Antioxidant Activity of *Nerium odorum* Soland and *Thevetia* neriifolia Juss

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Summary

The aqueous extract of *Nerium odorum* Soland and methanol extract of *Thevetia neriifolia* Juss were evaluated for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Gallic acid and butylated hydroxyanisole (BHA) were used as reference standards. They exhibited strong antioxidant radical scavenging activity with IC_{50} value of 0.4 µg/ml, 1.15 µg/ml, 15.45 µg/ml and 43.79 µg/ml for Gallic acid, BHA, *Nerium odorum* Soland and *Thevetia neriifolia* Juss respectively. The strongest antioxidant activity of the extracts could be due to the presence of flavonoids.

Key words: *Nerium odorum* Soland; *Thevetia neriifolia* Juss; antioxidant activity; DPPH; gallic acid; butylated hydroxyanisole.

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Introduction

Nerium odorum Soland (Apocynaceae), known as Kaner, Karavira, is a small shrub distributed in the Mediterranean regions, Subtropical Asia. It is found in Himalayas from Nepal westwards to Kashmir up to 1950 m and in upper Gangetic plain, wild or cultivated in Punjab, Madhya Pradesh, West Bengal, Chota Nagpur, Bihar, Orissa, Deccan, Central India and South India. The plants are grown as ornamental shrub for their showy flowers. It is used as cardiokinetic, diuretic, cardiotonic, anti-stress, antibiotic, antifungal, insecticidal, hypotensive, spasmodic, antipyretic, anti-inflammatory, adaptogenic, anticancer and analgesic [1]. The plant *Thevetia neriifolia* Juss (Apocynaceae), known as yellow oleander, is a large evergreen shrub native to Tropics of America and West Indies. In India, it is grown throughout the plains [2]. In Traditional System of Medicine, the plant is used as cardio tonic [3, 4, 5], antipyretic, antimalarial [6], abortifacient, intermittent fever [7], tumor, rheumatism, dropsy and as purgative [8].

Free radicals are reactive molecules involved in many physiological processes and human diseases such as cancer, aging, arthritis, Parkinson syndrome, ischemia, toxin induced reactions, alcoholism, liver injury etc. Research in finding a natural antioxidant from the plant source is therefore important as plants are potential source of immense chemicals for the treatment of number of aliments. With this view, the present study was undertaken to evaluate *in vitro* antioxidant DPPH free radical scavenging activity of leaves of *Nerium odorum* Soland and stem bark of *Thevetia neriifolia* Juss as these plants are useful for number of aliments as per Traditional System of Medicine.

Materials and Methods

Chemicals: 1, 1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid and butylated hydroxyanisole was purchased from Loba Chemie Pvt Ltd., Mumbai. All the chemicals and reagents used were of analytical grade.

Plant Material: The leaves of *Nerium odorum* Soland and stem bark of *Thevetia neriifolia* Juss were collected in the month of July 2007 and September 2007 respectively and authenticated by Prof. Gajendra Rao, Central Council for Research in Ayurveda and Siddha, Bangalore. Voucher specimens (12477RRCBI and 12476RRCBI) are deposited in the Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore.

Preparation of aqueous and methanol extract: The shade dried powdered leaves of of *Nerium odorum* (450 g) and coarsely powdered stem bark of *Thevetia neriifolia* (750 g) were exhaustively extracted with petroleum ether (60^0-80^0C) followed by chloroform and methanol using soxhlet apparatus. The marc was extracted on water bath with water for 24 hr. The crude extracts were evaporated to dryness in a rotary film evaporator, with the percentage yield being 4.21, 0.85, 4.82 and 11.91 % w/w for *Nerium odorum* and 4.1, 0.95, 4.93 and 5.9 % w/w for *Thevetia neriifolia* respectively in term of dry plant

material. All the extracts were subjected to various chemical tests to detect the presence of different phytoconstituents [9]. The aqueous extract of *Nerium odorum* (NOAE) and methanol extract of *Thevetia neriifolia* (TNME) only were chosen for further antioxidant activity on the basis of the presence of flavonoids as phytoconstituent in the phytochemical test.

Evaluation of Antioxidant Activity

The free radical scavenging activity of the aqueous extract of *Nerium odorum* and methanol extract of *Thevetia neriifolia* were measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) [10].Briefly, 0.1 mM solution of DPPH in ethanol was prepared. 1 ml of the solution was added to 3 ml of NOAE and TNME solution in methanol at different concentration (100 – 1.56 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a spectrophotometer (UV- VIS Shimadzu). Reference standard compound being used were gallic acid and butylated hydroxyanisole (0.125-1 µg/ml and 1-5 µg/ml) respectively. The experiment was done in triplicate. The IC_{50} value is the concentration of sample required to inhibit 50 % of the DPPH free radical. The IC_{50} value for the sample was calculated using log-dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated using the following equation:

DPPH scavenging effect (%) = $100 \times A_1 / A_0$

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in presence of the standards or samples.

Result and Discussion

Preliminary phytochemical screening revealed the presence of steroids, flavonoids, glycosides, saponin, carbohydrates and tannins in the different extracts of the both plants. Only aqueous extract of *Nerium odorum* and methanol extract of *Thevetia neriifolia* were chosen as it contains flavonoids which are generally potent inhibitors of free radicals [11].

The model for scavenging the stable DPPH radical is widely used model to evaluate antioxidant activities in a relatively short time to compare with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accept an electron or hydrogen radical to become a stable diamagnetic molecule and therefore inhibit the propagation phase of lipid peroxide [12, 13].

The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progresses, which results in scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants.

Table: 1 illustrate the percentage inhibition of DPPH radical by standards, NOAE and TNME. The IC_{50} value was found to be 0.4, 1.15, 15.45 and 43.79 µg/ml for gallic acid, butylated hydroxyanisole, *Nerium odorum* aqueous extract and *Thevetia neriifolia* methanol extract respectively. Aqueous and methanol extracts has shown potent DPPH radical activity which could be due to the flavonoids present in the extract. The components responsible for the antioxidant activity of the extract are unknown. Further research is therefore needed for the isolation and identification of the antioxidant components in the extract.

Table 1: DPPH free radical scavenging activity of standard and extracts [NOAE and TNME]

Extract/standard	IC_{50} value (µg/ml)
Gallic acid	0.40
Butylated hydroxyanisole	1.15
Nerium odorum aqueous extract (NOAE)	15.45
Thevetia neriifolia methanol extract (TNME)	43.79

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