IN VITRO RADICAL SCAVENGING EFFICACY OF DIFFERENT ORGANIC EXTRACTS OF *Nerium indicum* LEAVES

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Summary

Many medicinal plants contain large amounts of antioxidants such as secondary metabolites, which can play an important role in absorbing and neutralizing free radicals. In the present study *in vitro* radical scavenging efficacy of methanolic, ethanolic, and ethylaceate extracts of *Nerium indicum* leaves were carried out. *In vitro* free radical scavenging efficacies of these extracts were assessed by studying its ability to scavenge DPPH, nitric oxide, hydrogen peroxide and hydroxyl radicals. The methanolic extract of *Nerium indicum* leaves showed IC_{50} with minimum concentration and more effective in scavenging DPPH, nitric oxide, hydrogen peroxide and hydroxyl radicals when compared to ethanol and ethyl acetate extracts. Thus, the methanolic extract of *Nerium indicum* leaves can be recommended as a potent antioxidant to the patients suffering from various oxidative degenerative diseases such as diabetes, arthritis, cardiovascular diseases and cancer. This radical scavenging activity might be due to the active antioxidants present in the methanolic extract of *Nerium indicum* leaves.

Keywords: *Nerium indicum,* Free radical scavenging activity

Introduction

In the human body the free radicals are continuously produced due to the oxygen utilization by the cells of the body. This generates a series of reactive oxygen species (ROS) like super oxide anion (O_2-) and hydroxyl (HO·) radicals and non-free radical species such as H_2O_2, singled oxygen (O_2) and nitric oxide (NO) (1). The free radicals are known to be scavenged by synthetic antioxidants, but due to their adverse side effects leading to carcinogenicity; search for effective and natural antioxidants has

become crucial (2). In recent times, focus on plant research has increased all over the world and a large body of evidences has collected to show immense potential of medicinal plants used in various traditional systems (3). Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites, which are rich in antioxidant activities (4). A number of plants and plant isolates have been reported to protect free radical induced damage in various experimental models (5).

Nerium indicum is a well known ornamental plant with leathery evergreen leaves and handsome clusters of red, pink or white flowers. The plant originates from the Mediterranean region and is indigenous to Indo-Pakistan subcontinent (6). The flowers are hermaphrodite (7). Leaves are powerful repellent and a decoction of the leaves has been applied externally in the treatment of scabies and to reduce swellings. The leaves and the flowers are cardio tonic, diaphoretic, diuretic, emetic, expectorant and sternutatory . It has also being reported to have antibacterial and antidiabetic activities (8). Whole plant believed to have anticancer properties (9, 10). .Hence the present study has been made to investigate the *in vitro* radical scavenging efficacy of different organic extracts of *Nerium indicum* leaves.

Materials and Methods

Collection of plant material

Fresh leaves of *Nerium indicum* were collected from the outskirts of Coimbatore district, Tamilnadu. The collected leaves were washed thoroughly in tap water, shade dried and finely powdered.

Preparation of methanolic, ethanolic and ethyl acetate extracts of *Nerium indicum* leaves

10g of powder of *Nerium indicum* was filled in the thimble and extracted with 150ml of methanol, ethanol and ethyl acetate using a soxhlet extractor for 24 hours. The extracts were then distilled and evaporated to dryness. The concentrated extracts were then accurately weighed and stored in small vials at -20^0C, for further studies.

Assessment of *in vitro* radical scavenging efficacy of different organic extracts of *Nerium indicum* leaves

DPPH radical scavenging assay

This was assayed as described by Elizabeth and Rao (1990) (11). The reaction mixture contained Methanol-50 ml. DPPH (Diphenyl-2-picryl hydrazyl radical)-1mM 3 ml of 1mM DPPH in methanol was added to 100µl of plant extract with concentrations ranging from 10µg to 100µg. DPPH solution with methanol was used as a positive control and methanol alone acted as a blank. When DPPH reacts with antioxidant in the sample, it was reduced and the color changed from deep violet to light yellow. This was measured at 518 nm. The percentage scavenging activity was calculated by the following formula.

$$\text{Scavenging activity (\%)} = \frac{\text{A518 (control)-A518 (sample)}}{\text{A518 (control)}} \times 100$$

Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat (1964) (12).The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25^0C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess illosvery reaction at 540 nm.

Hydrogen peroxide scavenging assay

Hydrogen peroxide assayed as described by (Ruch *et al.,* 1989) (13) proposed an assay for the determination of antioxidant activity of compounds by their ability to scavenge the oxidant hydrogen peroxide. The reaction mixture contained Phosphate buffer (pH-7.4) hydrogen peroxide in phosphate buffer (40mM). A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer. Plant extracts at the concentration of 10mg/10µl was added to a hydrogen peroxide solution (0.6ml,40mM).The total volume was made up to 3ml.The absorbance of the reaction mixture was recorded at 230nm.The blank solution contained phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by the plant extract was calculated as follows:

$$\text{Percentage of scavenged } H_2O_2 = \frac{A0 - A1}{A0} \times 100$$

A0- Absorbance of control
A1- Absorbance in the presence of plant extract

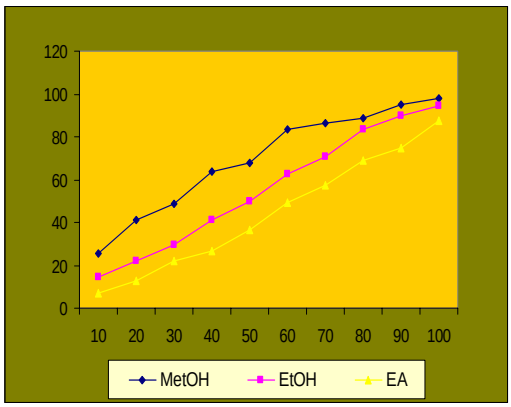
Hydroxyl radical scavenging activity

Hydroxyl radical assayed as described by Elizabeth and Rao (1990) (14). The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} -Ascorbate – EDTA – H_2O_2 system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM),0.1 ml EDTA (0.1 mM), 0.1 ml H_2O_2 (1mM), 0.1 ml Ascorbate (0.1mM), 0.1 ml KH_2PO_4-KOH buffer, pH 7.4 (20mM) and various concentrations of plant extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37^0 C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

Results

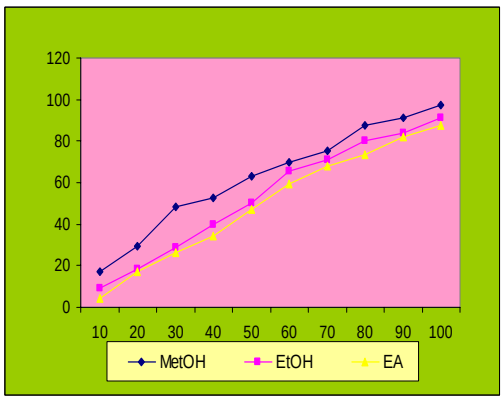
In vitro radical scavenging efficacy of various organic extracts of *Nerium indicum* leaves were assessed by its ability to scavenge DPPH, NO, H_2O_2 and OH radicals. Fig 1 illustrates the dose response curve of DPPH radical scavenging activity of methanolic, ethanolic and ethyl acetate extracts of *Nerium indicum* leaves. The IC_{50} values of methanolic, ethanolic and ethyl acetate extracts were found to be 31µg, 50µg and 61µg respectively. It was observed that methanolic extract of *Nerium indicum* leaves had higher activity for scavenging DPPH than that of ethanolic and ethyl acetate extracts.

Fig 1. DPPH radical scavenging efficacy of various organic extracts of *Nerium indicum* leaves



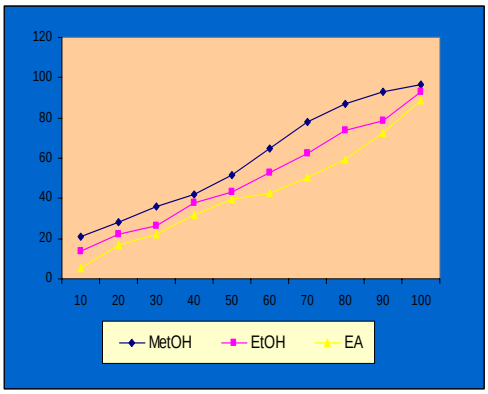
Methanolic extract of *Nerium indicum* leaves showed the highest NO scavenging activity when compared to ethanolic and ethyl acetate extracts in a moderate dose dependent manner with an IC_{50} value of 34 µg as shown in the Fig 2. The IC_{50} values of ethanolic and ethyl acetate extracts were found to be 49µg and 53µg respectively.

Fig 2. Nitric oxide radical scavenging efficacy of various organic extracts of *Nerium indicum* leaves



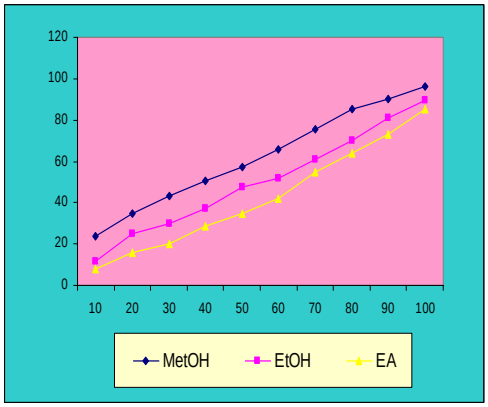
Scavenging of H_2O_2 and its percentage inhibition of methanolic, ethanolic and ethyl acetate extracts of *Nerium indicum* leaves showed the IC_{50} values of 49µg, 57 µg and 70 µg respectively. The results are shown in the Fig 3. Here also the methanolic extract of *Nerium indicum* leaves showed IC_{50} with minimum concentration followed by ethanolic and ethyl acetate extracts.

Fig 3. Hydrogen peroxide radical scavenging efficacy of various organic extracts of *Nerium indicum* leaves



The OH radical scavenging assay shows the ability of methanolic, ethanolic and ethyl acetate extracts to inhibit OH radical mediated deoxyribose degradation in an Fe^{3+} -EDTA- Ascorbic acid - H_2O_2 reaction mixture. The results are shown in the Fig 4. The IC_{50} values of the methanolic, ethanolic and ethyl acetate extracts were 39 µg, 54 µg and 66 µg respectively. Here also the methanolic extracts of *Nerium indicum* leaves showed IC_{50} with minimum concentration followed by ethanol and ethyl acetate extracts.

Fig 4. Hydroxyl radical scavenging efficacy of various organic extracts of *Nerium* indicum leaves



Discussion

DPPH radical scavenging is considered a good in vitro model widely used to assess antioxidant efficacy within a very short time. In its radical form, DPPH has disappears on reduction by an antioxidant compound or a radical species to become a stable diamagnetic molecule resulting the colour changes from purple to yellow, which could be taken as an indication of the hydrogen donating ability of the tested sample (15, 16). DPPH radical scavenging ability of the methanolic extract of *Nerium indicum* leaves were significantly higher than that of ethanolic and ethyl acetate extracts.

It is well known that nitric oxide has an important role in various inflammatory processes. Sustained levels of production of this radical are directly toxic to tissues and contribute to the vascular collapse associated with septic shock, whereas chronic expression of nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (17). The toxicity of NO increases greatly when it reacts with super oxide radical, forming the highly reactive peroxy nitrite anion ($ONOO^-$) (18). The nitric oxide generated from sodium nitro prusside reacts with oxygen to form nitrite. The methanolic extract of *Nerium indicum* leaves inhibits the nitrite formation by directly competing with oxygen in the reaction with NO. This study proved that the mehanolic extract has more potent NO scavenging activity when compared to ehanolic and ethyl acetate extracts.

H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells (19). The results showed that the methanolic extract of *Nerium indicum* leaves had an effective H_2O_2 scavenging activity.

Hydroxyl radicals are most reactive species, initiating the peroxidation of the cell membrane (20). The lipid radical, thus generate would initiate chain reaction in the presence of oxygen, giving rise to lipid peroxide, which break down to aldehydes such as malondialdehyde. The IC_{50} value indicates that the methanolic extract of *Nerium indicum* leaves are better hydroxyl radical scavenger than that of ethanolic and ethyl acetate extracts.

Conclusion

On the basis of the results obtained in the present study, it concludes radical scavenging efficacy of methanolic extract of *Nerium indicum* leaves and thus gives scientific basics for its traditional uses as potent antioxidant to the individuals under oxidative stress such as inflammation, aging, mutagenicity and carcinogenicity. In future we look forward to check the potency of methanolic extract of *Nerium indicum* leaves by means of *in vivo* antioxidant studies and also to isolate, identify and characterize the active constituents responsible for these effects.

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