EVALUATION OF *IN-VITRO* ANTIOXIDANT ACTIVITY OF *OUGEINIA OOJEINENSIS* LEAVES

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Summary

The present investigation was undertaken to evaluate the *in-vitro* antioxidant activity of the methanol and aqueous extracts of leaves of *Ougeinia oojeinensis*. The antioxidant activity *in vitro* was measured by means of the 1, 1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO) and superoxide (SOD) free radical scavenging assays. The methanol and aqueous extracts exhibited scavenging potential with IC_{50} values of 125.31 and 146.12 µg/ml respectively for DPPH radical and 189.75 µg/ml and 223.77 µg/ml for inhibition of nitric oxide respectively. The methanol extract and aqueous extract has scavenged superoxide radical with the IC_{50} values of 177.85 and 214.69 µg/ml respectively. All the parameters were found to dose dependent.

Keywords *Ougeinia oojeinensis*, DPPH, superoxide, nitric oxide

Introduction

It is increasingly being realized that a majority of the disease of today are due to the shift in the balance of the pro-oxidant and the antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals or due to the excessive oxidative stress of the current life or due to the poor scavenging/quenching in the body due to the depletion of the dietary antioxidants. Free radicals have been implicated in causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes *etc.* Reactive oxygen species (ROS) have been known to cause tissue injury through covalent binding and lipid peroxidation. Lipid peroxidative process has been shown to augment collagen synthesis and fibrosis. Hence antioxidants have a role in inhibiting the fibrotic process induced during the cell damage of liver. As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity[1-3]. Therefore, there is an increasing interest in searching for antioxidants of natural origin.

Ougeinia oojeinensis (Roxb.) Hochr (Fabaceae) known in Hindi as Tinsa and in Sanskrit as Ratha is a deciduous trees, found in the outer Himalayas and sub-Himalayan tracts from Jammu to Bhutan up to an altitude of 1500m and extending through the whole of northern and central India into the greater part of Deccan peninsula[4,5]. The extract of the whole plant *O. oojeinensis* were scientifically evaluated for anti-inflammatory and analgesic activities in previous studies. The 50% of ethanolic extract of stem bark has been reported exhibit antispasmodic action[6]. The hepatoprotective, in-vitro anti-inflammatory and wound healing activity of *O. oojeinensis* bark have been reported[7-9]. Phytochemical investigated on *O. oojeinensis* have reported the presence of lupeol, hydroxlupeol, betulin and isoflavanones such as dalbergioidin, homoferreirin and ougenin[10-12]. We report here the results of a screening for antioxidant activity of methanol and aqueous extracts of *O. oojeinensis* leaves.

Material and Methods

Plant materials: The leaves of *Ougeinia oojeinensis* were collected from Betul district, Madhya Pradesh, India, during the months of January and February 2007. The species was identified by the local people during the time of collection and later on authentication was made by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai, India. The leaves were shade dried, reduced to coarse powder and stored in airtight container till further use.

Preparation of extract: 1 Kilogram of powdered drug was packed in soxhlet apparatus and extracted with petroleum ether to defat the drug. Defatted powdered drug was then extracted with methanol. The methanol extract was separated and the marc was further extracted with distilled water. The solvents were removed by distillation and the last traces of solvent being removed under reduced pressure.

Hydrogen-Donating Activity: Hydrogen donating activity was quantified in presence of stable DPPH radical on the basis of Blois method. Briefly, to a methanolic solution of DPPH (100m M, 2.95 ml), 0.05 ml of both extracts dissolved in methanol was added at different concentrations (50-400 µg/ml). Reaction mixture was shaken and after 30 min at room temperature, the absorbance values were measured at 518 nm and converted into percentage of antioxidant activity (% AA). Ascorbic acid was used as standard[13,14]. The degree of discoloration indicates the scavenging efficacy of the extract, was calculated by the following equation:

$$\% AA = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{DPPH}\}$$

Nitric Oxide Scavenging: Nitric oxide scavenging activity was measured spectrophotometrically. Sodium nitroprusside (5mM) in phosphate buffered saline was mixed with different concentrations of both extracts (50-400 µg/ml) dissolved in methanol and incubated at 25 °C for 30 min, then 1.5 ml of the incubation solution were removed and diluted with 1.5 ml of Griess reagent (1% Sulfanilamide, 2% phosphoric acid, and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylene diamine was measured at 546 nm along with a control [15,16].

Superoxide Scavenging Activity: Superoxide scavenging was carried out by using alkaline DMSO. Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use. Filtrate (200 ml) was added to 2.8ml of an aqueous solution containing nitroblue tetrazolium (56 mM), EDTA (10 mM) and potassium phophate buffer (10 mM, pH 7.4). Sample extract (1 ml) at various concentrations (50-400 µg/ml) in water was added and the absorbance was recorded at 560 nm against a control in which pure DMSO has been added instead of alkaline DMSO[17,18].

Statistics Analysis: The data were reported as mean values ± standard deviation (SEM). Values representing the concentrations of investigated extracts that cause 50% of neutralization/inhibition (IC_{50}) were determined by the linear regression analysis.

Results and Discussions

Antioxidant ability of *O. oojeinensis* was assessed by establishing its efficacy in hydrogen-donating, nitric oxide scavenging and superoxide scavenging activity models. DPPH is stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, then losing colour stoichometrically with the number of electrons consumed, which is measured spectrophotometricallty at 517 nm. As shown in table 1, *O. oojeinensis* of methanol and aqueous extracts strongly scavenged DPPH radical with the IC_{50} being 125.31 and 146.12 µg/ml respectively (Figure 1a, 1b). The scavenging was found to dose dependent. The standard drug ascorbic acid scavenged DPPH radical with the 96.24 µg/ml.

O. oojeinensis of methanol and aqueous extracts also moderately inhibited nitric oxide in dose dependent manner (Table 2, Figure 2a, 2b) with the IC_{50} being 189.75 and 223.77 µg/ml respectively. The standard drug ascorbic acid inhibited nitric oxide radical with the 91.04. Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities. Studies in animal models have suggested a role for NO in the pathogenesis of inflammation and pain and NOS inhibitors have been shown to have beneficial effects on some aspects of the inflammation and tissue changes seen in models of inflammatory bowel disease. Thus establishing the usage of the plant in the Indian indigenous system as an anti-inflammatory agent.

SOD is an important cellular antioxidant enzyme, which converts superoxide radical into H_2O_2 and O_2. We also looked for the protective effect of the both extract on antioxidant enzyme SOD in mitochondria exposed to H_2O_2. Table 3 gives the changes in the activity of SOD upon treatment with the both extracts. The chloroform and ethanol extracts also moderately scavenged superoxide radical with the IC_{50} values of 177.85 and 214.69 µg/ml respectively (Figure 3a, 3b). The standard drug ascorbic acid scavenged superoxide radical with the 91.43 µg/ml.

The methanol and aqueous extract of *O. oojeinensis* possess potent antioxidant activity and further investigations are in progress in our laboratory to identity the active principles involved in this antioxidant activity.

Table 1: Free radical scavenging capacity of methanol and aqueous extracts of *O. oojeinensis*

Concentration (µg/ml)	DPPH Scavenging %		
	Methanol Extract	Aqueous Extract	Ascorbic Acid
50	21.45±1.24	18.61±1.42	96.24±2.12
100	36.71±1.59	34.82±2.16	-
150	58.32±0.98	50.18±1.19	-
200	81.52±2.01	65.24±2.74	-
250	104.15±2.31	88.37±1.97	-
IC_{50}	125.31	146.12	-

Values are means ± SEM of six determinations

Table 2: Nitric oxide scavenging capacity of chloroform and ethanol extracts of *O. oojeinensis*

Concentration (µg/ml)	Nitric oxide Scavenging %		
	Methanol Extract	Aqueous Extract	Ascorbic Acid
50	11.51±2.01	6.15±2.19	91.04±1.85
100	19.43±1.74	16.27±1.69	-
150	35.12±1.83	27.61±2.41	-
200	55.26±2.51	45.85±2.73	-
250	69.32±2.37	57.11±1.38	-
IC_{50}	189.75	223.77	-

Values are means ± SEM of six determinations

Table 3: Super oxide scavenging capacity of chloroform and ethanol extracts of *O. oojeinensis*

Concentration (µg/ml)	Superoxide Scavenging %		
	Methanol Extract	Aqueous Extract	Ascorbic Acid
50	14.56±2.14	9.23±3.17	91.43±1.96
100	25.31±3.04	18.65±2.84	-
150	40.41±1.79	31.38±1.37	-
200	58.16±2.34	48.19±2.43	-
250	71.26±1.95	59.43±1.49	-
IC_{50}	177.85	214.69	-

Values are means ± SEM of six determinations.

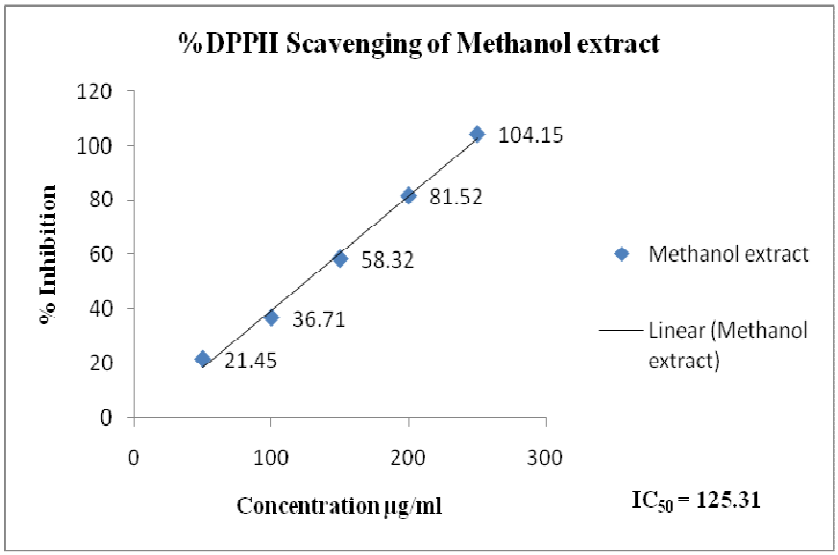

Fig. 1a- IC_{50} values, from the data, were calculated by regression analysis for DPPH scavenging of methanol extract

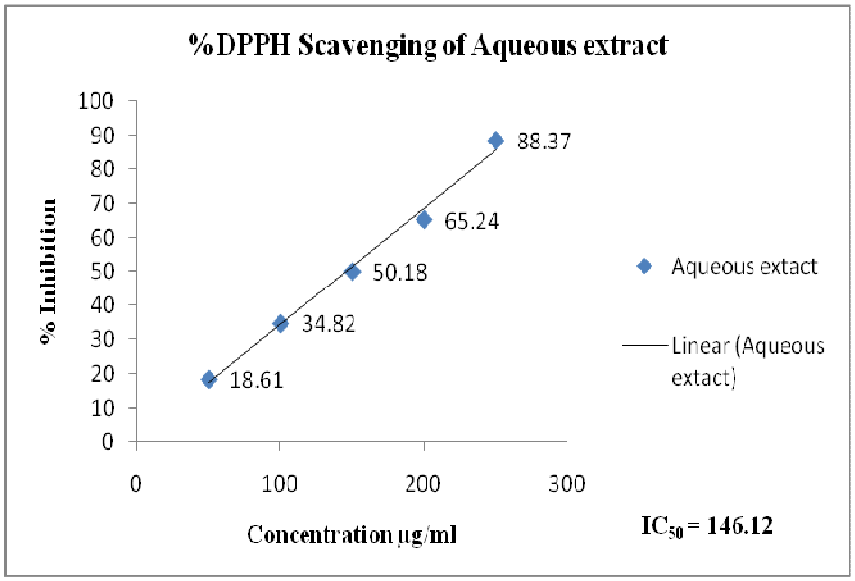

Fig. 1b- IC_{50} values, from the data, were calculated by regression analysis for DPPH scavenging of aqueous extract

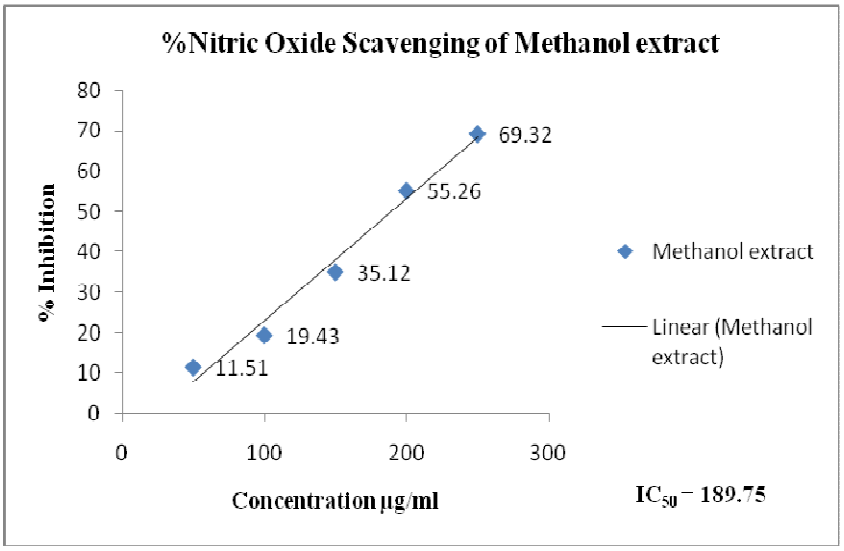

Fig. 2a- IC_{50} values, from the data, were calculated by regression analysis for nitric oxide scavenging of methanol extract

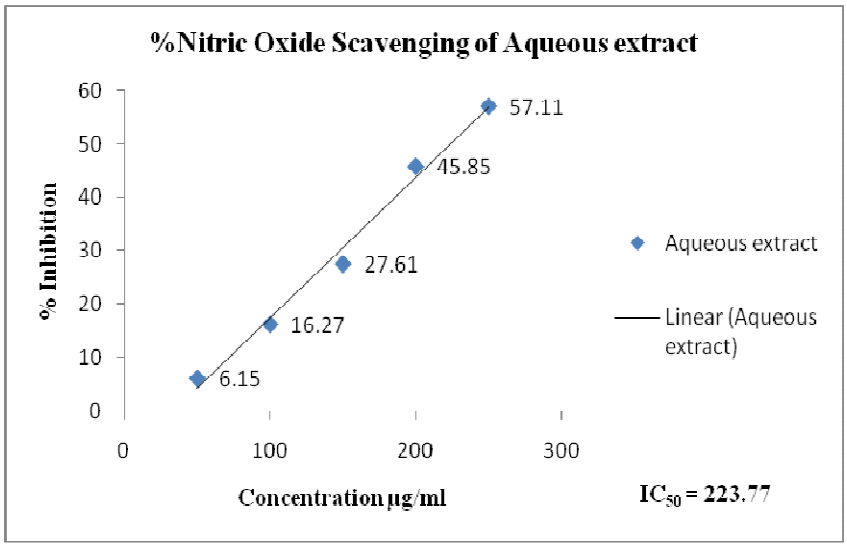

Fig. 2b- IC_{50} values, from the data, were calculated by regression analysis for nitric oxide scavenging of aqueous extract

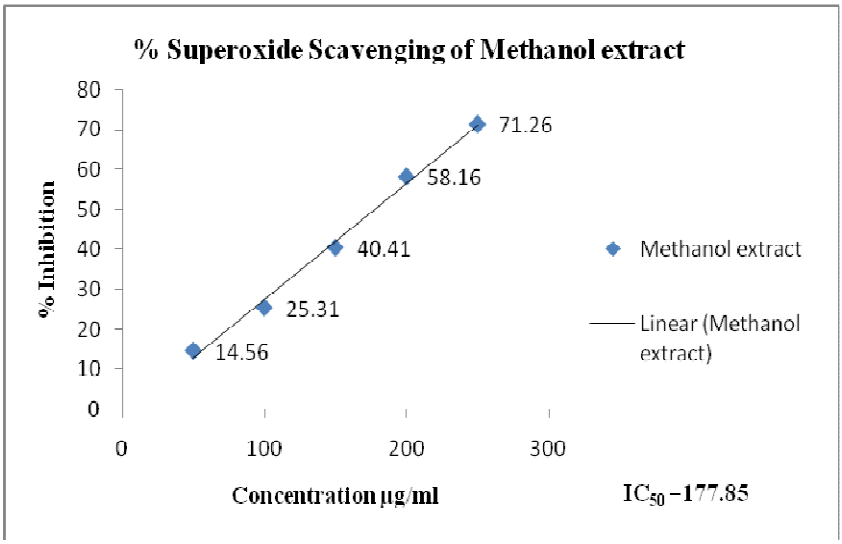

Fig. 3a- IC_{50} values, from the data, were calculated by regression analysis for superoxide scavenging of methanol extract

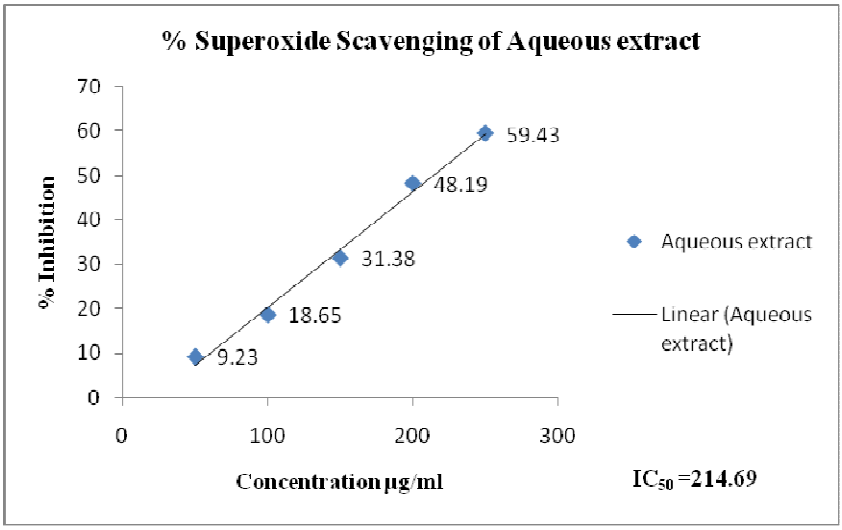

Fig. 3b- IC_{50} values, from the data, were calculated by regression analysis for superoxide scavenging of aqueous extract

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