

**NOTE****Free Radical Scavenging Assay of *Thevetia nerifolia* Leaf Extracts**

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The petroleum ether, benzene, chloroform, acetone and ethanol extract of *Thevetia nerifolia* (Apocynaceae) leaves were investigated for free radical scavenging properties. The antioxidant activities of extracts were investigated using 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging assay. Butylated hydroxytoluene and L-ascorbic acid were employed as a positive control. All extracts showed DPPH radical scavenging activities, but among them petroleum ether extract shows maximum activity as compared to other extracts.

**Key Words:** *Thevetia nerifolia*, Free radical scavenger.

Free radicals are known to be a major cause of various chronic and degenerative diseases, ageing, coronary heart diseases, inflammations, stroke, diabetes mellitus, cancer<sup>1</sup>, cellular injuries, initiate per-oxidation of polyunsaturated fatty acids in biological membranes<sup>2</sup>, DNA damage<sup>3</sup> and oxidation of important enzymes<sup>4</sup> in human body. Recently, natural food and food derived antioxidants such as vitamins and phenolic phytochemical have received growing attention, because they are known to function as chemo preventive agent's oxidative damage. Determination of natural sources of antioxidant of plants is important. There is no information above free radical scavenging assay of extracts of *Thevetia nerifolia*. Present study was done to evaluate its free radical scavenging properties of *Thevetia nerifolia*.

The plant material was obtained from the Yellagiri Hills, Vellore District, identified in School of Biosciences, VIT University. The collected leaves were air-dried for a week at room temperature and stored for further use.

**Preparation of extracts:** About 50 g of the air dried leaves was extracted using petroleum ether, benzene, chloroform, acetone and ethanol in a soxhlet apparatus. After each extraction the solvents are removed by rotary flash evaporator and to obtain green solid mass, except petroleum ether extract which was obtained as brownish yellow gummy residue.

**Free radical scavenging assay:** The scavenging activity of crude extracts of dried leaves was determined by the DPPH\* assay and the results are shown as graph. 0.01mM of DPPH (Stigma-Aldrich) in absolute methanol was prepared and stored in refrigerator for further use. 3 mL of extract solution (0.2 g in 10 mL absolute

methanol) and 1 mL of DPPH solution were taken in cuvette and the absorbance was noted at 517 nm for interval of each 5 min for 0.5 h. Butylated hydroxytoluene (BHT) and ascorbic acid at  $100 \mu\text{g mL}^{-1}$  in methanol were used as standard. Decreasing the absorbance of DPPH solution indicates an increase in DPPH radical scavenging activity. This activity is given as per cent of DPPH radical scavenging which is calculated with the following equation,

$$\% \text{ DPPH Radical Scavenging} = \frac{\text{DPPH}_{\text{Abs}} - S_{\text{Abs}}}{\text{DPPH}_{\text{Abs}}} \times 100$$

where,  $S_{\text{Abs}}$  = Sample absorbance.

The measurements of DPPH radical scavenging activity were carried out for the extracts for 3 replications and values are an average of 3 replications.

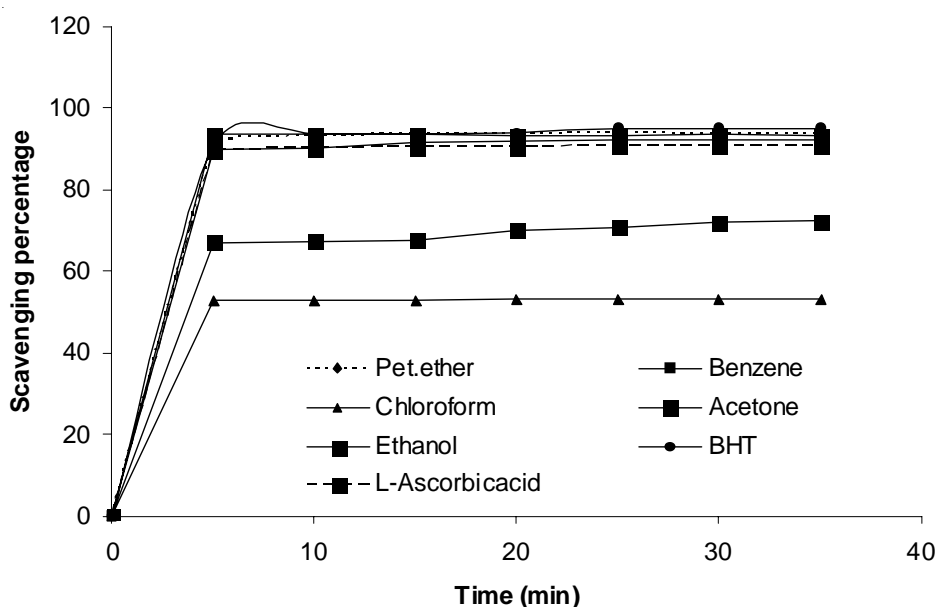


Fig. 1. Antioxidant activity of various solvent extracts of *Thevetia neriifolia* leaves

All the solvent extracts of leaf samples had a good radical scavenging activity. The orders of scavenging activities were ethanol, petroleum ether, benzene, acetone and chloroform respectively. When compared to all extracts, ethanol, petroleum ether and benzene have comparable radical scavenging capacity than butylated hydroxytoluene. Benzene extracts has low scavenging activity than L-ascorbic acid. Though the acetone and chloroform extracts has radical scavenging property, they are less active than standards. Among all the extracts ethanol and petroleum ether extract from the leaves of *Thevetia neriifolia* was found to have free radical scavenging ability. Further studies are necessary to elucidate the compound responsible

for antioxidant activity of extracts and results suggest that the component from petroleum ether and ethanol extracts of this species can be potent antioxidants comparable in activity with the widely used synthetic antioxidants butylated hydroxytoluene and L-ascorbic acid.

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