

**ANTIOXIDANT SCREENING OF *SHOREA THUBUGGAIA ROXB.*
AN ENDEMIC AND ENDANGERED SPECIES OF SESHACHALLUM
HILLS**

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ABSTRACT

Shorea thubuggaia Roxb. belongs to the family Dipterocarpaceae is globally highly threatened and vulnerable medicinal plant. The aim of this study was to assess, antioxidant activity for the first time from different parts of leaves, stem and root of *shorea thubuggaia* by using stable free radical 1,1Diphenyl-2- Picryl Hydrazyl(DPPH), Hydrogen Peroxide (H_2O_2) and Nitric oxide (NO) Scavenging activity. The plant extracts showed antioxidant effect which is expected mainly due to phenolic components such as flavonoids, phenolic acids and phenolic diterpenes. This phenolic component possesses many hydroxyl groups including O-dihydroxyl group which have very strong radical scavenging effect and antioxidant power. In DPPH assay, the

antioxidant was able to reduce the stable radical DPPH to yellow colour. The observed scavenging effect of plant extracts and standards on the DPPH radical is very high in leaves at $75\mu\text{g/ml}$ when compare to stem and root respectively. The DPPH assay provides an easy rapid way to evaluate potential antioxidants. The H_2O_2 scavenging activity is also high in leaves when compare to stem and root. The Hydrogen peroxide sometime it can be toxic to living cells, because in living cells it is converted into free radical called hydroxyl radicals(OH), react with biomolecules, causes tissue damage and cell death. Nitric oxide is a potent pleiotropic mediator of physiological processes. Its activity is high in stem and leaf when compare to root. Hence, it can be concluded that *S. thubuggaia* could be pharmaceutically exploited for antioxidant properties.

KEYWORDS: Antioxidants, methanolic extract, free radical, DPPH Scavenging.

INTRODUCTION

Antioxidants may be defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Antioxidants can also protect the human body from free radicals and ROS effects. They retard the progress of many chronic diseases as well as lipid peroxidation. Also, antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods. There are numerous antioxidant methods for evaluation of antioxidant activity. For in vitro antioxidant screening, DPPH, nitric oxide, hydrogen peroxide and reducing power assay are most commonly used. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecule and radical progresses, results in the scavenging of the radical by hydrogen donation¹⁸. It is visually noticeable as a discoloration from purple to yellow. Figure 1 indicates noticeable effect of extracts and fractions on scavenging of free radicals. The antioxidants are now known to be protective or therapeutic agents. The applications of antioxidants are industrially widespread in order to prevent polymers oxidative degradation, auto-oxidation of fats, synthetic and natural pigments discoloration, etc. There is an increased interest of using antioxidants for medical purposes in the recent years, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs (Vaya J, Aviram M. 2001). In the past few years, there is an increased preference for antioxidants from natural sources rather than from synthetic sources because of the health risks and toxicity of synthetic antioxidants (Buxiang S, Fukuhara M 1997). Plants are being utilized frequently as sources of natural antioxidants, and some of the compounds present in plants have significant antioxidative properties and health benefits (Abdalla A E, Roozen J P. 1999). The potential of the antioxidant constituents of plant materials for the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists and food manufacturers as consumers move toward functional foods with specific health effects (Lo liger J 1991). At present, most of the natural antioxidants such as traditional nutrients, polyphenols, and flavonoids are obtained from plants. Although many plant species have been investigated in the search for novel antioxidants, but there is still a demand to find more information concerning the antioxidant potential of medicinal plant species. However, there is not even a single report of antioxidant activity for the leaves of this highly threatened and vulnerable medicinal plant from North Indian Himalayan region. Therefore, the chief objective of this present study is to assess the antioxidant activity for first time from different extracts of leaves, stem and root of Shorea

tumbuggaia by the use of stable free radical diphenyl picryl hydrazyl (DPPH), Hydrogen peroxide (H₂O₂) scavenging activity and Nitric oxide (NO) scavenging activity.

10.1. MATERIAL AND METHODS

Plant Material

The sampling site was Tirumala tirupathi, where we have collected Leaf, Stem and Root material from mother plant. While the research activities for Antioxidant screening (*in vitro*) were done at UGC laborator at S. V. University.



Figure 3: Shorea tumbuggaia Roxb in flowering stage in Tirumala forest.

- A. *Shorea tumbuggaia* Roxb in tirumal forest
- B. *Shorea tumbuggaia* Roxb in flowering stage

DPPH radical scavenging activity: The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution of 1, 1-diphenyl-1-picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 ml of various concentrations of the plant extracts Leaf, stem (bark) and roots (25, 50, 75, mL) in methanol was added to 4 ml of 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. The percent of inhibition (I %) of free radical production from DPPH was calculated by the following equation.

$$\% \text{ of scavenging} = \frac{(A \text{ control} - A \text{ sample})}{(A \text{ control})} \times 100$$

Where A control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. Tests were carried in triplicate.

Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activity was measured by slightly modified methods. Nitric oxide radicals (NO) were generated from sodium nitroprusside. 1 ml of sodium nitroprusside (10 mM) and 1.5 ml of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50, 75 $\mu\text{g/mL}$) of the plant extracts Leaf, stem (bark) and roots, and incubated for 150 min at 25 $^{\circ}\text{C}$ and 1 ml of the reaction mixture was treated with 1 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore was measured at 546 nm. Nitric oxide scavenging activity was calculated using the above equation.

Hydrogen peroxide (H_2O_2) scavenging activity

H_2O_2 scavenging ability of the test compound was determined according to the literature method. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 25, 50, 75 $\mu\text{g/mL}$ concentrations of the plant extracts Leaf, stem (bark) and roots in 3.4 ml phosphate buffer were added to H_2O_2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. The percent of scavenging of H_2O_2 was calculated using the above equation.

10.2. RESULTS AND DISCUSSION

Like other medicinal plants, different extracts from leaves of *Shorea tumbuggaia* showed antioxidative effect which is expected mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. These phenolic components possess many hydroxyl groups including o-dihydroxy group which have very strong radical scavenging effect and antioxidant power. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 1, 1-diphenyl-1, 2-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in methanol solution centred at 517 nm. The observed scavenging effect of plant extracts and standard on the DPPH radical is very high in leaf (52.2 ± 1.47) at 75 $\mu\text{g/mL}$ when compared to stem and root respectively (Table-1a,b,c). Hydrogen peroxide scavenging activity

peroxide non reactive, but sometimes it can be toxic to living cells, because in living cell it is converted into free radical called hydroxyl radicals ($\bullet\text{OH}$), react with biomolecules, cause tissue damage and cell death. Table shows (43.44 ± 0.56) at $75\mu\text{g/ml}$, it is less than the $75\mu\text{g/ml}$ of leaf. Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes. The NO at $75\mu\text{g/ml}$ shows 36.61 ± 1.09 . It is very less when compared to leaf and stem.

Table 1.

A. *In vitro* antioxidant activity by DPPH method

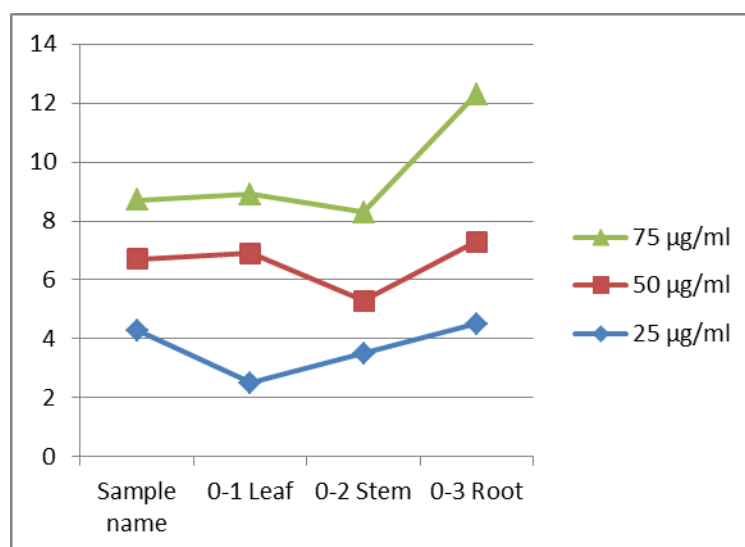
Sample name	25 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$	75 $\mu\text{g/ml}$
0-1 Leaf	21 ± 0.44	42.8 ± 0.37	52.2 ± 1.47
0-2 Stem	16.25 ± 1.25	31.46 ± 1.84	41.4 ± 1.66
0-3 Root	11.35 ± 0.66	23.96 ± 0.11	36.44 ± 0.58

B. *In vitro* antioxidant activity by H₂O₂ method.

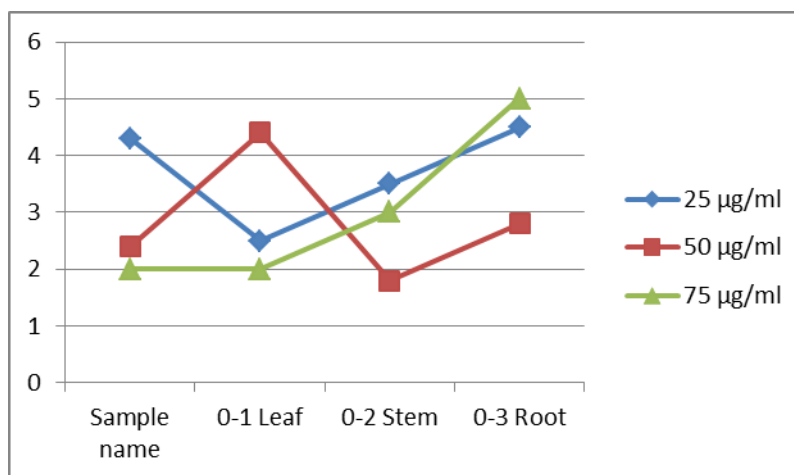
Sample name	25 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$	75 $\mu\text{g/ml}$
0-1 Leaf	14.7 ± 0.29	36.12 ± 0.7	43.44 ± 0.56
0-2 Stem	10.31 ± 1.7	23.53 ± 1.57	39.48 ± 1.46
0-3 Root	7.21 ± 0.41	21.3 ± 1.22	29.88 ± 1.62

C. *In vitro* antioxidant activity by nitric oxide (NO) method.

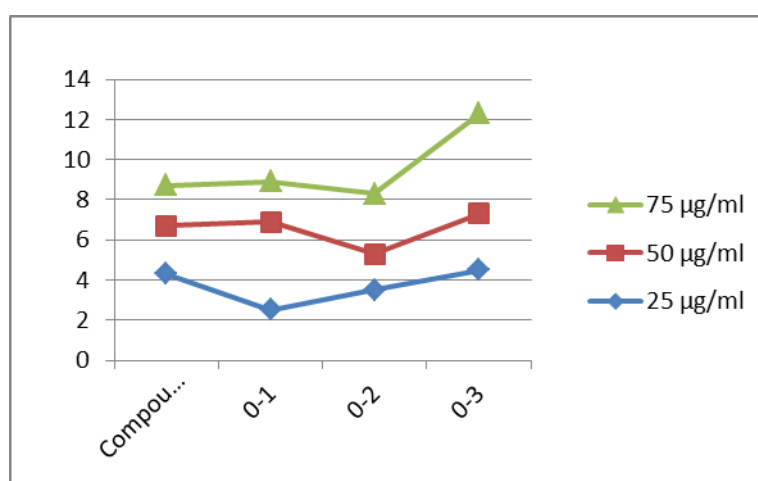
Compound name	25 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$	75 $\mu\text{g/ml}$
0-1	15.9 ± 1.91	20.4 ± 1.46	36.61 ± 1.09
0-2	10.69 ± 1.1	24.6 ± 0.19	39.26 ± 0.1
0-3	9.1 ± 1.4	17.7 ± 1.48	24.46 ± 1.52



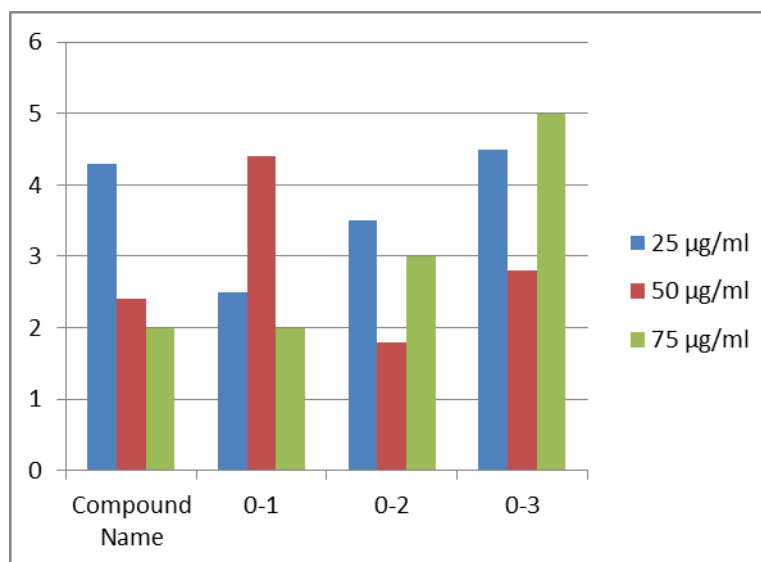
Graph 1: In vitro antioxidant activity by DPPH method.



Graph 2: In vitro antioxidant activity by H₂O₂ method.



Graph 3: In vitro antioxidant activity by nitric oxide (NO) method.



Graph 4: Invitro antioxidation activity of 1.leaf 2.Stem 3. Root by DppH, H₂O₂ and No method.

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