



## In vitro free radical scavenging of *Andrographis lineata* Wallich ex. Nees an endemic healing plant

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**Abstract:** Antioxidants conjointly called free radical scavengers interact and neutralize free radicals thereby preventing cellular injury within the biological system. During this research for antioxidant activities from *A. lineata*, carried out bioassay-guided fractionation with free radical scavenging activity. The ethanolic extract of aerial parts was screened via different free radicals viz., DPPH, reducing power assay, total antioxidant, hydrogen peroxide scavenging and nitric oxide scavenging activity were studied. Various fractions of *A. lineata* aerial parts showed significant activities against the tested reactive free radicals. The *A. lineata* was shown to have the lowest  $IC_{50}$  values for all antioxidant activities moreover; *A. lineata* had the best activities in total antioxidant activity. These results suggest the potential of *A. lineata* aerial parts as a medicine against free-radical-associated oxidative damage.

**Keywords:** *Andrographis lineata*, free radical scavenging, antioxidant, DPPH

### Introduction

Plants are well-known excellent perspectives for the discovery of new therapeutical products. In recent years, an ample interest has been developed in finding natural antioxidants from commonly available in plants was generally mistreated (Rehman, 2006; Khan *et al.*, 2011; Jerutoet *et al.*, 2008). According to (Khan *et al.*, 2010; Khan *et al.*, 2011; Kareruet *et al.*, 2007; Afolayan and Jimoh, 2008 ) revealed it is believed that they possess a remarkable potential to overwhelm the deadly diseases of the modern world. Numerous reports of crude extracts and purely natural compounds have appeared for antioxidant and radical-scavenging activities. Amicet *et al.*, 2003; Balasundramet *et al.*, (2006) estimated due to the presence of the conjugated ring structures and hydroxyl groups and many phenolic compounds have the potential to function as antioxidants by stabilizing free radicals involved in oxidative processes through hydrogenation with oxidizing species. Antioxidant activity is characterized as the capacity of given mixes to diminish responsive species, including free radicals. Numerous strategies are accessible for its estimation (Antolovichet *et al.*, 2002; Moon and Shibamoto 2009; Alamet *et al.*, 2013; López-Alarcón and Denicola 2013; Apaket *et al.* 2016).

*Andrographis lineata* Wallich ex Nees belongs to the family Acanthaceae. *A. lineata* commonly known as "Periyanagai" is distributed in southwest India, Sri Lanka, Tamilnadu, Kerala, Andhra Pradesh at altitudes ranging from 1025m to 2500m (Alagesaboopathi, 2012). The genus *Andrographis* is comprised of annual herbs, shrubs, including about 40 species, distributed in tropical Asia (Anonymous, 1948). *Andrographis* is a large genus of herbs distributed in Indo-Malaya, Africa, Brazil, and Central America Northward into Mexico (Lawrence, 1969). On hilly habitats nearly all the plants of a species flower as a rule in the same season (Fyson, 1915). The genus *Andrographis* used in snake-bites, constipation, skin diseases, and lung diseases. These plants are also claimed to possess snakebites and this leaf paste is locally applied for skin diseases in cattle (Alagesaboopathi, 1993) as well as three flavonoids were isolated from the leaf extract (Kishore *et al.*, 2003). This study evaluates the various *in vitro* antioxidant properties of ethanol extract of *A. lineata*.

### Materials and Methods

#### Collection of plant materials

Fresh plant parts of the studied species of *Andrographis lineata* was collected from Chitteri hills, the Eastern Ghats, Dharmapuri district, Tamil Nadu, India. They were clean, shade dried and coarsely powdered in a Willy Mill to 60 mesh size for extraction.

#### Preparation of crude plant extract

Aerial parts of the studied species were collected from Chitteri hills, the Eastern Ghats, Dharmapuri district, Tamil Nadu, India. Fifty grams of coarsely powdered plant samples were extracted with ethanol using soxhlet apparatus. The extract was filtered and concentrated with rotary vacuum evaporator and it was dried.

## In Vitro Antioxidant

### DPPH radical scavenging activity

DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. Various concentrations of the plant extract (4.0 ml) were mixed with 1.0 ml of a 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 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as a control. The percentage of inhibition in DPPH radical scavenging activity was calculated as follows: % Inhibition =  $A_0 - A_1 / A_0 \times 100$ .

### Reducing power assay

Reducing power was measured by direct electron donation in the reduction of  $Fe^{3+} (CN)_6$  to  $Fe^{2+} (CN)_6$ . The reaction mixture contained 2.5 ml of various concentrations of the extract, 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 0.2 M sodium phosphate buffer. The mixture was incubated at 50°C for 20 min. and it was terminated by the addition of 2.5 ml of 10% (w/v) of trichloroacetic acid, followed by centrifugation at 3000 rpm for 10 min. To take 5.0 ml of the supernatant was mixed with 5.0 ml of deionized water and add 1.0 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm. Increased absorbance indicates increased reducing power of the sample.

### Total Antioxidant capacity

Antioxidant capacity was measured by spectrophotometric method. 0.1 ml of the sample (10 µg / ml) dissolved in water was combined with 1.0 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution was measured at 695 nm. Ascorbic acid was used as standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid.

### Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity was measured in terms of a decrease in the absorbance by spectrophotometrically. A solution of  $H_2O_2$  was prepared in phosphate buffer.  $H_2O_2$  concentration was determined spectrophotometrically from its absorption at 230 nm. Various concentrations of the extract were added to  $H_2O_2$  and incubated for 10 min. The absorbance at 230 nm was determined against a blank containing phosphate buffer without  $H_2O_2$ . The percentage of scavenging of  $H_2O_2$  and standard compounds were calculated using the formula: Percentage of radical scavenging activity =  $(Control - Sample) / Control \times 100$

### Nitric oxide scavenging assay

The interaction of the sample with nitric oxide was assessed by the nitrite detection method. Nitric oxide was generated from sodium nitroprusside and measured by the Griess-Illiosvay reaction. The reaction mixture (6.0 ml) containing sodium nitroprusside (4.0 ml), phosphate buffer saline (PBS, 1.0 ml) and different concentrations (100 to 500 µg / ml) of a leaf extract (1.0 ml) in DMSO was incubated at 25°C for 15 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was removed, 1.0 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotization and 1.0 ml of naphthyl ethylenediamine dihydrochloride was added, mixed well and allowed to stand for 30 minutes in diffused light. A pink colored chromophore was formed. The absorbance of these solutions was measured at 540 nm. Rutin was used as a standard. The inhibition was calculated according to the equation:  $I = A_0 - A_1 / A_0 \times 100$ , Where,  $A_0$  is absorbance of control reaction,  $A_1$  is absorbance of the test compound.

## Statistical analysis

Values were analyzed using the statistical analysis system (SAS). Comparisons between different groups were carried out by analysis of variance, ANOVA ( $P < 0.05$ ). Means differences were separated using Duncan's Multiple Range Test.

## Results and Discussion

### DPPH scavenging activity

The DPPH radical scavenging activity results are shown in (Table.1) as comparable with known antioxidant Vitamin C. From the analysis we can conclude that the scavenging effects of aerial parts of ethanolic extracts on DPPH radicals were excellent, especially in the case of *A. lineata* at various concentrations like 100, 200, 300, 400 and 500 (µg/ml). Table 1 shows antioxidant activity with  $IC_{50}$  values of standard vitamin C and *A. lineata* these aerial parts measured by DPPH radical scavenging assays. Overall, *A. lineata* exposed the best antioxidant properties (significantly lower  $IC_{50}$  values = 447.11 µg/ml) and the standard was also revealed. According to (Nuutila *et al.*, 2003) revealed the DPPH radical was widely used to evaluate the free radical scavenging ability of antioxidants.

**Table 1: DPPH scavenging activity of *A. lineata* ethanol extract**

| Plant Extract | % of Inhibition |             |             |             |             | $IC_{50}$ value (µg/ml) |
|---------------|-----------------|-------------|-------------|-------------|-------------|-------------------------|
|               | 100 (µg/ml)     | 200 (µg/ml) | 300 (µg/ml) | 400 (µg/ml) | 500 (µg/ml) |                         |
| Aerial parts  | 10.3 ± 0.4      | 20.5 ± 0.5  | 32.1 ± 0.1  | 45.2 ± 0.55 | 54.5 ± 0.3  | 447.11                  |

|           |          |          |          |         |          |        |
|-----------|----------|----------|----------|---------|----------|--------|
| Vitamin C | 15.4±0.1 | 29.5±0.3 | 40.4±0.5 | 51±0.45 | 63.1±0.1 | 389.41 |
|-----------|----------|----------|----------|---------|----------|--------|

The experiment was conducted in triplicates (n=3)

#### Reducing power activity

The various concentrations of aerial parts of ethanol extracts and the standard vitamin C used at 100, 200, 300, 400 and 500 ( $\mu\text{g/ml}$ ). (Fig.1) showed the result of the reducing power activity of *A. lineata*. On the other hand, the activity of antioxidants has been assigned to different mechanisms such as preclusion of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, reductive capacity and radical scavenging (Diplock, 1997; Yildirim *et al.*, 2000). In this investigation, *A. lineata* had activity values corresponding to (12.5<21.3<34.55<45.25<54.5  $\mu\text{g/ml}$ ) respectively. Whereas at the standard activity values of were (16.4<32.5<46.7<60.85<68.3  $\mu\text{g/ml}$ ) respectively.

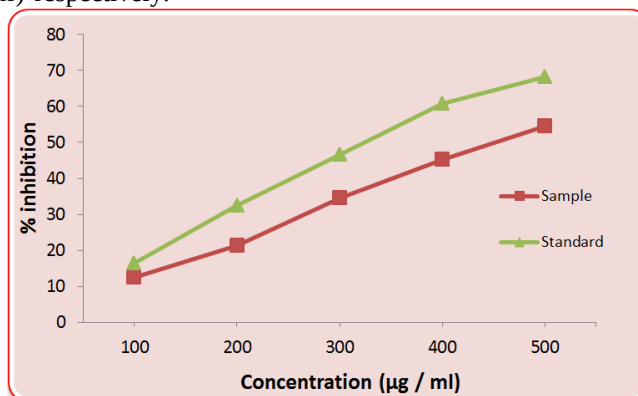


Fig 1 Reducing power activity of *A. lineata* ethanol extract

#### Total antioxidant activity

Total antioxidant capacity (TAC) of the ethanolic extract of *A. lineata* aerial parts were determined (Figure.2). The assay is based on the fact that molybdenum is reduced in the presence of a reducing agent (antioxidant), forming a green phosphomolybdate complex, which can be evaluated spectrophotometrically at 765 nm (Jan *et al.*, 2013; Mashwani *et al.*, 2013). The assay involves an electron transfer mechanism. Many natural products, including phenols and flavonoids, can cause this reduction. As Figure-2 reveals, the antioxidant activity is dose-dependent. The ethanolic extract showed a much higher  $\text{IC}_{50}$  activity (417.66  $\mu\text{g/ml}$ ) than known vitamin C (310.74  $\mu\text{g/ml}$ ) was the least active.

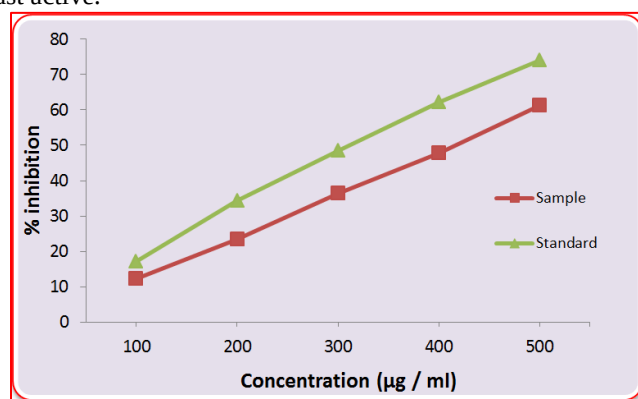


Fig 2. Total antioxidant activity of *A. lineata* ethanol extract

#### Hydrogen peroxide scavenging activity

Table-2 showed hydrogen peroxide scavenging activity of the ethanol extract of the aerial plant and standard vitamin C. The studied plant *A. lineata* extract caused a strong dose-dependent inhibition of hydrogen peroxide activity and it can cross cell membranes hastily, once inside the cell,  $\text{H}_2\text{O}_2$  can perhaps react with  $\text{Fe}^{2+}$  and possibly  $\text{Cu}^{2+}$  ions to form hydroxyl radical and this may be the origin of many of its toxic effects (Miller *et al.*, 1993). The present study showed good scavenging ability compared to the standard compound. The  $\text{IC}_{50}$  values for the extract were found to be 470.19  $\mu\text{g/ml}$  compared to standard vitamin C 362.27  $\mu\text{g/ml}$ . At a concentration of 100 to 500  $\mu\text{g/ml}$ , the scavenging percentages were 9.34 to 53.6 and 16.1 to 68.1 for ethanolic extract and standard antioxidant respectively.

**Table 2. Hydrogen peroxide scavenging activity of *A. lineate* ethanol extract**

| Plant Extract | % of Inhibition |             |             |             |             | IC <sub>50</sub> value (µg/ml) |
|---------------|-----------------|-------------|-------------|-------------|-------------|--------------------------------|
|               | 100 (µg/ml)     | 200 (µg/ml) | 300 (µg/ml) | 400 (µg/ml) | 500 (µg/ml) |                                |
| Aerial parts  | 9.34±0.25       | 18.65±0.10  | 29.50±0.10  | 41.50±0.65  | 53.60±0.70  | 470.19                         |
| Vitamin C     | 16.10±0.50      | 31.10±0.10  | 42.10±0.25  | 55.10±0.50  | 68.10±0.25  | 362.27                         |

The experiment was conducted in triplicates (n=3)

#### Nitric oxide radical scavenging activity

It is a diffusible free radical which plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumor activities (Miller *et al.*, 1993). Based on this study aerial parts of *A. lineate* ethanol extract moderately inhibited (Figure.3) nitric oxide in dose-dependent manner at the concentration of 100, 200, 300, 400 and 500 range the plant extract showed 9.85<20.1<32.15<44.38<57.5 as well as standard rutin ranging from 13.5<27.5<40.75<51.5<64.5 (Figure.3) respectively. In the plant extract IC<sub>50</sub> has been 444.01 µg/ml than the standard rutin 386.65 µg/ml.

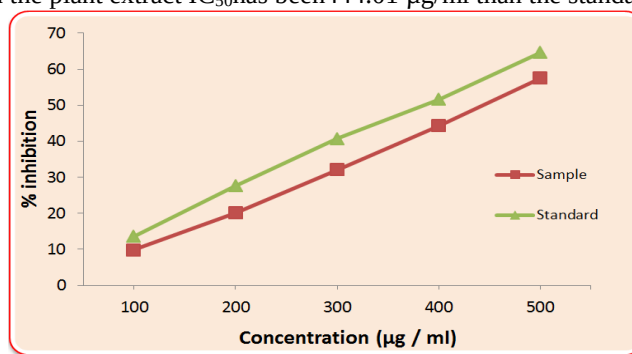


**Fig 3. Nitric oxide radical scavenging activity of *A. lineate* ethanol**

#### Conclusion

The present study elevated that the ethanol extract of *A. lineate* aerial parts possesses various *in vitro* antioxidant activities. The potential antioxidant activity of *A. lineate* aerial parts is due to the presence of flavonoids and polyphenols. Thus, it can be concluded that *A. lineate* aerial parts can be used as an accessible source of natural antioxidants with consequent health benefits.

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