



## DPPH radical scavenging activity of three medicinal plants from simlipal biosphere reserve: A study on antioxidant activity

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### ABSTRACT

The free radical scavenging activity of aqueous extracts of three medicinal plant species (*Saraca indica*, *Withania somnifera* and *Asparagus racemosus*) were assayed by DPPH (2,2-Diphenyl-1-Picrylhydrazyl) radical scavenging assay at concentrations 50, 75 and 100µg/ml with a view to assess the antioxidant properties of the medicinal plants. The DPPH activities of all plant species increased with the increase in concentrations. While aqueous extract of *S. indica* bark showed significantly higher DPPH activity, *A. racemosus* and *W. somnifera* showed comparatively moderate activity at all concentrations. Results revealed the presence of antioxidant properties borne by the different plant parts in different amounts which has got promising future prospect for their use in pharmaceuticals for replacement of synthetic antioxidants. © 2010 Trade Science Inc. - INDIA

### INTRODUCTION

Medicinal plants constitute a very important 'natural resource' used by indigenous medicinal systems for the last 300 years. The Central Council of Research on Ayurveda and Sidha medicine have drawn a list of 243 commonly used medicinal plants having greater demand for manufacture of gelanicals, mixtures, compound formations and potent medicines<sup>[1]</sup>. The medicinal plants also provide raw materials for modern medicines and pharmaceutical industries. Because of growing interest in herbal medicine, there is a great demand of medicinal plants from developing countries in the global market. As per an estimate, international export of medicinal plants is dominated by China, which exports 1, 21,900 tons of materials a year, whereas India, exports 32,600 tons annually<sup>[2]</sup>. In recent years there has been depletion of medicinal plants due to biotic interferences of various categories and different magnitudes. It is im-

perative, therefore that before we stand to loose valuable economic resource, we need to document the existing medicinal plants, their ethnomedicinal uses and explore their medicinal potential for biotechnological exploitation.

Reactive oxygen species (ROS) are generated in human body endogenously as a result of normal metabolic activity and also due to exogenous sources like UV radiation etc.<sup>[3]</sup> and which are neutralized through our antioxidant system. In pathological conditions, the antioxidant mechanisms are often inadequate, as excessive quantities of ROS generally get generated. The ROS formed often results in causing cellular and sub-cellular damage by peroxidation of membrane lipids, denaturation of DNA strands and cellular proteins<sup>[3]</sup>. In a multicellular organism including human beings, the cell protects itself from the damage caused by the ROS system by various enzymatic and non-enzymatic ways. The common enzymes those work efficiently in the re-

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duction of ROS are catalase, glutathione peroxidase, glutathione reductase, superoxide dismutase, lipid peroxidase and many more. Beta carotene, vitamin A, C and E constitutes the non- enzymatic pathways<sup>[4]</sup>. Recently, there is growing urge to discover natural antioxidants as it has been believed that the consumption of plant products reduces the risk of developing chronic diseases as a result of side effects since the phytochemicals are generally safer than the chemical and synthetic formulations<sup>[5]</sup>.

Tropical forests and many other tropical ecosystems are rich sources of a diversity of plant derived chemical compounds. A greater portion of the tropical plant species contains secondary compounds, potentially useful as models for/as medicines<sup>[6]</sup>.

Similipal biosphere reserve (SBR), located in Mayurbhanj district of Orissa (India), is a unique habitat of mixed tropical forest which is rich in medicinal plants<sup>[7,8]</sup>. Although more than 500 medicinal plants exist in SBR, limited studies have been made to evaluate the phytochemical constituents and medicinal properties which could be exploited for pharmaceutical applications. Keeping these in view, the present study is aimed at evaluating the antioxidant activity of three important medicinal plants having ethnomedicinal uses<sup>[9]</sup> viz. *Asparagus racemosus* (root), *Withania somnifera* (root) and *Saraca indica* (bark) by DPPH (2,2-Diphenyl-1-Picrylhydrazyl) radical scavenging activity.

## MATERIALS AND METHODS

### Plant material

Three plant species namely *Saraca indica* L., *Withania somnifera* Dunal and *Asparagus racemosus* Willd. were collected from the forests of Similipal biosphere reserve (20° 17' -22° 10' N latitude and 85° 57' -85° 47' E longitude), Orissa, India in the March and April 2009. The plants were identified by Dr. A.K.Biswal, Department of Botany, North Orissa University. The dried bark and root was homogenized to powder and further subjected to extraction.

### Crude extraction

The bark and roots of plant were shade dried for 15 days and then pulverized into fine powder using pestle and mortar. Twenty five grams of fine powder

was added to a soxhlet apparatus along with a solvent (water) for extraction of chemicals. The liquid extracts were evaporated to dryness by vacuum distillation and stored at 4°C for further analysis.

### Scavenging of DPPH radical

The scavenging effect of crude aqueous extract of *Saraca indica*, *Withania somnifera* and *Asparagus racemosus* was determined by following standard method<sup>[10]</sup>, with little modification. Briefly, 2.0ml of 0.1 mM DPPH (2, 2- diphenyl-1-picrylhydrazyl) solution (in methanol) was added to the test tube containing 0.1 ml aliquote of aqueous plant extract (50-100µg/ml). The mixture was vortexed for 1 minute and kept at room temperature for 30 minutes in the dark. The absorbance of all the sample solutions was measured at 517 nm. The percentage scavenging effect was calculated from the following equation.

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

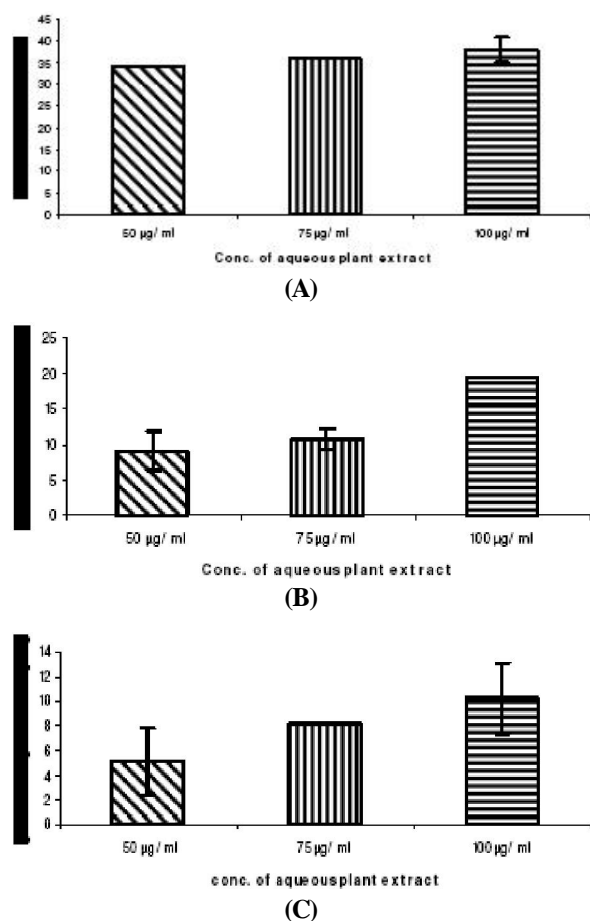
where  $A_0$  = Absorbance of control,  $A_1$  = Absorbance of test sample.

### Statistical analysis

Each data point was obtained by making at least 3 independent measurements. The results were expressed as mean  $\pm$  SD (Standard deviation).

## RESULTS AND DISCUSSION

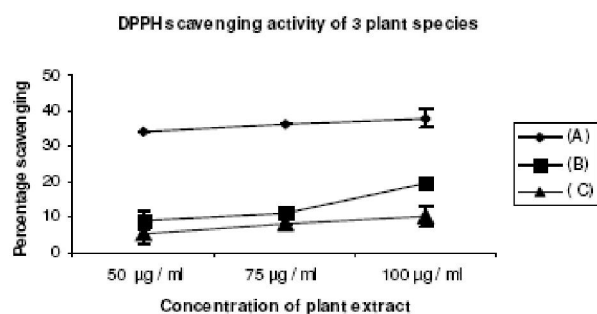
The antioxidant property of the aqueous extracts of three medicinal plants viz. *Saraca indica*, *Withania somnifera* and *Asparagus racemosus* was evaluated using DPPH scavenging assay (Figure 1(A, B, C)). Aqueous extract of *S. indica* (bark) showed significantly higher DPPH activity (i.e. 34 %, 36 % and 38 %) at all three concentrations (50, 75 and 100µg/ml) which increased with increase in concentration (Figure 1(A)), where as *A. racemosus* and *W. somnifera* showed moderate activity at all the three concentrations (Figure 1 (B & C)) with an increasing trend in increased concentrations. Out of the three plant species tested, *S. indica* showed comparatively higher DPPH radical scavenging activity (Figure 2). Earlier studies on antioxidant activity of methanolic extracts of *A. racemosus* and *W. somnifera* roots have shown to in-



**Figure 1 : DPPH radical scavenging activity of aqueous extracts of (A) *S. indica* (B) *A. racemosus* and (C) *W. somnifera* from Similpal Biosphere reserve**

crease antioxidant defense i.e enzyme superoxide dismutase, catalase and ascorbic acid in rats<sup>[11,12]</sup> reported dose dependent DPPH scavenging activity of aqueous extract of *A. racemosus* root with IC<sub>50</sub> of 60.7 microg/ml.

Cellular damage or oxidative injury arising from free radicals or reactive oxygen species (ROS) now appears to be one of the fundamental mechanism for a number of human diseases, such as ischemic injury, inflammation, cancer, atherosclerosis, neurodegenerative diseases (Parkinson's and Alzheimer's) and ageing<sup>[13-15]</sup>. Recent clinical and experimental data showed the involvement of reactive oxygen species in many human pathophysiological conditions<sup>[12]</sup>. DPPH has been used extensively as a free radical to evaluate reducing substances<sup>[16]</sup> and is a useful reagent for investigating the free radical scavenging activities of compounds<sup>[17]</sup>. This assay provides information on the reactivity of tested compounds (plant extracts) with a stable free radical



**Figure 2 : Comparison of DPPH radical scavenging activity of the aqueous extracts of (A) *S. indica* (B) *A. racemosus* and (C) *W. somnifera* from Similpal biosphere reserve**

(DPPH). Because of its odd electron, DPPH gives a strong absorption band at 517nm in visible spectroscopy (deep violet colour). As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes, and the resulting decolorization is stoichiometric with respect to the number of electrons taken up<sup>[18]</sup>. The result of the present study shows that aqueous extracts of *Saraca indica*, *Withania somnifera* and *Asparagus racemosus* possess promising antioxidant property. The present study although revealed possession of antioxidant property of the three tested medicinal plants, more assays are required to fully establish this property. Further determination of antioxidant compounds from the extracts of these plants is essential for exploring their pharmaceutical application.

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