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In vitro Antioxidant Activity of Methanolic Leaves Extract of *Nerium indicum*

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Abstract: The study was designed to investigate the *in vitro* antioxidant activity of methanolic leaves extract of *Nerium indicum*. *In vitro* antioxidant activity was determined by using DPPH free radical scavenging, superoxide anion scavenging and reducing power assay. The results suggest that methanolic leaves extract of *Nerium indicum* had shown very significant radical scavenging activity compared to the control. The results concluded that the extract have a potential source of antioxidants of natural origin.

Key words: *Nerium indicum*, antioxidant assay, superoxide, DPPH, reducing power, India

INTRODUCTION

Nerium indicum is an erect (Begum *et al*., 1999) smooth shrub, 1.5-3 m high with a cream colored sticky resinous juice (Al-Farwacchi *et al*., 2008). Leaves are in whorls of 3 or 4, linear-lanceolate, 10-15 cm long with numerous horizontal nerves (Vijayvergia and Kumar, 2007). Flowers are showy, sweet-scented (Wang *et al*., 2006) single or double, 4-5 cm in diameter, white, pink or red (Tiwari and Singh, 2004), borne in terminal inflorescence (cymes) (Begum *et al*., 1997). Fruit is cylindrical, paired with deep linear striations, 1.5-2.0 cm long (Abe *et al*., 1996). Seeds are numerous and compressed with a tuft of fine, shining, white, silky hairs.

MATERIALS AND METHODS

The plant material *Nerium indicum* Mill were collected from Sagar, Madhya Pradesh, India. The sample was identified Prof. Madhuri Modak, plant taxonomist, Department of Botany, M.V.M. College, Bhopal, Madhya Pradesh and the voucher specimen was deposited at Department of Botany, M.V.M. College, Bhopal.

Extraction: The plant material was dried in a shade. The powdered drug (100 g) was treated using soxhlet extractor with petroleum ether (300 mL) for about 48 h. The extract was dried under vacuum rotary evaporator. The remaining marc was extracted with methanol (300 mL) for about 24 h. The extract was filtered and the filtrate thus obtained was concentrated with a rotary evaporator (Ahmed *et al*., 2006). The dried extract was stored in airtight container for further experiments.

Antioxidant assay: The antioxidant activity of plant extract were determined by different *in vitro* methods such as the DPPH free radical scavenging, superoxide anion scavenging and reducing power assay methods. All the assays were carried out in triplicate and average values were considered.

DPPH free radical scavenging activity: The free radical scavenging capacity of the methanolic leaves extract of *Nerium indicum* was determined using DPPH method. (Krishnaraju *et al*., 2009; Naznin and Nur, 2009). The DPPH solution (0.004% w/v) was prepared in 95% methanol. Methanolic leaves extract of *Nerium indicum* were mixed with 95% methanol to prepare the stock solution (10 mg/100 mL). The concentration of this *Nerium indicum* methanolic leaves extract solution was 10 mg/100 mL. From stock solution 2, 4, 6, 8 and 10 mL of this solution were taken in five test tubes and by serial dilution with same solvent were made the final volume of each test tube up to 10 mL whose concentration was then 20, 40, 60, 80 and 100 μg mL^{-1} respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing *Nerium indicum* methanolic leaves extract (20, 40, 60, 80 and 100 μg mL^{-1}) and after 10 min, the absorbance was taken at 517 nm using a spectrophotometer. Control sample was prepared containing the same volume without any extract was used as blank. Percentage scavenging of the DPPH free radical was measured using the following equation:

$$\text{DPPH radical scavenging (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

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Superoxide anion scavenging activity: The scavenging activity of the *Nerium indicum* leaves extract towards superoxide anion radicals was measured. Superoxide anions were generated in a non-enzymatidc phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS, NADH and oxygen (Basniwal *et al*., 2009; Ghasemi *et al*., 2009). It was assayed by the reduction of Nitroblue Tetrazolium (NBT). Various concentrations of test solutions were taken in a test tube. To this reaction mixture consisting of 1 mL of sodium carbonate, 0.4 mL of NBT, 0.2 mL of EDTA solution were added to the test tube and 0 min reading was taken at 560 nm. The reaction was initiated by the addition of 0.4 mL of hydroxylamine hydrochloride to the above solution. Reaction mixture was incubated at 25°C for 15 min; the reduction of NBT was measured at 560 nm. All extracts were treated in the similar manner. Absorbance was recorded and percentage of inhibition was calculated according to the following equation:

$$\text{Inhibition}(\%) = \frac{A_0 - A_1}{A_0} \times 100$$

Where:

A_0 = The absorbance of the control (blank, without extract)

A_1 = The absorbance in the presence of the extract

Reducing power assay: Different doses of *Nerium indicum* leaves extract were mixed in 1 mL of distilled water so as to get 10, 25 and 50 μg concentration. This was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium frricyanide (2.5 mL 1%). The mixture was incubated at 50°C for 20 min which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measure at 700 nm. (Shyur *et al*., 2005; Saleem *et al*., 2003; Ripa *et al*., 2009).

RESULTS AND DISCUSSION

The DPPH antioxidant assay is based on the ability of DPPH a stable free radical to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The methanol leaves extract of *Nerium indicum* exhibited a significant dose dependent inhibition of DPPH activity.

It is well known that superoxide anions damage biomolecules directly or indirectly by forming H_2O_2,. OH, peroxy nitrite or singlet oxygen during aging and pathological events such as ischemic reperfusion injury. Superoxide has also been observed to directly initiate lipid peroxidation. The superoxide anion radical scavenging activity of *Nerium indicum* leaves extract assayed by the PMS-NADH system. The superoxide scavenging activity of *Nerium indicum* was increased markedly with the decrease in absorbance.

For the measurements of the reducing ability, the Fe^{3+}-Fe^{2+} transformation was investigated in the presence of *Nerium indicum* leaves extract. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. The reducing power activity of *Nerium indicum* leaves extract increased with increasing dosage.

CONCLUSION

In present investigation, antioxidant activity of methanolic leaves extract of *Nerium indicum* was carried out. The results suggest that methanolic leaves extract of *Nerium indicum* had shown very significant radical scavenging activity compared to the control. The results concluded that the extract have a potential source of antioxidants of natural origin.

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