

IN VITRO ANTIOXIDANT ACTIVITY OF CISSUS VITIGINEA LEAVES AND ITS SILVER NANOPARTICLES

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Article Received on
08 April 2018,

Revised on 29 April 2018,
Accepted on 20 May 2018,

DOI: 10.20959/wjpr201811-12483

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ABSTRACT

Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery. Various methods are used to investigate the antioxidant property of samples. Antioxidant activity of *Cissus vitifolia* 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 and reducing power 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: *Cissus vitifolia*, Silver nanoparticles, Antioxidant activity.

INTRODUCTION

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants (Badarinath *et al.*, 2010). Free radicals are responsible for causing a large number of diseases including cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, aging and atherosclerosis (Velavan, 2011; Smith *et al.*, 2000). Protection against free radicals can be

enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention. There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term (Blokhina *et al.*, 2003). Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery. Various methods are used to investigate the antioxidant property of samples (diets, plant extracts, commercial antioxidants etc.) (Nur Alam *et al.*, 2013). This present study was to investigate the antioxidant activity of *Cissus vitiginea leaf* extract and its silver nano particles (AgNPs).

MATERIALS AND METHODS

Chemicals

All the experiments were conducted at room temperature. Chemicals used in this study are AR grade silver nitrate (AgNO_3) obtained from Merck, India.

Collection of plant materials

The *Cissus vitiginea* leaves were collected in March 2016 from Thanjavur, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Dr. S. John Britto, Director, Rapinat Herbarium and center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu, India. A Voucher specimen has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of leaf extract

The dried leafs 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 leaf extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4°C for further use.

Synthesis of Ag nanoparticles using leaf extract

45 ml of 1 mM aqueous AgNO_3 solution taken in a 250 ml Erlenmeyer flask and add 5 ml of *Cissus vitiginea* leaf extract. 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 leaf extract. The obtained Ag nanoparticle solution 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 and dried for using SEM analysis (Arunachalama *et al.*, 2012).

***In vitro* antioxidant activity**

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity was determined by the method of Shimada *et al.* (1992). The scavenging activity of the *Cissus vitifolia* towards superoxide anion radicals was measured by the method of Liu *et al.* (1997). The total antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.* (1999).

RESULTS

DPPH radical scavenging activity

DPPH radical scavenging activity of *Cissus vitifolia* leaves extract, AgNPs and standard ascorbic acid are presented in Fig.1. The half inhibition concentration (IC₅₀) of *Cissus vitifolia* leaves extract, AgNPs and ascorbic acid were 52.20, 37.18 and 34.89µg/ml respectively. The AgNPs exhibited a significant dose dependent inhibition of DPPH activity (Table 1) as compared to *Cissus vitifolia* leaves extract. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentrations. AgNPs has potential antioxidant activity than *Cissus vitifolia* extract and near to standard.

Table 1: DPPH radical scavenging activity of *Cissus vitifolia* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Concentrations	20 µg/ml	40µg/ml	60µg/ml	80µg/ml	IC ₅₀ (µg/ml)
	% of inhibition				
<i>Cissus vitifolia</i>	22.27±1.55	37.27±2.60	59.55±4.17	73.18±5.12	52.20
AgNPs	29.54±2.06	57.27±4	74.09±5.19	89.54±6.26	37.18
Ascorbic acid (Std.)	25.6±2.04	61.26±4.90	88.98±7.11	99.34±7.94	34.89

Values are expressed as Mean± SD for triplicates.

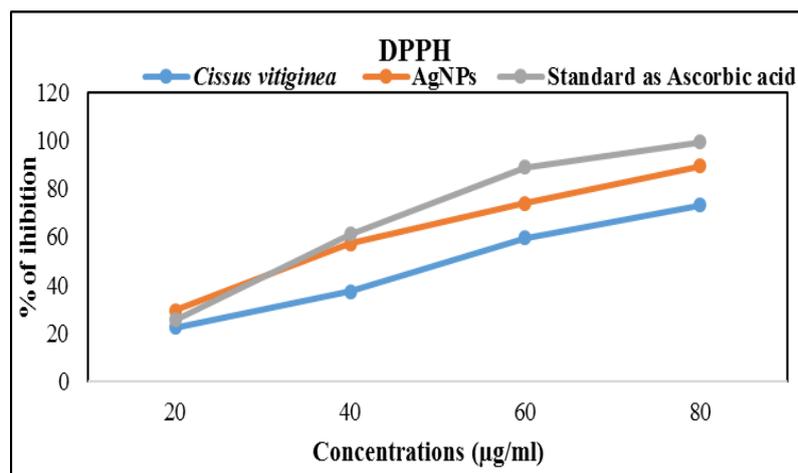


Figure 1: DPPH scavenging activity of *Cissus vitiginea* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Total antioxidant activity

The total antioxidant capacity of *Cissus vitiginea* leaves extract, AgNPs and standard ascorbic acid is presented in Fig.2. The total antioxidant activity of *Cissus vitiginea* leaves extract, AgNP sand ascorbic acid was dose dependent manner. The half inhibition concentration (IC_{50}) of *Cissus vitiginea* leaves extract, AgNPs and ascorbic acid were 48.17, 38.49 and 42.38µg/ml respectively. The AgNPs exhibited a significant dose dependent inhibition of TAA activity (Table 2). AgNPs has potential antioxidant activity than *Cissus vitiginea* extract and near to standard.

Table 2: Total antioxidant activity of *Cissus vitiginea* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Concentrations	20 µg/ml	40µg/ml	60µg/ml	80µg/ml	IC_{50} (µg/ml)
	% of inhibition				
<i>Cissus vitiginea</i>	23.43±1.64	41.25±2.89	63.75±4.46	78.43±5.49	48.17
AgNPs	33.43±2.34	51.25±3.58	69.68±4.88	86.56±6.05	38.49
Ascorbic acid (Std.)	22.35± 1.80	51.23± 4.09	72.54± 5.80	86.35± 6.91	42.38

Values are expressed as Mean± SD for triplicates

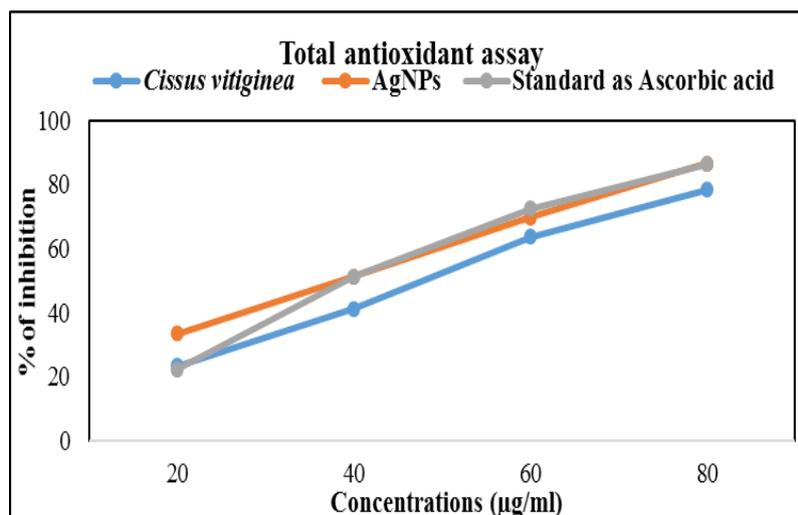


Figure 2: Total antioxidant activity of *Cissus vitifolia* leaves extract, AgNPs and Ascorbic acid at different concentration.

Superoxide Scavenging Activity

The superoxide anion radical scavenging activities of the *Cissus vitifolia* 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 *Cissus vitifolia* leaves extract, AgNPs and ascorbic acid was increased markedly with the increase of concentrations (Table 3). The half inhibition concentration (IC_{50}) of *Cissus vitifolia* leaves extract and AgNPs were 49.59, 41.52 and ascorbic acid was 31.60 µg/ml respectively. AgNPs has potential superoxide anion scavenging activity than *Cissus vitifolia* extract and was near to standard.

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

Concentration	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	IC_{50} (µg/ml)
	% of inhibition				
<i>Cissus vitifolia</i>	23.57±1.65	41.07±2.87	62.50±4.38	74.28±5.20	49.59
AgNPs	29.28±2.05	52.85±3.70	66.07±4.62	79.64±5.57	41.52
Ascorbic acid (Std.)	31.25±2.50	64.23±5.13	89.54±7.16	98.51±7.88	31.60

Values are expressed as Mean± SD for triplicates.

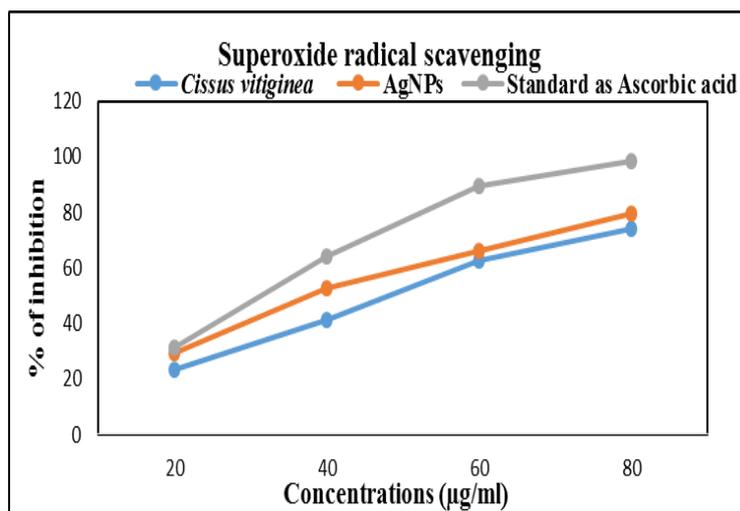


Figure 3: Superoxide radical scavenging activity of *Cissus vitiginea* leaves extract, AgNPs and Ascorbic acid at different concentrations.

The ferrous ion chelating activity

The formation of the ferrozine – Fe²⁺ complex is interrupted in the presence extract of *Cissus vitiginea* leaves extract, AgNPs and ascorbic acid was increased markedly with the increase of concentrations (Table 4). The half inhibition concentration (IC₅₀) of *Cissus vitiginea* leaves extract and AgNPs were 53.81, 40.13 and ascorbic acid was 30.93 µg/ml respectively. AgNPs has potential ferrous ion chelating activity than *Cissus vitiginea* extract and was near to standard. (Fig. 4).

Table 4: Iron chelating activity of *Cissus vitiginea* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Concentration	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	IC ₅₀ (µg/ml)
	% of inhibition				
<i>Cissus vitiginea</i>	22.30±1.56	33.84±2.37	53.84±3.76	76.15±5.33	53.81
AgNPs	31.92±2.23	48.84±3.42	69.23±4.85	86.15±6.03	40.13
Ascorbic acid (Std.)	35.23±2.81	65.21±5.28	78.51±6.28	98.65±7.89	30.93

Values are expressed as Mean ± SD for triplicates.

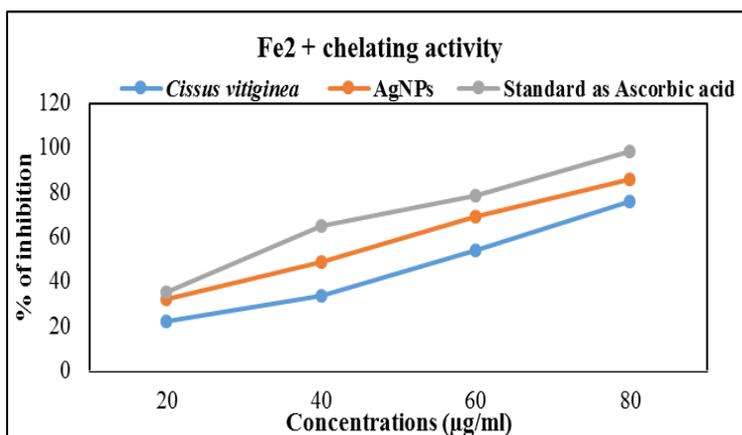


Figure 4: Iron chelating activity of *Cissus vitiginea* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Reducing power activity

Fig. 5 depicts the reductive effect of *Cissus vitiginea*. Similar to the antioxidant activity, the reducing power of *Cissus vitiginea* 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 *Cissus vitiginea* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

Table 5: Reducing power activity of *Cissus vitiginea* leaves extract, AgNPs and Ascorbic acid at different concentrations.

Concentration	20 µg/ml	40µg/ml	60µg/ml	80µg/ml
<i>Cissus vitiginea</i>	0.23±0.02	0.41±0.03	0.67±0.05	0.78±0.05
AgNPs	0.32±0.02	0.53±0.04	0.72±0.05	0.85±0.06
Ascorbic acid (Std.)	0.41±0.03	0.71±0.05	0.89±0.07	0.98±0.08

Values are expressed as Mean± SD for triplicates.

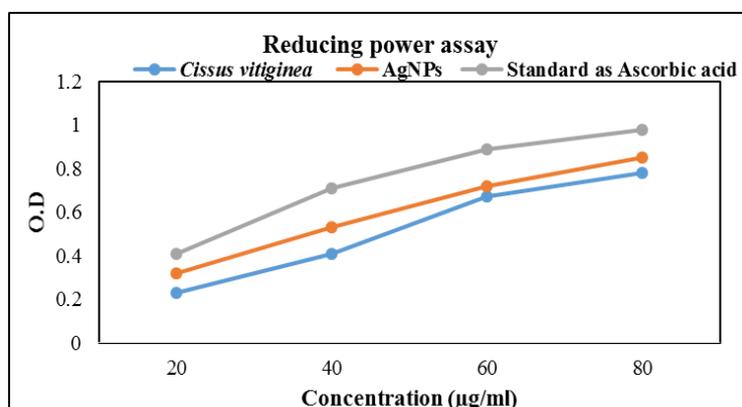


Figure 5: Reducing power activity of *Cissus vitiginea* leaves extract, AgNPs and Ascorbic acid at different concentrations.

DISCUSSION

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 *Cissus vitifolia* leaf extract, AgNPs and standard as ascorbic acid were investigated. The half inhibition concentration (IC₅₀) of *Cissus vitifolia* leaf extract, AgNPs and ascorbic acid were 52.20, 37.18 and 34.89 µg/ml respectively. The AgNPs exhibited a significant dose dependent inhibition of DPPH activity as compared to *Cissus vitifolia* leaf extract. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentrations. AgNPs has potential antioxidant activity than *Cissus vitifolia* extract and near to standard. Similar results observed in Soumya menon *et al.*, (2017) studies.

Total antioxidant activity

Total antioxidant capacity of AgNPs and *Cissus vitifolia* leaf 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). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The total antioxidant activity of *Cissus vitifolia* leaf extract, AgNPs and ascorbic acid was dose dependent manner. The half inhibition concentration (IC₅₀) of *Cissus vitifolia* leaf extract, AgNPs and ascorbic acid were 48.11, 38.49 and 42.38 µg/ml respectively. The AgNPs exhibited a significant dose dependent antioxidant activity. AgNPs has potential antioxidant activity than *Cissus vitifolia* extract and near to standard.

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 scavenging activity of *Cissus vitifolia* leaf extract, AgNPs and ascorbic acid was increased markedly with the increase of concentrations. The half inhibition concentration (IC₅₀) of *Cissus vitifolia* leaf extract and AgNPs were 49.59, 41.52 µg/ml and ascorbic acid was 31.60 µg/ml respectively. AgNPs has potential superoxide anion scavenging activity than *Cissus vitifolia* extract and near to standard.

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 – Fe²⁺ complex is interrupted in the presence extract of *Cissus vitifolia* leaves extract, AgNPs and ascorbic acid was increased markedly with the increase of concentrations. Thus the decrease in the absorbance at 562 nm indicated high levels of iron binding potential and antioxidant activity of the nanoparticles. The half inhibition concentration (IC₅₀) of *Cissus vitifolia* leaves extract and AgNPs were 53.81, 40.13 and ascorbic acid was 30.93 µg/ml respectively. AgNPs has potential antioxidant activity increased proportionally to the polyphenol content. The phenols contain hydroxyls that are responsible for the radical scavenging effects mainly due to the redox properties (Adedapo *et al.*, 2008). Present results agreement with Abdul-Rehman Phull *et al.*, (2016) studies who reported that Antioxidant, cytotoxic and antimicrobial activities of green synthesized silver nanoparticles from crude extract of *Bergenia ciliata*.

CONCLUSION

The greatest *in vitro* antioxidant activity of silver nanoparticles was observed as compared with *Cissus vitifolia* leaf extract.

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