EVALUATION OF *IN VITRO* ANTI OXIDANT POTENTIAL OF METHANOLIC EXTRACTS OF THE FERNS *SELAGINELLA WIGHTII HIERON* AND *ANEMIA WIGHTIANA* GARD.

Moorthy Durai Samy*[1] , Rekka Raja[2] and Nirubama Kumar[3]

[1&2]*Department of Botany, Kongunadu Arts and Science College, Coimbatore, GN Mills Post, Tamil Nadu, India.*

[3]*Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore, GN Mills Post, Tamil Nadu, India.,*

***Corresponding Author:** ycdferns@gmail.com

PG and Research Department of Botany, Kongunadu Arts and Science College (Autonomous), G.N. Mills Post, Coimbatore – 641029, Tamil Nadu, India.

ABSTRACT

Free radicals or highly reactive oxygen species are capable to bring out the oxidative stress, which leads to damage of carbohydrates, proteins, lipids and DNA and Causes the diseases and cellular injuries. In the present study to evaluate the *in-vitro* antioxidant activity of methanolic extracts *Selaginella wightii* and *Anemia wightiana*. Scavenging activity was carried by DPPH (2, 2-diphenyl-picryl-1-picryl-hydrazyl radical), $ABTS^{\bullet+}$, Ferrous ion chelating assay and Reducing power assay. From this analysis, *Selaginella wightii* and *Anemia wightiana*, were found to have potent antioxidant activity against DPPH IC_{50} value of 13.83 - 72.51 and 23.40 - 90.44 respectively. The plant species *Selaginella wightii* had the highest values for $ABTS^{\bullet+}$ radical scavenging activity (1716.19) in trolx equivalent and reducing power assay (0.23) while the fern, *A. wightiana* exhibited higher ferrous iron chelating activity (62.55 at 5000 µg/mL) than *Selaginella wightii*. Thus the results obtained in the present study indicate that these plants have the potential as natural sources of antioxidants, capable of protecting against free radical mediated damage and may have applications in preventing and curing various diseases.

Keywords: Antioxidant activity, Pteridophytes, *Anemia wightiana* and *Selaginella wightii*.

INTRODUCTION

The several medicinal plants are used with chronic degenerative diseases including cancer, coronary artery diseases, hypertension and diabetes, obesity etc. There are some speculations that the generation of free radicals inside the body in some physiological conditions is resulted in the cellular changes and the development of cancer etc. and this could be neutralized by the antioxidants from different medicinal plants

[1]. Research reports suggest that higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases particularly cardiovascular diseases and cancer [2]. Several studies have shown that plant derived antioxidant neutraceuticals scavenge free radicals and modulate oxidative stress-related degenerative effects [3].

Antioxidants appear naturally in plant parts such as leaf, stem, bark, fruit and Seeds in the form of ascorbic acid, vitamin E and phenolic compounds, which acquire the strength to reduce the oxidative damage associated with many diseases. So far, many researchers have focused and evaluated on natural antioxidants properties from the medicinal plants Antioxidants appear naturally in plant parts such as leaf, stem, bark, fruit and Seeds in the form of ascorbic acid, vitamin E and phenolic compounds, which acquire the strength to reduce the oxidative damage associated with many diseases. So far, many researchers have focused and evaluated on natural antioxidants properties from the medicinal plants [4].

The pteridophytes considered being the primitive vascular cryptogams and more than 1200 species of fern and fern allies have been reported from India [5]. In several years ago, Pteridophytes have been used successfully in of Ayurveda, Homeopathy and Unani systems of medicine. The usage of pteridophytes with respect to its medicinal values and several pharmacological activates have been reported by many scientists such as antimicrobial, anti-tumor, anti-diabetic, anticancer, and most important of all the antioxidant activity [6].

In addition to the unique and specific active biochemical ingredients pteridophytic plants houses innumerable Halder and Chakraborty 16 minerals, vitamins, alkaloids, saponins, phenols, tannins, phytosterols, triterpenes and terpenoids in a substantial amount [7]. Though Pteridophytes are known for their biological and pharmacological properties but very less attention is given to this group of species. In this present study we have measured antioxidant activity of various extracts like *Anemia wightiana* and *Selaginella wightii* employing various *in vitro* assay methods, such as scavenging activity of DPPH, $ABTS^{\bullet+}$, Ferrous ion chelating assay and Reducing power assay.

MATERIALS AND METHODS

Plant material

The clean and healthy study plants *Selaginella wightii* and *Anemia wightiana* were collected from the Shevaroyan hills. The fresh whole plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in air tight bottles.

Preparation of extract

50g of coarsely powdered materials of *Selaginella wightii* and *Anemia wightiana* were extracted with 250 ml methanol through soxhlet apparatus separately for 8 to 10 hours. The extracts obtained were then concentrated and finally dried to a constant weight.

DPPH radical scavenging activity

The 2, 2-diphenyl-picryl-1-picryl-hydrazyl radical (DPPH) scavenging activity was measured according to the method of Blois. Methanol extract of the samples at various concentrations (50, 100, 150,200 and 250µg/mL,) was added separately to each 5mL of 0.1mM methanolic solution of DPPH and allowed to stand for 20min. Absorbance at 517nm using spectrophotometer was measured. BHT was used as standard. The corresponding blank reading was also taken and DPPH radical scavenging activity was calculated by using the following formula:

DPPH radical scavenging activity (%) = Control OD-Sample OD/Control OD×100

Reducing power assay

Reducing power assay was determined according to the method of [8], Different concentrations of methanolic extracts of the two study species (300, 400, 500, 600 and 700 µg/mL) were mixed with 2.5mL of 200mM sodium potassium ferric cyanide separately and incubated at 50^0C for 20 min. After adding 2.5mL of 10% trichloro acetic acid, the mixture was centrifuged at 3000rpm for 10min. The supernatant was taken out and immediately mixed with 5mL of distilled water and 0.5mL of 1% ferric chloride. After incubation for 10min. the absorbance was measured at 700nm. Higher absorbance of the reaction mixture indicates reductive potential of the extract.

Ferrous ion chelating assay

The chelating of ferrous ions by whole plant methanolic extracts of the two study species were estimated by the method of [9], Briefly the extract samples (250 µL) were added to a solution of 2 mmol/L FeCl2 (0.05 mL). The reaction was initiated by the addition of 5 mmol/L ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g extract.

Antioxidant activity by the $ABTS^{\bullet+}$ assay

The total antioxidant activity of the samples was measured by $ABTS^{\cdot+}$ [2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)] radical cation decolorization assay according to the method of [10], $ABTS^{\bullet+}$ was produced by reacting 7mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30˚C to give an absorbance of 0.700±0.02 at 734 nm. The stock solution of the sample extracts was diluted such that after introduction of 10 µL aliquots into the assay, which have been produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1 mL of diluted ABTS solution to 10 µL of sample or trolox standards (final concentration 0–15 µM) in ethanol, absorbance was measured at 30˚C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition

was calculated from the blank absorbance at 734 nm and then it was plotted as a function of trolox concentration. The unit of total antioxidant activity (TAA) is defined as the concentration of trolox having equivalent antioxidant activity expressed as µmol/g sample extract on dry matter.

RESULTS AND DISCUSSION

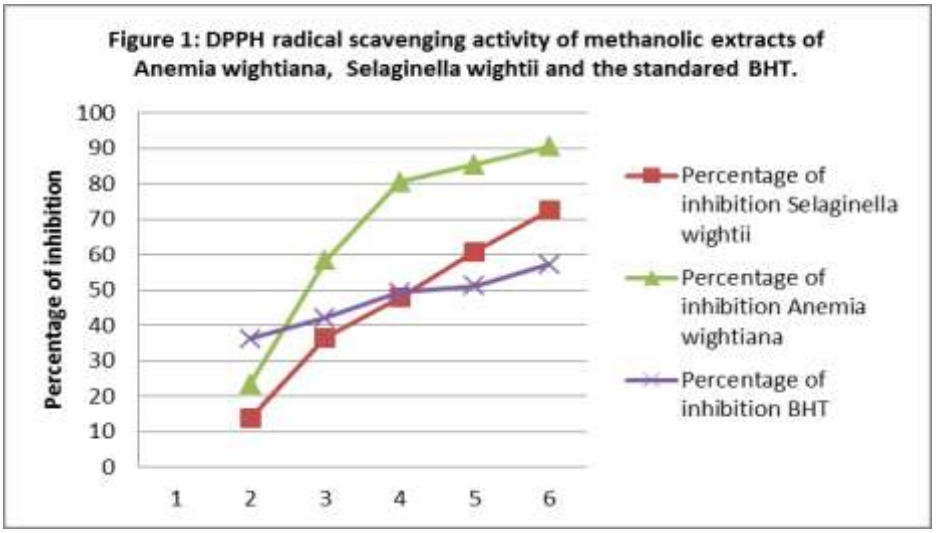
DPPH radical scavenging activity

DPPH, a relatively stable organic radical with a characteristic strong absorption band at 517 nm in visible spectroscopy (deep violet colour) was used to evaluate the free radical scavenging ability of the investigated samples. The best known natural and synthetic antioxidant standards, *viz*., BHT were used as positive control for comparison. The free radical scavenging activity of two study species, *Selaginella wightii* and *Anemia wightiana* was increased with the increase of concentrations (Table-1). Among the plants examined, the methanolic extracts of *Anemia wightiana* (23.40 - 90.44) demonstrated effective DPPH radical quenching capacity. *Selaginella wightii* extracts were contributed fairly outstanding antiradical capacity. The key role of phenolic compounds as scavengers of free radical is emphasized in several reports. Recent works also examined the influences of different drying pretreatments of *Dryopteris erythrosora* leaves on total flavonoid contents, antioxidant activity, and flavonoid ingredients.

Table: 1. DPPH radical scavenging activity of methanolic extracts of *Anemia wightiana*, *Selaginella wightii* and the standard BHT.

Sample concentration (µg/ml)	Percentage of inhibition		
	Selaginella wightii	***Anemia wightiana***	**BHT**
50	$13.83^{a} \pm 0.72$	$23.40^{a} \pm 0.33$	$36.24^{a} \pm 0.31$
100	$36.50^{b} \pm 0.86$	$58.42^{b} \pm 0.18$	$42.21^{b} \pm 0.38$
150	$47.89^{c} \pm 0.15$	$80.49^{c} \pm 0.25$	$49.39^{b} \pm 0.34$
200	$60.79^{d} \pm 0.20$	$85.36^{d} \pm 0.47$	$51.16^{bc} \pm 0.40$
250	$72.51^{e} \pm 0.41$	$90.44^{d} \pm 0.33$	$57.15^{c} \pm 0.24$

BHT used as reference standard
Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).



Reducing Power assay

Table-2 shows the reductive capabilities of different concentrations of methanolic extracts *Selaginella wightii* and *Anemia wightiana* in comparison to that of the standard, ascorbic acid. It was found that the reducing power increased with the increasing of the concentrations of the extracts. In the present study, *Selaginella wightii* extract showed the highest reducing ability (absorbance 0.23 at 700µg/ml) than the other fern studied. However, the activity was lesser than the standard, ascorbic acid (absorbance 1.05 at 700µg/ml) presence of reducers. Accordingly, it can be suggested that the polyphenolic richness of the extracts might appear to function as good electron and hydrogen atom donors and therefore could terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products.

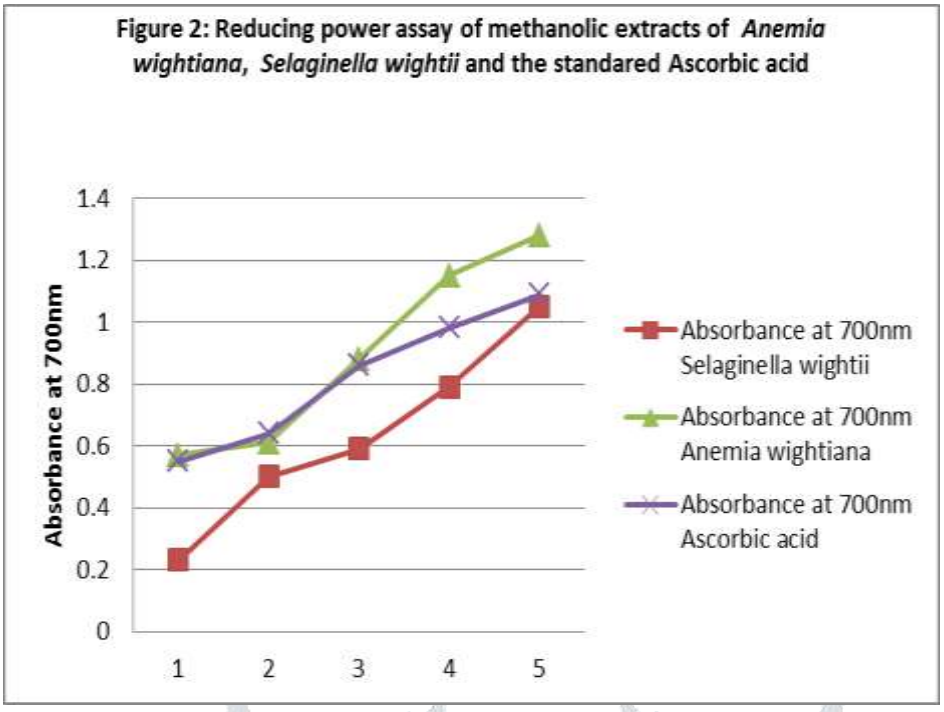
Table: 2.

Reducing power assay of methanolic extracts of *Anemia wightiana*, *Selaginella wightii* and the standard Ascorbic acid

Sample concentration	Absorbance at 700nm		
	Selaginella wightii	***Anemia wightiana***	**Ascorbic acid**
300	$0.23^{a} \pm 0.25$	$0.57^{a} \pm 0.16$	$0.55^{a} \pm 0.03$
400	$0.50^{b} \pm 0.68$	$0.61^{b} \pm 0.18$	$0.64^{b} \pm 0.04$
500	$0.59^{b} \pm 0.35$	$0.88^{c} \pm 0.85$	$0.86^{c} \pm 0.03$
600	$0.79^{c} \pm 0.91$	$1.15^{de} \pm 0.76$	$0.98^{d} \pm 0.02$
700	$1.05^{d} \pm 0.25$	$1.28^{e} \pm 0.23$	$1.09^{e} \pm 0.04$

Values were performed in triplicates and represented as mean ± SD.

Mean values followed by different superscript in a column are significantly different ($p<0.05$).



Ferrous ion chelating assay

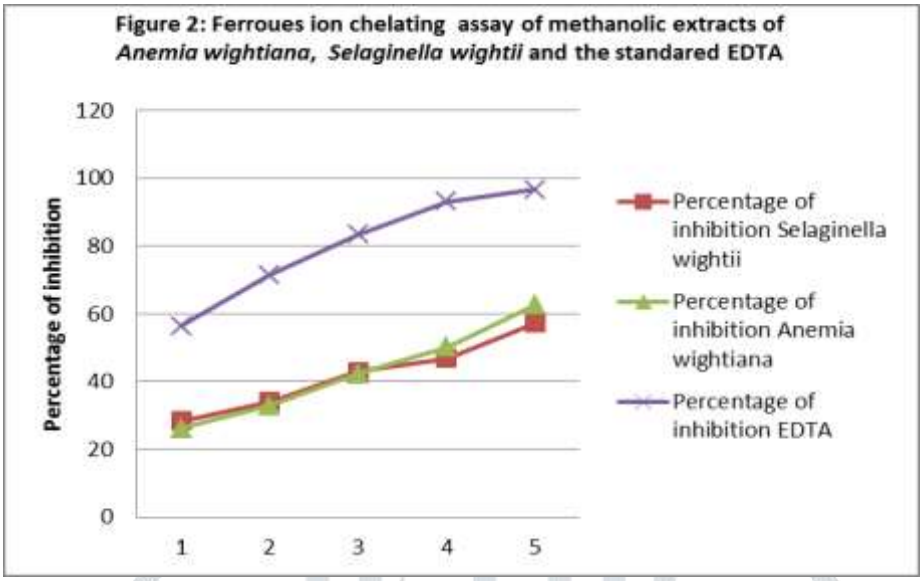
The chelating effect on the ferrous ions by methanolic extract of *Selaginella wightii* and *Anemia wightiana* are presented in (Table -3). All the samples exhibited the ability to chelate metal ions. Among the extracts *A. wightiana* showed higher activity (62.55 at 5000 µg/mL) than that of the other studied fern. From the above Fe_2^+ chelating data, it is evident that the extracts, due to the presence of polyphenolic compounds, may be able to play a protective role against oxidative damage by sequestering iron (II) ions that may otherwise become catalyst for Fenton-type reactions or participate in metal-catalyzed hydroperoxide decomposition reactions [11]. Hence, it is suggested that the Fe_2^+ chelating properties of the different solvent extracts of the test samples would be a positive response against the damages caused by oxidation.

Table: 3. Ferrous ion chelating assay of methanolic extracts of *Anemia wightiana*, *Selaginella wightii* and the standard EDTA.

Sample concentration (µg/mL)	Percentage of inhibition		
	Selaginella wightii	***Anemia wightiana***	**EDTA**
1000	$28.26^{a} \pm 0.65$	$26.15^{a} \pm 0.37$	$56.28^{a} \pm 0.19$
2000	$34.09^{b} \pm 0.26$	$32.89^{b} \pm 0.09$	$71.55^{b} \pm 0.33$
3000	$42.89^{c} \pm 0.18$	$42.25^{c} \pm 0.26$	$83.46^{c} \pm 0.20$
4000	$46.79^{cd} \pm 0.38$	$50.18^{d} \pm 0.45$	$93.19^{d} \pm 0.21$
5000	$57.28^{d} \pm 0.46$	$62.55^{e} \pm 0.34$	$96.69^{de} \pm 0.15$

EDTA reference standard Values were

performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).



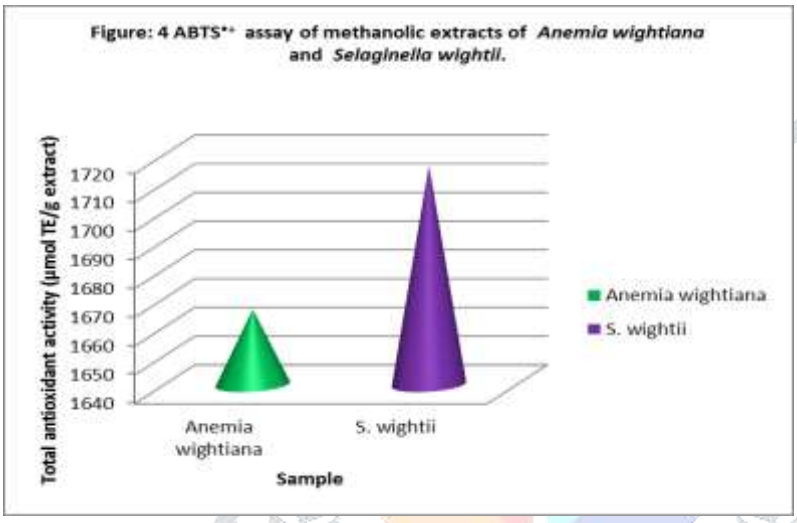
ABTS$^{\bullet+}$ assay

In the present investigation, (Table-4) the methanolic extract of *S. wightii* registered the highest total antioxidant activity (1716.19 µmol/g) followed by *A. wightiana* (1666.17 µmol/g). ABTS$^{\cdot+}$, a protonated radical has characteristic absorbance maxima at 734nm which decreases with the scavenging of the proton radicals. ABTS$^{\cdot+}$ was generated by incubating it with potassium persulfate. The presence of chemical compounds in the tested extracts that inhibit the potassium persulfate activity may reduce the production of ABTS$^{\cdot+}$ the activity may be contributed by the hydrogen-donating compounds which are most likely to be present in the polar solvents [12]. Due to their effective ABTS$^{\bullet}$+scavenging property they can be classified as agreeable antiradical agents, equally effective as Trolox.

Table: 4. ABTS$^{\bullet+}$ assay of methanolic extracts of *Anemia wightiana* and *Selaginella wightii*.

Sample	Total antioxidant activity (µmol TE/g extract)
Anemia wightiana	1666.17 ± 0.68^{e}
S. wightii	1716.19 ± 1.20^{d}

Total antioxidant activity (µmol equivalent trolox)
Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).



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