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P. Uma Maheswari
PG and Research Department of Botany, Kongunadu arts and science college (Autonomous), Coimbatore-641029, Tamilnadu, India.

A. Shalimol
PG and Research Department of Botany, Kongunadu arts and science college (Autonomous), Coimbatore-641029, Tamilnadu, India.

K. Arumugasamy
PG and Research Department of Botany, Kongunadu arts and science college (Autonomous), Coimbatore-641029, Tamilnadu, India.

Correspondence:
P. Uma Maheswari
PG and Research Department of Botany, Kongunadu arts and science college (Autonomous), Coimbatore-641029, Tamilnadu, India.

Free Radical Scavenging Activity of *Smilax wightii* A. DC. (Smilacaceae), an Endemic Medicinal Plant from Western Ghats

P. Uma Maheswari, A. Shalimol and K. Arumugasamy

ABSTRACT
The present study was aimed to evaluate the free radical scavenging activity of methanol extract of *Smilax wightii* by using DPPH, Nitric oxide and ABTS radical scavenging assays. The extract had scavenging activities on DPPH radical in a dose-dependent manner with an IC_{50} value of 31.68 µg/ml. The extract showed its highest nitric oxide radical scavenging activity 95.16% in 50 µg/ ml. A steady increase in the percentage inhibition of the ABTS radicals by extract was observed in 872.52±0.08 trolox equivalence in µMol/g extract at the dose of 50 µg/ml. The results suggest that the methanol extract of *S. wightii* has very strong antioxidant activities and could be used as effective natural antioxidant resources.

Keywords: *Smilax wightii*, DPPH, Nitric oxide and ABTS radical scavenging assays.

1. Introduction
Medicinal plants continue to be an important therapeutic aid for alleviating ailments of humankind. Search for eternal health and longevity and to seek remedy to relieve pain and discomfort prompted the early man to explore his immediate natural surrounding and tried many plants, animal products and minerals and developed a variety of therapeutic agents. The ancient civilization of India, China, Greece, Arab other countries of the world developed their systems of medicine independent of each other but all of them were predominantly plant based. But the theoretical foundation and the insights and indepth understanding on the practice of medicine that we find in Ayurveda is much superior among organized ancient systems of medicine [1].
Smilax wightii belongs to the family Smilacaceace. It is a large climber with slender, sparsely spiny, striate and quadrangular branches [2]. The plants of genus *Smilax* are nutritionally important as they have nectar-rich flowers. Smilax rhizomes have different kinds of pharmacological behaviours such as antibacterial, antifungal, antioxidant and other activities [3]. Hence, the present study was under taken to evaluate the free radical scavenging activity of methanolic extract of *Smilax wightii* by DPPH, Nitric oxide and ABTS radical scavenging assays.

2. Materials and Methods
2.1 Preparation of plant sample
Smilax wightii, the whole plant was collected from Kodanadu, The Nilgiri Hills, The Western Ghats, Southern India, Tamil Nadu. The plant was identified and authenticated by a plant taxonomist, SACON, Coimbatore. The whole plant materials were dried in shade after washing with cold water and then powdered using pulveriser and passed through sieve. About 100 g of dried plant powder was extracted with petroleum ether using soxhlet apparatus for 18 hours. The petroleum ether was evaporated from the extract and then the residue was re-extracted with methanol. This extract after evaporation of methanol, the filtered residue was stored at 4 ^{0}C in refrigerator for further use.

2.2 DPPH radical scavenging assay
The scavenging effect of extracts on DPPH radicals was determined according to the method of Shimada *et al.*, [4]. Various concentrations of sample (4 ml) were mixed with 1ml of methanol solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture was shaken vigorously and left to stand for 30min and the absorbance was measured at 517 nm.

The percentage of inhibition was calculated according to the formula: (A0-A1)/A0]×100, where A0 was the absorbance of the control and A1 was the absorbance of the sample.

2.3 Nitric oxide radical scavenging activity

The scavenging effect of extracts on nitric oxide radicals was determined according to the method of Marcocci *et al*., [5]. Different concentrations (10-50 µg/ml) of the methanol extracts of plant sample were dissolved in 2.5 ml of sodium nitroprusside and made up to 3 ml with PBS. Then the mixture was incubated for 13 minutes at 25 ^{0}C. After incubation, 0.5 ml of the reaction mixture was mixed with 0.5 ml of Griess reagent. Then the absorbance was measured at 546 nm. The experiment was repeated in triplicate. The percentage of inhibition was calculated by comparing the results of the test with those of control as per the following formula: Inhibition % = Control-test/control ×100).

2.4 ABTS radical cation scavenging activity

The ABTS radical cation scavenging activity was performed with slight modifications described by Re *et al.,* [6]. The ABTS-+ cation radicals were produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. Prior to use, the solution was diluted with ethanol to get an absorbance of 0.700 ± 0.025 at 734 nm. Free radical scavenging activity was assessed by mixing 10 µl of test sample with 1.0 ml of ABTS working standard in a microcuvette. The decrease in absorbance was measured exactly after 6min. The percentage inhibition was calculated according to the formula: [(A0-A1)/A0]×100, where A0 was the absorbance of the control and A1 was the absorbance of the sample.

3. Results and Discussion

The free radical scavenging effects of *S.wightii* methanolic extract was examined by DPPH, Nitric oxide and ABTS radical scavenging assays. The results showed that the DPPH, Nitric oxide and ABTS scavenging assays exhibited potent scavenging activity in a concentration dependent manner. The extract had scavenging activities on DPPH radical in a dose-dependent manner with an IC_{50} value of 31.68 µg/ ml. In DPPH assay, the extract exhibited 72.16% inhibition at the higher concentration of 50 µg/ ml (Table 1).

The effect of nitric oxide radical scavenging activity at different concentrations (10, 20, 30, 40 and 50 µg/ ml) of *S.wightii* extracts were measured. The radical scavenging effects were found to be increased with increasing concentration of *S.wightii* extracts. The methanolic extract of *S.wightii* showed its highest radical scavenging activity 95.16% in 50 µg/ ml. The IC_{50} value was expressed in 23.90 µg/ml concentration of the plant extract (Table 2).

Table 1: DPPH radical-scavenging activity of different concentrations of methanolic extract of *Smilax wightii*.

S. No	Concentrations of sample (µg/ml)	% inhibition ± SD	IC_{50} value (µg/ml)
1	10	26.47	31.68
2	20	38.12	
3	30	49.32	
4	40	64.14	
5	50	72.16	

Table 2: Nitric oxide radical-scavenging activity of different concentrations of methanolic extract of *Smilax wightii*.

S. No	Concentrations of sample (µg/ml)	% inhibition ± SD	IC_{50} value (µg/ml)
1	10	38.32±0.12	23.90
2	20	48.37±0.28	
3	30	66.14±0.46	
4	40	85.23±0.08	
5	50	95.16±0.76	

Table 3: ABTS r adical-scavenging activity of different concentrations of methanolic extract of *Smilax wightii*.

S. No	Concentrations of sample (µg/ml)	ABTS radical scavenging activity
1	10	254.36±0.56
2	20	368.14±0.22
3	30	543.27±0.48
4	40	736.43±0.74
5	50	872.52±0.08

Values are expressed as Trolox equivalence in µMol/g extract

Table 3 shows the ABTS radical scavenging activity at different concentrations of methanolic extract of *S. wightii*. The percentage of ABTS radical scavenging activity was found to increase with increasing concentrations of the plant extract. A steady increase in the percentage inhibition of the ABTS radicals by extract was observed in 872.52±0.08 trolox equivalence in µMol/g extract at the dose of 50 µg/ml.

Recently, there has been a growing interest in the search for natural antioxidants for the following reasons. First, numerous clinical and epidemiological studies have demonstrated that the consumption of fruits and vegetables is associated with reduced risks of developing chronic diseases such as cardio vascular disorders and diabetes/second, the public's perceptions have expected that natural antioxidant is safer than synthetic analogues because of potential harmful effects of the chronic consumption of synthesis antioxidant in food. Now-a-days there is an increase in aromatic and medicinal plants as source of natural antioxidants [7].

Antioxidants are substances that delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions [8]. Phenolic constituents, such as flavonoids, phenolic acids and tannins are well known for their high antioxidant activity [9]. The free radicals scavenging effects of *S. wightii* methanolic extract were examined by DPPH, Nitric oxide and ABTS radicals scavenging assays. The results showed that the DPPH, Nitric oxide and ABTS radicals scavenging assays exhibited potent scavenging activity in a concentration dependent manner. The results showed close agreement with antioxidant activity of *Smilax china* root [10] and *S. excelsa* leaf [3]. The results indicated that the methanol extract of *S. wightii* had very strong antioxidant activities and could be used as effective natural antioxidant resources.

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