolic extract (from 21 to 40 µg/mL in DMSO solution) or standard was added to 4 mL of DPPH in methanol solution (100 µM). After incubation at 37ºC for 0.5 h, the absorbance of each solution was determined at 490 nm the corresponding blank readings were also taken and remaining DPPH was calculated. IC_{50} values 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[5]. Scavengers of nitric oxide complete with oxygen leading to reduced production of nitric oxide[6]. The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL), phosphate buffer saline (0.5) and extract or standard solution (0.5 mL) was incubated at 25ºC for 2.5 h. After incubation, 0.5 mL of the reaction mixture containing nitric was pipette out 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 1-naphthylamine (5 %) was added, mixed and allowed standing for 0.5 h, a pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nM against the corresponding blank solutions. The IC_{50} value is the concentration of sample required to inhibit 50 % of nitric oxide radical.

RESULTS AND DISCUSSION

In vitro assay: The successive extracts of *Grewia asiatica* exhibited antioxidant activity in the DPPH and the nitric oxide radical inhibition assay as evidenced by the low IC_{50} values (Tables 1 and 2). The successive extracts such as petroleum ether, benzene, ethyl acetate, methanol, water and 50 % crude methanol extracts exhibited IC_{50} values of 249.60 ± 7.37, 16.19 ± 2.132, 26.17 ± 1.49, 27.38 ± 1.80, 176.14 ± 5.53 and 56.40 ± 3.98 µg/mL, respectively in DPPH and 22.12 ± 02.65, 27.00 ± 01.62, 47.38 ± 05.88, 56.85 ± 06.16, 152.75 ± 5.76 and 72.75 ± 13.76 µg/mL, respectively in nitric oxide radical inhibition assays. These values were more than those obtained for ascorbic acid and quercetin, used as standards.

TABLE-1
ANTIOXIDANT ACTIVITY OF *Grewia asiatica* LEAVES EXTRACTS USING DPPH METHOD

Test compounds	IC_{50} values ± SE* (µg/mL)
Petroleum ether extract	249.60 ± 7.37
Benzene extract	16.19 ± 2.13
Ethyl acetate extract	26.17 ± 1.49
Methanol extract	27.38 ± 1.80
50 % Methanol crude extract	56.40 ± 3.98
Aqueous crude extract	176.14 ± 5.53
Ascorbic acid	78.17 ± 4.05
Quercetin	53.60 ± 1.79

*Average of 10 determinations.

TABLE-2
ANTIOXIDANT ACTIVITY OF *Grewia asiatica* LEAVES EXTRACTS USING NITRIC OXIDE RADICAL INHIBITION ASSAY

Test compounds	IC_{50} values ± SE (µg/mL)
Petroleum ether extract	22.12 ± 2.65
Benzene extract	27.00 ± 1.62
Ethyl acetate extract	47.38 ± 5.88
Methanol extract	56.85 ± 6.16
50 % Methanol crude extract	72.75 ± 13.76
Water extract	152.75 ± 5.76
Ascorbic acid	20.50 ± 1.16
Quercetin	19.50 ± 1.85

*Average of 10 determinations.

Free radical oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient natural antioxidant defenses[7]. Among the 6 extracts *Grewia asiatica* leaves and 2 standards tested for antioxidant activity using DPPH method, the benzene and ethyl acetate successive extracts showed the maximum antioxidant activity with IC_{50} values of 16.19 ± 2.13 and 26.17 ± 1.49 µg/mL, respectively. The methanol extract also showed antioxidant activity with IC_{50} values 27.38 ± 1.80 µg/mL. The 50 % methanol and distilled water crude extracts showed IC_{50} values of 56.40 ± 3.98 and 176.14 ± 5.53 µg/mL, respectively. However, petroleum ether extract showed lowest anti oxidant activity with an IC_{50} value of 249.60 ± 7.37 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.

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