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# *In -Vitro* Antioxidant Potential of Asiatic acid

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**ABSTRACT**
*Centella asiatica, a plant widely grown in India, China, Sri Lanka, and Africa as a vegetable or spice, contains Asiatic acid as its primary constituent. Current research was focus on identification of antioxidant potential of Asiatic acid. DPPH, Capacity for Total Reductions, ABTS+ Radical Cation, Hydroxygen peroxide's Radical Scavenging Activity, Assay for Nitric Oxide were studied with respect to asiatic acid against standard ascorbic acid and Trolox at 4µg/ml, 8 µg/ml, 16 µg/ml, 32 µg/ml, 64 µg/ml concentration. In -vitro antioxidant potential of asiatic acid was interpreted from results of all above parameters tests.*
***Keywords****: Antioxidant Potential, Asiatic Acid, DPPH, Capacity for Total Reductions, ABTS+ Radical Cation, Hydroxygen peroxide's Radical Scavenging Activity, Assay for Nitric Oxide*

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## INTRODUCTION

It's long been known that natural substances have been used in medicine. Natural triterpenoids have gained a lot of attention in recent years, particularly in terms of extraction, isolation, structural analysis, and a wide range of biological activities. Phytoconstituents known as pentacyclic triterpenoids show great promise as potential therapeutics. The antioxidant, organ-protective, and anticancer properties of these foods are well-documented. Chemotherapy-sensitizing agents such as these are well-known. They are safe and have been used in diets since the beginning of time. Pentacyclic triterpenes, which are found in the stem, leaves, bark, and fruit peel, are ancillary plant elements. *Centella asiatica*, a plant widely grown in India, China, Sri Lanka, and Africa as a vegetable or spice, contains Asiatic acid as its primary constituent. Animal studies have demonstrated Asiatic acid's anticancer, antidiabetic, anti-inflammatory, organotropic, and antioxidant properties in suitable models.

## MATERIAL AND METHODS

Extrasynthese provided the chemical standards for asiatic acid, madecassic acid, asiaticoside, and madecassic acid (Genay, France). Fisher Scientific supplied HPLC-grade phosphoric acid and acetonitrile for this experiment (Pittsburgh, PA). In addition, Sigma Aldrich provided the sirius red, collagen type I, L-glutamine, and penicillin-streptomycin antibiotics (St. Louis, USA). In addition to DMEM and heat-inactivated foetal bovine serum, Difco supplied the necessary supplies (Detroit, Michigan, USA). CCD-1114Sk (CRL 2450) SW-480 and HCT116 were obtained from the ATCC (American Type Culture Collection, Manassas, VA, USA). S D Fine-Chemicals supplied glycerol and Eudragit E 100. HP-B-CD was provided by Marck while copovidone copolymer by JRS pharma. There were no non-analytical reagents used.

### Assay for Radical Scavenging Activity of 2, 2-Diphenyl-1- Picrylhydrazyl (DPPH)

The approach was used to determine the free radical scavenging activity of the samples. The UV measurements were made using a newly produced solution of DPPH in methanol. The 2, 2-Diphenyl-1-Picrylhydrazyl solution was vortexed after samples with concentrations ranging from 4–64 g/mL were added in a 1:1 ratio. Then, in the dark and at room temperature, it was allowed to proceed. For a standard, both ascorbic acid and trolox were used: The following equation was used to get the inhibition % for DPPH radical scavenging activity. [1, 2]

$$\text{Inhibition (\%)} = [(A_0 - A)/A_0] \times 100$$

It's important to note that $A_0$ and A are DPPH's absorbances in their respective absence and presence, respectively. Sample solution concentrations were plotted against the percentage of inhibition to determine the IC50 values (the concentration necessary to remove 50% of free radicals).

**Capacity for Total Reductions**

The approach was used to determine the total reduction capacity of the samples. Mixing was done by adding 2.5 mL of phosphate buffer (pH 6.6) and 1 percent potassium ferric cyanide to the samples (4–64 g/mL), which were then gently mixed. For 20 minutes, the mixture was kept at a temperature of 50°C in a water bath. After stopping the reaction with 2.5 mL of 10% Trichloroacetic acid, the liquid was centrifuged for 10 minutes at 4000 rpm. The 2.5 mL of distilled water and 0.5 mL of 0.1 percent ferric chloride ($FeCl_3.6H_2O$) were stirred vigorously in a separate tube. [2,3] Using 700 nm against a blank, the absorbance was measured after 5 minutes. Both trolox and ascorbic acid were considered to be a baseline.

**Toxicological Assay for ABTS+ Radical Cation**

The $ABTS^+$ [2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation scavenging ability of the extracts was used to measure their antioxidant activity. It was created by combining 7 mM. 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2.45 mM potassium persulfate ($K_2S_2O_8$) in a dark, room-temperature environment and incubating for 24 hours at ambient temperature. [5,6] Different concentrations of AA were mixed thoroughly with ABTS+ solution to test the radical scavenging activity. Ascorbic acid and trolox were used as a benchmark. For six minutes, the reaction mixture was kept at room temperature.

**Hydroxygen peroxide's Radical Scavenging Activity (H2O2)**

The procedure was used to determine the extracts' radical scavenging abilities. Water extracts (4–64 g/mL) of AA were added to $H_2O_2$ with the prepared phosphate buffer (0.6 mL) and $H_2O_2$ (40 mM) (pH 7.4). For 10 minutes, the reaction mixtures were kept at room temperature. Incubation resulted in a 230-nm reading of the reaction mixture against phosphate buffer blank (pH 7.4). Ascorbic acid and trolox were used as a benchmark. [7, 8]

$$\text{Percentage (\%) of inhibition} = (A_1 - A_2)/A_1 \times 100.$$

was used to compute the inhibition percentage. $H_2O_2$ absorbance is A1 and the reaction mixture with extracted AA absorbance is A2.

**Radical Scavenging Assay for Nitric Oxide**

The approach was used to conduct a nitric oxide (NO) radical scavenging experiment. Extracted AA at various concentrations (4–64 g/mL) was combined with 0.6 mL of 10 mM sodium nitroprusside. A pre-prepared 1.0 mL of Griess reagent was added to the mixture after it had been incubated for 150 minutes at 25°C (1 percent sulfanilamide, 0.1 percent naphthyl ethylenediamine dichloride, and 2 percent phosphoric acid). To ensure consistency, we used ascorbic acid and trolox as a reference point. [9, 10]

## RESULT AND DISCUSSION

**Radical Scavenging in DPPH**

It was determined by the decrease in absorbance at 517nm caused by antioxidant extracts that DPPH radicals could be reduced. [11] Due to the extract's hydrogen-donating ability, hydrazine was synthesised by converting unpaired electrons to paired electrons. At a concentration of 64 µg/mL, extracted of AA, trolox, and ascorbic acid had IC50 values. Studying extracted AA ability to neutralise free radicals, found that it had far greater free radical scavenging activity equivalent standard Trolox.

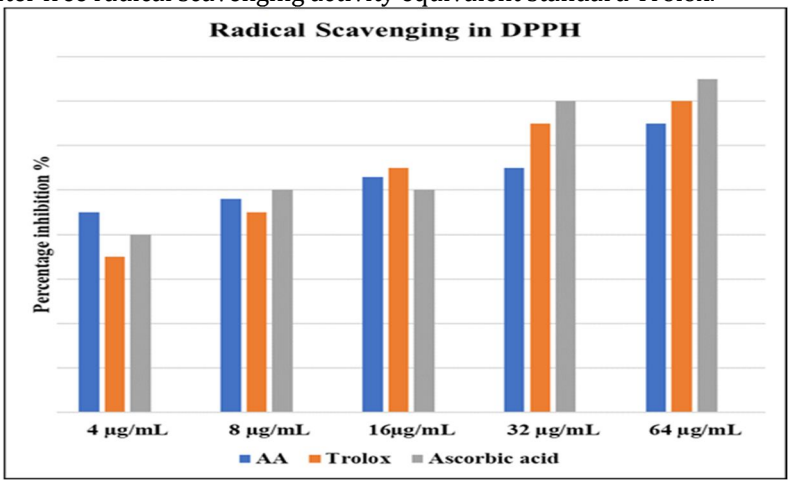


**Figure 1: 2-2- Diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging activity**

**Capacity for total reduction.**

Methods were used to determine the extract's reducing ability. The tested plant extract's reduction ability improved as trolox and ascorbic acid equivalent concentrations. According to the results of the analysis, the absorbance of extracted AA at 64 µg/mL was 0.18. Trolox and ascorbic acid were used as positive control and its reducing power at 64 µg/mL were 0.21 and 0.28. The test samples' reductive power increased significantly (p 0.05) as concentration increased. Compared to ascorbic acid, extracted AA had a marked difference in the ability to reduce ferric ions (Fe3+).

**Table 1: Total reduction capacity**

| Concentration | Absorbance | | |
|---|---|---|---|
| | Extracted AA | Trolox | Ascorbic acid |
| 4µg/ml | 0.09 | 0.07 | 0.06 |
| 8 µg/ml | 0.10 | 0.09 | 0.11 |
| 16 µg/ml | 0.11 | 0.09 | 0.17 |
| 32 µg/ml | 0.13 | 0.12 | 0.22 |
| 64 µg/ml | 0.18 | 0.21 | 0.28 |

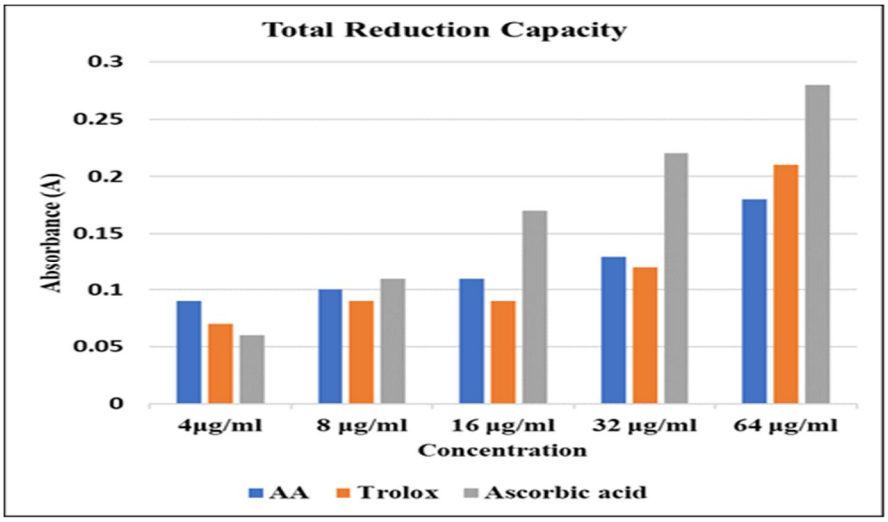


**Figure 2: Total reductive capability.**

**Radical Cation Decolorization Analyze using ABTS Radicals**

Radical scavenging is an important way to measure antioxidant capacity. The scavenging capacity of ABTS, a protonated radical, is inversely proportional to its absorbance maxima at 734 nm. Extracted AA (IC50 = 27.21 µg/mL), ascorbic acid (IC50 = 12.06 µg/mL) and trolox (IC50 = 12.76 µg/mL) were found to have the highest ABTS+ radical scavenging ability. By varying the concentrations, the three antioxidants showed dose-dependent effectiveness.

**Table 2: Radical Cation Decolorization Analyze using ABTS Radicals**

| Concentration | Percentage inhibition % | | |
|---|---|---|---|
| | Extracted AA | Trolox | Ascorbic acid |
| 4µg/ml | 37 | 37 | 40 |
| 8 µg/ml | 42 | 45 | 40 |
| 16 µg/ml | 45 | 55 | 48 |
| 32 µg/ml | 53 | 60 | 55 |
| 64 µg/ml | 60 | 65 | 68 |

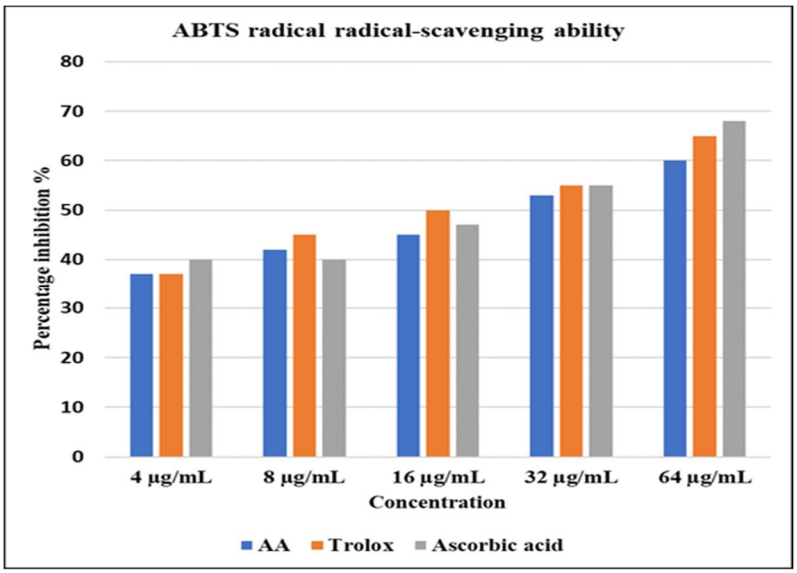


**Figure 3: ABTS radical radical-scavenging ability**

**Hydrogen peroxide ($H_2O_2$)**

Scavenging to the extreme Free radicals known as hydroxyl radicals are extremely reactive and there are no specific enzymes in humans to protect against them. [112] Trolox, ascorbic acid, and extracted AA scavenging abilities can be seen in. At 64 µg/mL, extracted AA extracts had a radical-scavenging ability of 55 %, ascorbic acid extracts had a radical-scavenging ability of 69.9%, and trolox extracts had a radical-scavenging ability of 65 %. The IC50 value indicates that the plant extract is a better hydroxyl radical scavenger than the standard ascorbic acid and trolox. The higher the concentration of the extracted AA, the greater the hydroxyl radical scavenging activity.

**Table 3: Hydrogen peroxide ($H_2O_2$).**

| Concentration | Percentage inhibition % | | |
|---|---|---|---|
| | Extracted AA | Trolox | Ascorbic acid |
| 4µg/ml | 40 | 42 | 50 |
| 8 µg/ml | 45 | 47 | 53 |
| 16 µg/ml | 45 | 49 | 55 |
| 32 µg/ml | 50 | 55 | 60 |
| 64 µg/ml | 55 | 65 | 69.9 |

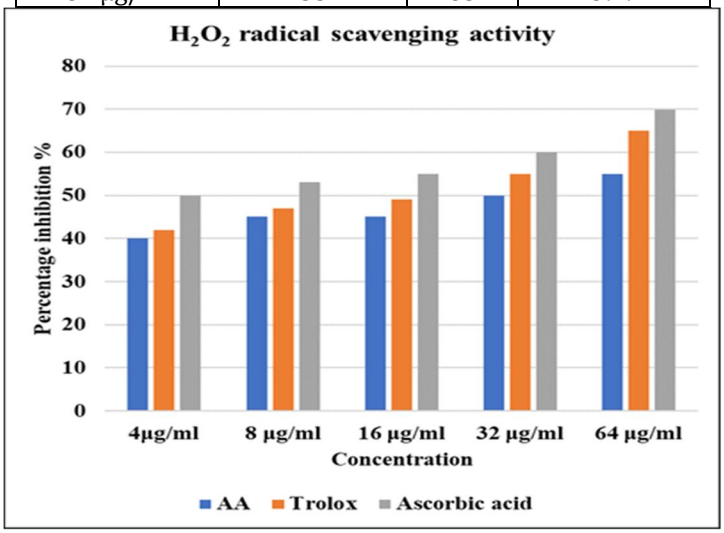


**Figure 4: $H_2O_2$ radical scavenging activity**

**Inhibition of Radical Scavenging by Nitric Oxide**

Analyses at 540 nm showed that extracted AA had the highest inhibitory effect. At 32 µg/mL of extracted AA, there was no difference in ascorbic acid and trolox's NO radical-scavenging abilities. Previous studies

have shown that phenolic compounds play a significant role in NO suppression, which may be the reason for the different inhibitory effects observed in this study.

**Table 4: Inhibition of Radical Scavenging by Nitric Oxide**

| Concentration | Percentage inhibition % | | |
|---|---|---|---|
| | Extracted AA | Trolox | Ascorbic acid |
| 4µg/ml | 24 | 35 | 34 |
| 8 µg/ml | 30 | 43 | 40 |
| 16 µg/ml | 35 | 50 | 45 |
| 32 µg/ml | 50 | 52 | 50 |
| 64 µg/ml | 60 | 65 | 63 |

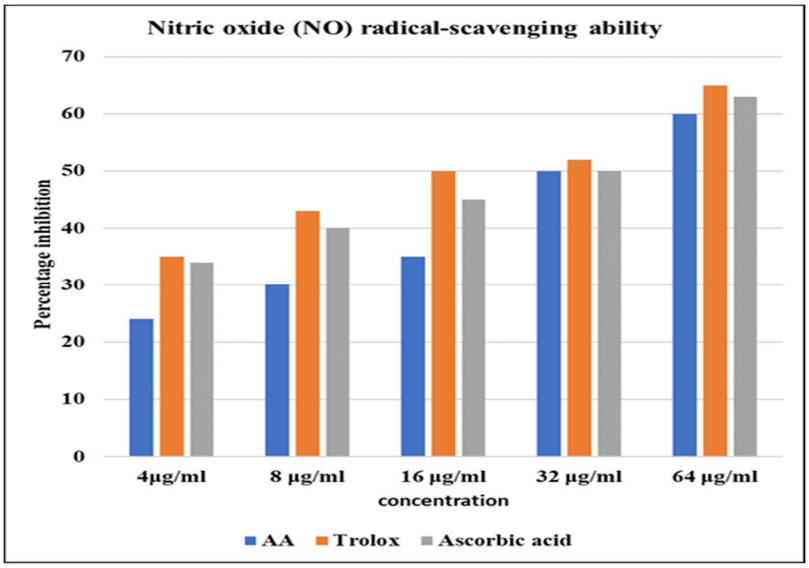


**Figure 5: Nitric oxide (NO) radical-scavenging ability**

## CONCLUSION

Asiatic acid as its primary constituent of *Centella asiatica.* Current research which indicated AA ability to neutralise free radicals, found that it had far greater free radical scavenging activity (DPPH) equivalent standard Trolox. In Capacity for total reduction, test samples' reductive power increased significantly (p 0.05) as concentration increased. Compared to ascorbic acid, extracted AA had a marked difference in the ability to reduce ferric ions (Fe3+). ABTS+ radical scavenging ability shows varying the concentrations, the three antioxidants showed dose-dependent effectiveness. The higher the concentration of the extracted AA, the greater the hydroxyl radical scavenging activity. At 32 µg/mL of extracted AA, there was no difference in ascorbic acid and trolox's NO radical-scavenging abilities. These all results indicates that Asiatic acid had good antioxidant potential which can further useful for anticancer formulation.

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