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A.S. Prabakaran and N. Mani

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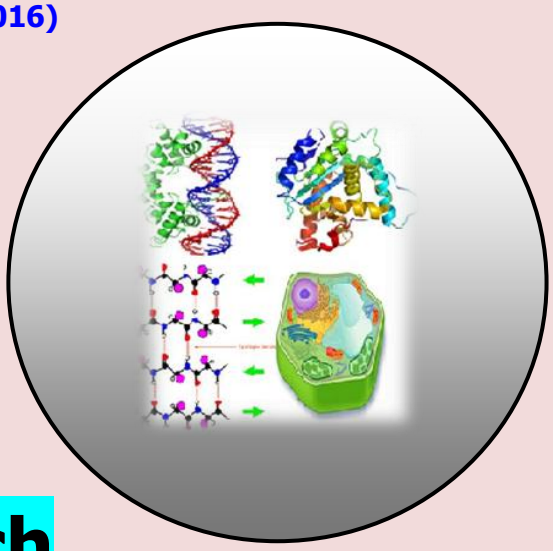

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A.S. Prabakaran
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RESEARCH PAPER

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In Vitro Antioxidant Activity of Silver Nanoparticle Synthesized from *Eichhornia crassipes* Leaves Extract

A.S. Prabakaran and *N. Mani

PG and Research Department of Chemistry, AVVM Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu, India
***PG and Research Department of Chemistry, AVVM Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu, India**

ABSTRACT

Antioxidant activity of Eichhornia crassipes leaves and AgNPs were carried out for proving its utility in free radical mediated diseases. The leaves extract and AgNPs were screened for in vitro antioxidant activity by oxygen radical scavenging such as DPPH, total antioxidant assay, superoxide radical scavenging, iron chelating, reducing power and nitric oxide scavenging activity at different concentrations. The antioxidant activity was found to be concentration dependent. Among this AgNPs possess potential antioxidant activity as compared with plant extract and close to the standard.
Keywords:Eichhornia crassipes, Antioxidant and Silvernanoparticle.

INTRODUCTION

Synthesis of silver nanoparticles through a green method has attracted great interest in this area (Niramathi *et al.,* 2013). Among the various methods the biological and green methods are considerably preferred for the biosynthesis of silver nanoparticles using the plant extract that possesses phytochemicals with strong antioxidant properties (Yoganannd *et al.,* 2008). The antioxidant effect of the plant extract is due to the presence of phenolic compounds such as flavonoids, phenolic acid, tannins and diterpenes. Antioxidants protect cells against cell damage causing molecules known as free radicals (Gupta and Sharma, 2010). The development of nanoparticles used as therapeutic agents has introduced new opportunities for the improvement of medical treatment. Oxidation of biomolecules for the regulation of oxidative chain reaction and plant plays an effective role in nanoparticle synthesis as they are free from toxic chemicals as well as provide natural capping agents (Arya and Yadavas, 2011). In the present study to investigate the*in vitro* antioxidant activity of *Eichhornia crassipes*leaves extract and AgNPs using DPPH, total antioxidant assay, superoxide radical scavenging, iron chelating, reducing power and nitric oxide scavenging activity at different concentrations.

MATERIALS AND METHODS

The *Eichhornia crassipes* leaves were collected in January 2015 from Koraiyaru River, Mannargudi, Thiruvarur district, Tamil Nadu. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the

Rapiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen (SJCBO12335) has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of leaves extract

The dried leaves were pulverized well with mortar and pestle to make a powder. Twenty grams of powder sample was mixed into 100 ml of deionized water and the mixture was boiled for 10 min. After cooling the leaves extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4◦C for further use. Doses such as 20, 40, 60 and 80µg/ml were chosen for *in vitro* antioxidant activity.

Synthesis of Ag nanoparticles using leaves extracts

For the Ag nanoparticles synthesis, 5 ml of *Eichhornia crassipes* leaves extract was added to 45 ml of 1 mM aqueous AgNO3 solution in a 250 mlErlenmeyer flask. The flask was then incubated in the dark at 5hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without leaves extract. The Ag nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the Ag nanoparticles were freeze dried using SEM analysis (Arunachalam *et al.,* 2012).

Antioxidant activity

The scavenging ability of the natural antioxidants of the plant extract towards the stable free radical DPPH was measured by the method of Shimada *et al*. (1992).The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*. (1999).The scavenging activity of the *Annona muricata* leaves towards superoxide anion radicals was measured by the method of Liu *et al*. (1997).The chelating activity of the AgNPs and plant extract for ferrous ions Fe^{2+} was measured according to the method of Dinis *et al*. (1994).Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964). The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu (1986).

Statistical analysis

Tests were carried out in triplicate for 3–5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a nonlinear regression algorithm.

RESULTS AND DISCUSSION

Synthesis of Silver nanoparticles

The use of plant and plant products in nanoparticle synthesis is considered advantageous over microbial based system because it reduces the elaborate process of maintaining cell cultures. The particle size growth can also be controlled by altering synthesis conditions like pH, reductant concentration, temperature, mixing ratio of the reactants etc. The plant based synthesis can be carried out either extracellular or intracellular. Intracellular synthesis takes place inside the plant whereas the extracellular synthesis occurs *in vitro*. Our earlier report indicates that UV-Vis spectral studies confirmed the surface plasmon resonance of green-synthesized silver nanoparticles. Biomolecules were responsible for reducing and capping of AgNPs, which were confirmed by FTIR measurements. SEM studies revealed spherical and uniform-shaped silver nanoparticles with size in the range 10-40 nm (Prabakaran and Mani, 2017).

***In vitro* antioxidant activity of *Eichhornia crassipes* leaves and Silver Nanoparticles**

DPPH radical scavenging activity

1, 1- Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical. DPPH is gained its stability as free radical molecules due to the delocalization of odd electron throughout the molecules. This more stabilized DPPH produce intense violet colour in ethanol solution. The antioxidant present in the extracts reacts with DPPH free radical solution and converts them into reduced form either by donating hydrogen atom or transferring electron followed by proton. This oxidation reaction is accompanied with loss of violet colour which can be measured quantitatively at 517 nm (Nuutila *et al.,* 2003). DPPH radical scavenging activity of *Eichhornia crassipes* leaves extract, AgNPs and standard as ascorbic acid are presented in Fig 1 The half inhibition concentration (IC_{50}) of *Eichhornia crassipes* leaves extract, AgNPs and ascorbic acid were 51.66, 52.27 and 34.89µg/ml respectively**.** The AgNPs exhibited a significant dose dependent inhibition of DPPH activity (Table 1) as compared to *Eichhornia crassipes* leaves extract. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentrations. AgNPs has potential antioxidant activity than *Eichhornia crassipes* extract and near to standard**.**

Table 1. DPPH radical scavenging activity of *Eichhornia crassipes* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Concentration (µg/ml)	% of inhibition		
	Eichhornia crassipes	AgNPs	Ascorbic acid (Std.)
20	19.55 ± 1.368	22.73 ± 1.5911	25.6 ± 2.04
40	38.64 ± 2.704	42.28 ± 2.959	61.26 ± 4.90
60	59.10 ± 4.137	62.28 ± 4.359	88.98 ± 7.11
80	76.37 ± 5.345	65.91 ± 4.613	99.34 ± 7.94
IC_{50} Value	51.66	52.27	34.89

Values are expressed as Mean± SD for triplicates

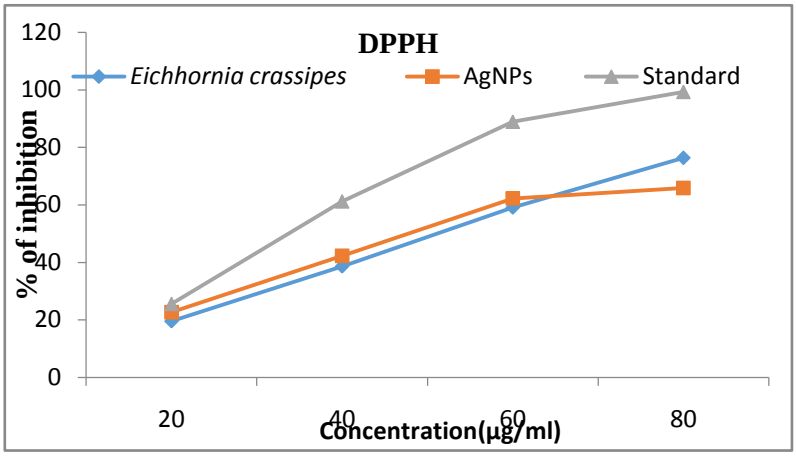

Figure 1. DPPH scavenging activity of *Eichhornia crassipes* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Total antioxidant activity

Total antioxidant capacity of AgNPs and *Eichhornia crassipes* leaves extract are expressed as the number of equivalents of ascorbic acid. The phospho molybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E and as it being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract (Prieto *et al.,* 1999). The total antioxidant capacity of *Eichhornia crassipes* leaves extract, AgNPs and standard ascorbic acid is presented in Fig 2 The total antioxidant activity of *Eichhornia crassipes* leaves extract, AgNPs and ascorbic acid was dose dependent manner. The half inhibition concentration (IC_{50}) of *Eichhornia crassipes* leaves extract, AgNPs and ascorbic acid were 51.94, 47.79 and 42.39µg/mlrespectively. The AgNPs exhibited a significant dose dependent inhibition of TAA activity (Table 2). AgNPs has potential antioxidant activity than *Eichhornia crassipes* extract and near to standard.

Table 2. Total antioxidant activity of *Eichhornia crassipes* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Concentration (µg/ml)	% of inhibition		
	Eichhornia crassipes	AgNPs	Ascorbic acid (Std.)
20	18.75 ± 1.312	21.25 ± 1.487	22.35 ± 1.80
40	38.12 ± 2.668	41.56 ± 2.909	51.23 ± 4.09
60	59.37 ± 4.155	61.25 ± 4.287	72.54 ± 5.80
80	76.25 ± 5.33	85.31 ± 5.971	86.35 ± 6.91
IC_{50} Value	51.94	47.79	42.39

Values are expressed as Mean± SD for triplicates

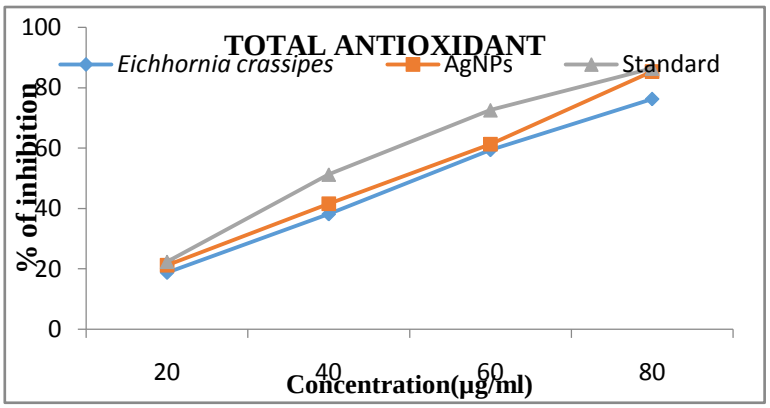

Figure 2. Total antioxidant activity of *Eichhornia crassipes* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Superoxide Scavenging Activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system (Korycka-Dahl and Richardson, 1978). The superoxide anion radical scavenging activities of the *Eichhornia crassipes* leaves extract, AgNPs and ascorbic acid was assayed by the PMS-NADH system and it was shown in Fig 3. The superoxide scavenging activity of *Eichhornia crassipes* leaves extract, AgNPs and ascorbic acid was increased markedly with the increase of concentrations (Table 3). The half inhibition concentration (IC_{50}) of *Eichhornia crassipes* leaves extract and AgNPs were 48.88, 47.06 and ascorbic acid was 31.61µg/ml respectively. AgNPs has potential superoxide anion scavenging activity than *Eichhornia crassipes* extract and was near to standard.

Table 3. Superoxide anion radical scavenging activity of *Eichhornia crassipes* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Concentration (µg/ml)	% of inhibition		
	Eichhornia crassipes	AgNPs	Ascorbic acid (Std.)
20	24.64 ± 1.724	20.35 ± 1.424	31.25 ± 2.50
40	38.21 ± 2.674	42.50 ± 2.97	64.23 ± 5.13
60	60.35 ± 4.224	63.92 ± 4.474	89.54 ± 7.16
80	81.07 ± 5.674	86.07 ± 6.024	98.51 ± 7.88
IC_{50} Value	48.88	47.06	31.61

Values are expressed as Mean± SD for triplicates

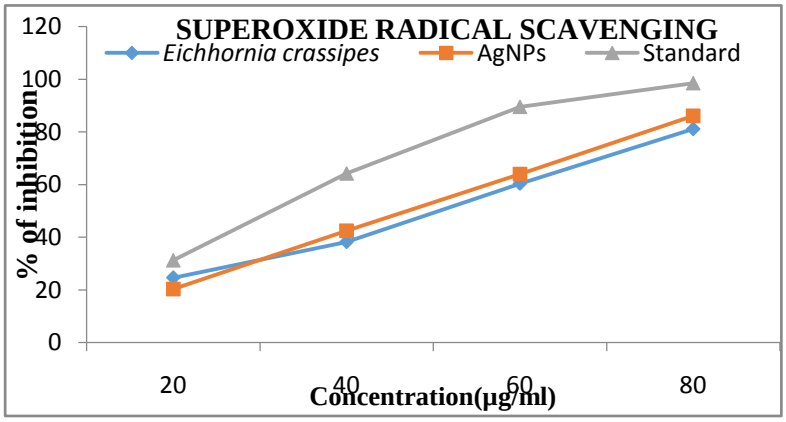

Figure 3. Superoxide radical scavenging activity of *Eichhornia crassipes* leaves extract and AgNPs and Ascorbic acid at different concentrations.

The ferrous ion chelating activity

The metal chelating assay involves color reduction which in turn determines their chelating ability of synthesized nanoparticles for ferrous ions. The formation of the ferrozine – Fe^{2+} complex is interrupted in the presence extract of *Eichhornia crassipes* leaves extract, AgNPs and ascorbic acid was increased markedly with the increase of concentrations (Table 4). The half inhibition concentration (IC_{50}) of *Eichhornia crassipes* leaves extract and AgNPs were 50.39, 45.92 and ascorbic acid was 30.94µg/ml respectively. AgNPs has potential ferrous ion chelating activity than *Eichhornia crassipes* extract and was near to standard (Fig 4).

Table 4. Iron chelating activity of *Eichhornia crassipes* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Concentration (µg/ml)	% of inhibition		
	Eichhornia crassipes	AgNPs	Ascorbic acid (Std.)
20	20.38±1.426	23.84±1.668	35.23 ± 2.81
40	39.61±2.77	43.84±3.068	65.21 ± 5.28
60	58.46±4.092	64.23±4.496	78.51 ± 6.28
80	80.00±5.6	84.61±5.922	98.65 ± 7.89
IC_{50} Value	50.39	45.92	30.94

Values are expressed as Mean± SD for triplicates

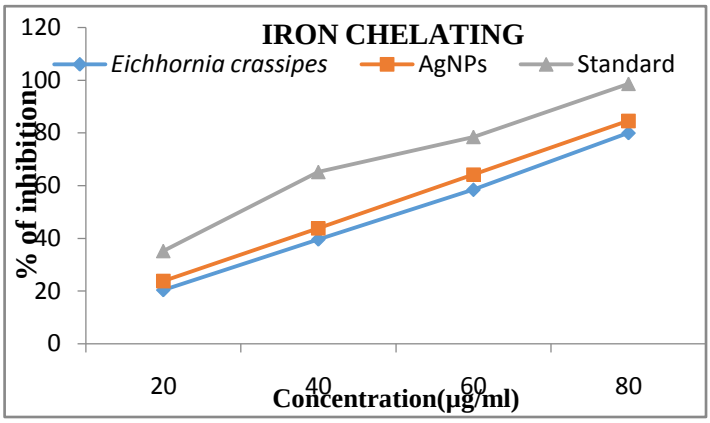

Figure 4. Iron chelating activity of *Eichhornia crassipes* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Reducing power activity

The reductive effect of *Eichhornia crassipes* leaves extract, AgNPs and ascorbic acid increased with increasing dosage. All the doses showed significant activities near to the control exhibited greater reducing power, indicating that *Eichhornia crassipes* consist of hydrophilic polyphenolic compounds that cause the greater reducing power. Fig. 5 depicts the reductive effect of *Eichhornia crassipes.* Similar to the antioxidant activity, the reducing power of *Eichhornia crassipes* leaves extract, AgNPs and ascorbic acid increased with increasing dosage (Table 5). All the doses showed significant activities near to the control exhibited greater reducing power, indicating that *Eichhornia crassipes* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

Table 5. Reducing power activity of *Eichhornia crassipes* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Concentration (µg/ml)	% of inhibition		
	Eichhornia crassipes	AgNPs	Ascorbic acid (Std.)
20	0.13 ± 0.0091	0.18 ± 0.0126	0.41 ± 0.03
40	0.27 ± 0.0189	0.32 ± 0.0224	0.71 ± 0.05
60	0.42 ± 0.029	0.49 ± 0.0343	0.89 ± 0.07
80	0.67 ± 0.046	0.72 ± 0.0504	0.98 ± 0.08

Values are expressed as Mean± SD for triplicates

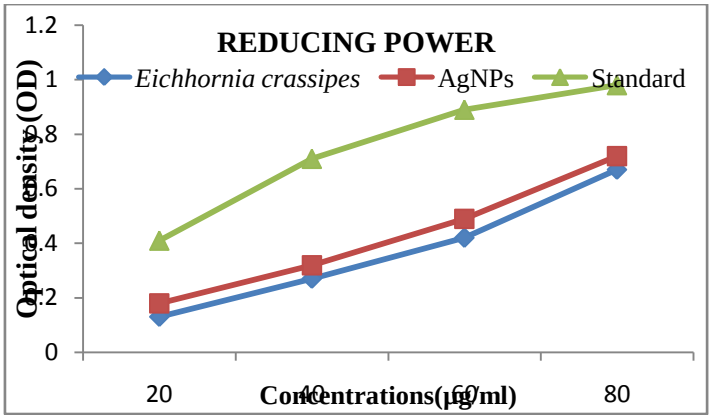

Figure 5. Reducing power activity of *Eichhornia crassipes* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Nitric oxide activity

The *Eichhornia crassipes* extract in SNP solution significantly inhibited the accumulation of nitrite, a stable oxidation product of NO• liberated from SNP in the reaction medium with time compared to the standard ascorbic acid. The toxicity of NO• increases when it reacts with superoxide to form the peroxynitrite anion (•ONOO-), which is a potential strong oxidant that can decompose to produce •OH and NO2 (Pacher *et al.,* 2007). The formation of the nitric oxide activity is interrupted in the presence extract of *Eichhornia crassipes* leaves extract, AgNPs and ascorbic acid was increased markedly with the increase of concentrations (Table 6). The half inhibition concentration (IC_{50}) of *Eichhornia crassipes* leaves extract and AgNPs were 49.35, 49.26 and ascorbic acid was 49.35µg/ml respectively**.** AgNPs has potential ferrous ion chelating activity than *Eichhornia crassipes* extract and was near to standard (Fig. 6).

Table 6. Nitric oxide activity of *Eichhornia crassipes* leaves extract, AgNPs and Ascorbic acid at different concentrations

Concentration (µg/ml)	% of inhibition		
	Eichhornia crassipes	AgNPs	Ascorbic acid (Std.)
20	22.85 ± 1.599	20.4 ± 1.432	26.21 ± 2.04
40	44.28 ± 3.099	35.71 ± 2.499	59.62 ± 4.65
60	56.19 ± 3.933	62.38 ± 4.366	84.23 ± 6.56
80	79.04 ± 5.532	84.76 ± 5.933	96.45 ± 7.52
IC_{50} Value	49.35	49.26	49.35

Values are expressed as Mean± SD for triplicates

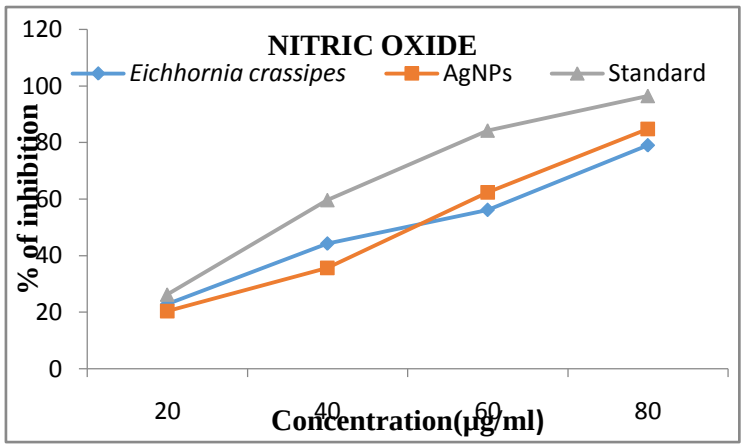

Figure 6. Nitric oxide activity of *Eichhornia crassipes* leaves extract, AgNPs and Ascorbic acid at different concentrations.

CONCLUSION

Antioxidant activity of *Eichhornia crassipes* leaves and AgNPs were carried out for proving its utility in free radical mediated diseases. The leaves extract and AgNPs were screened for *in vitro* antioxidant activity by oxygen radical scavenging such as DPPH, total antioxidant assay, superoxide radical scavenging, iron chelating, reducing power and nitric oxide scavenging activity at different concentrations. The antioxidant activity was found to be concentration dependent. Among this AgNPs possess potential antioxidant activity as compared with plant extract and close to the standard.

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Corresponding author: A.S. Prabakaran, PG and Research Department of Chemistry, AVVM Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu, India Email: aspind333@yahoo.com

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