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Antioxidant activity of *Commelina maculata* leaves and its silver nanoparticles: An *in vitro* study

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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 *Commelina maculata* 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: *Commelina Maculata*, 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) [1]. 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) [2, 3]. 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) [4]. 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) [5]. This present study was to investigate the antioxidant activity of *Commelina maculata* leaf extract and its silver nano particles (AgNPs).

Materials and methods
Chemicals
All the experiments were conducted at room temperature. Chemicals used for the production of silver nanoparticles are Analytical grade silver nitrate ($AgNO_3$) purchased from Merck, India.

Collection of plant materials
The *Commelina maculata* leaves were collected from Kathattipatti (Palaiyapatti North) Thanjavur, Tamil Nadu, India from a herb. The plant were identified and authenticated by Dr. S. John Britto, The Director, the Rapinat Herbarium and center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen (TCV001) 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. 20 grams of *Commelina maculata* leaves powder was mixed into 100 ml of deionized water and the mixture was boiled for 10 min. The leaf extract was filtered with Whatman No. 1 filter paper after cooling. The extract was kept at 4°C for further study.

Synthesis of Ag nanoparticles using leaf extracts
Five ml of *Commelina maculata* leaf extract was added to 45 ml of 1 mM aqueous $AgNO_3$ solution in a 250 ml Erlenmeyer flask and incubated in the dark at 5hrs at room temperature. A control setup as without leaf extract also maintained. The Ag nanoparticle solution thus achieved was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. (Arunachalama *et al* 2012) [6].

In vitro antioxidant activity
DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical-scavenging activity was determined by the method of Shimada *et al.* (1992) [7]. The scavenging activity of the *Commelina maculata* towards superoxide anion radicals was measured by the method of Liu *et al.* (1997) [8]. The total antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.* (1999) [9].

Results
Our earlier reported that the synthesized silver nanoparticles are evaluated the antimicrobial activity Vedhanayaki and Ramkumar (2020) [10].

DPPH radical scavenging activity

DPPH radical scavenging activity of *Commelina maculata* leaves extract, AgNPs and standard as ascorbic acid are presented in Fig.1. The half inhibition concentration (IC_{50}) of *Commelina maculata* leaves extract, AgNPs and ascorbic acid were 50.94, 47.15 and 44.28µg/ml respectively**.** The AgNPs exhibited a significant dose dependent inhibition of DPPH activity (Table 1) as compared to *Commelina maculata* leaves extract. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentrations. AgNPs has potential antioxidant activity than *Commelina maculata* extract and nearest to standard**.**

Table 1: DPPH radical scavenging activity of *Commelina Maculata* 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				
Commelina maculate (%)	19.54±1.36	31.36±2.19	63.63±4.45	81.36±5.69	50.94
AgNPs %)	22.72±1.59	35.9±2.51	70.9±4.96	82.72±5.79	47.15
Standard ascorbic acid (%)	24.09±1.68	40.45±2.83	75±5.25	85.45±5.98	44.28

Values are expressed as Mean± SD for triplicates

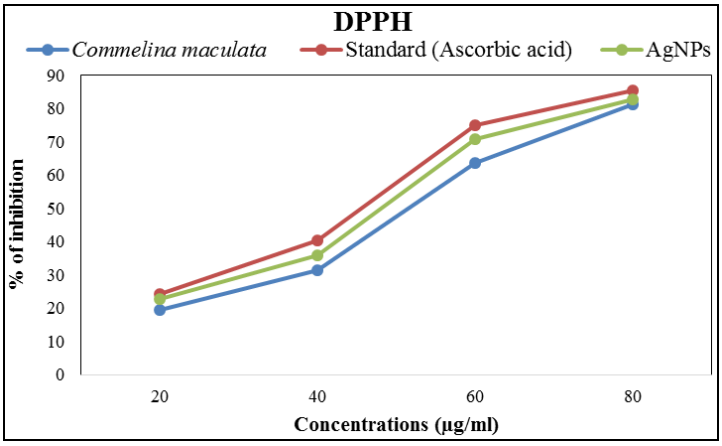

Fig 1: DPPH scavenging activity of *Commelina maculata* leaves extract, AgNPs and Ascorbic acid at different concentrations

Total antioxidant activity

The total antioxidant capacity of *Commelina maculata* leaves extract, AgNPs and standard ascorbic acid is presented in Fig.2. The total antioxidant activity of *Commelina maculata* leaves extract, AgNP sand ascorbic acid was dose dependent manner. The half inhibition concentration (IC_{50}) of *Commelina maculata* leaves extract, AgNPs and ascorbic acid were 59.30, 53.69 and 50.22µg/ml respectively**.** The AgNPs exhibited a significant dose dependent inhibition of TAA activity (Table 2). AgNPs has potential antioxidant activity than *Commelina maculata* extract and near to standard.

Table 2: Total antioxidant activity of *Commelina maculata* 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				
Commelina maculate (%)	18.43±1.29	24.68±1.72	56.56±3.95	67.18±4.70	59.30
AgNPs (%)	20.62±1.44	29.06±2.03	61.25±4.28	74.68±5.22	53.69
Standard ascorbic acid (%)	21.25±1.48	32.5±2.27	64.37±4.50	80.93±5.66	50.22

Values are expressed as Mean± SD for triplicates

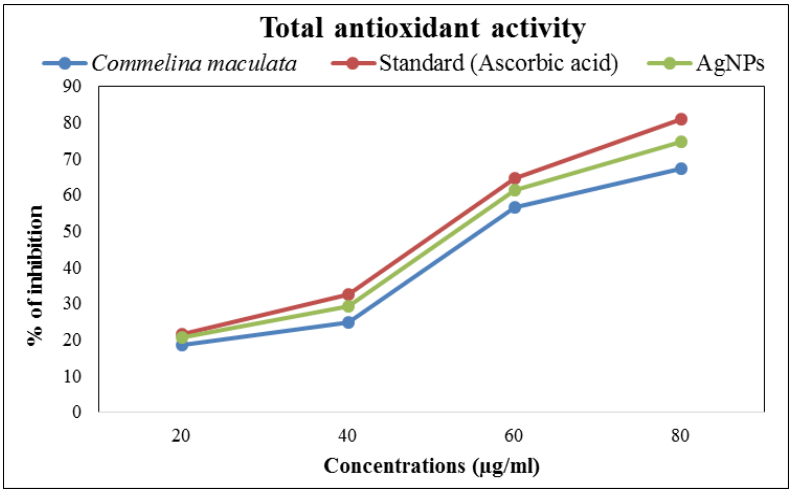

Fig 2: Total antioxidant activity of *Commelina maculata* leaves extract, AgNPs and Ascorbic acid at different concentration

Superoxide Scavenging Activity

The superoxide anion radical scavenging activities of the *Commelina maculata* 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 *Commelina maculata* leaves extract, AgNPs and ascorbic acid was increased markedly with the increase of concentrations (Table 3). The half inhibition concentration (IC_{50}) of *Commelina maculata* leaves extract and AgNPs were 52.25, 48.64 and ascorbic acid was 42.97µg/ml respectively**.** AgNPs has potential superoxide anion scavenging activity than *Commelina maculata* extract and was near to standard.

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

Concentration	20 µg/ml	40µg/ml	60µg/ml	80µg/ml	IC_{50}(µg/ml)
	% of inhibition				
Commelina maculate (%)	16.07±1.12	33.21±2.32	63.92±4.47	77.14±5.39	52.25
AgNPs (%)	20.71±1.44	36.42±2.54	68.21±4.77	80.35±5.62	48.64
Standard ascorbic acid (%)	23.21±1.62	45.71±3.19	75.71±5.29	86.07±6.02	42.97

Values are expressed as Mean± SD for triplicates

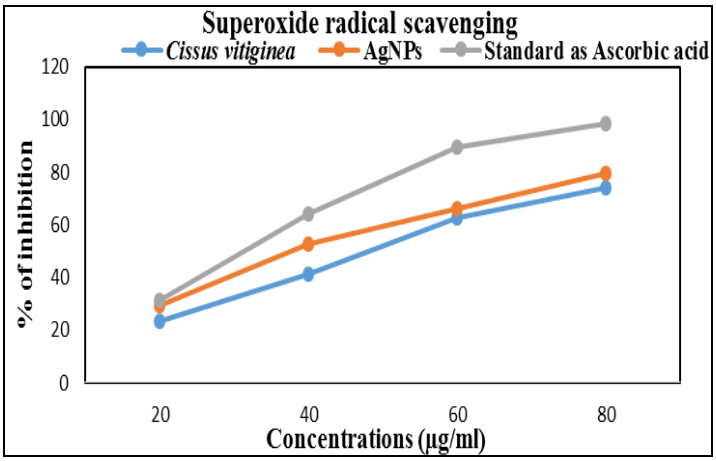

Fig 3: Superoxide radical scavenging activity of *Commelina maculata* leaves extract, AgNPs and Ascorbic acid at different concentrations

Nitric oxide scavenging activity

The nitric oxide scavenging activity of *Commelina maculata leaves* extract, AgNPs and ascorbic acid was increased markedly with the increase of concentrations (Table 4). The half inhibition concentration (IC_{50}) of *Commelina maculata* leaves extract and AgNPs were 56.76, 52.74 and ascorbic acid was 46.29µg/ml respectively**.**

AgNPs has potential nitric oxide scaavenging activity than *Commelina maculata* extract and was near to standard (Fig. 4).

Table 4: Nitric oxide scavenging activity of *Commelina maculata* leaves extract, AgNPs and Ascorbic acid at different concentrations

Concentration	20 µg/ml	40µg/ml	60µg/ml	80µg/ml	IC_{50} (µg/ml)
	% of inhibition				
Commelina maculate (%)	14.76±1.03	29.52±2.06	58.09±4.06	70.95±4.96	56.76
AgNPs (%)	18.57±1.29	35.71±2.49	61.42±4.29	73.8±5.16	52.74
Standard ascorbic acid (%)	21.9±1.53	40.47±2.83	70.95±4.96	82.38±5.76	46.29

Values are expressed as Mean± SD for triplicates

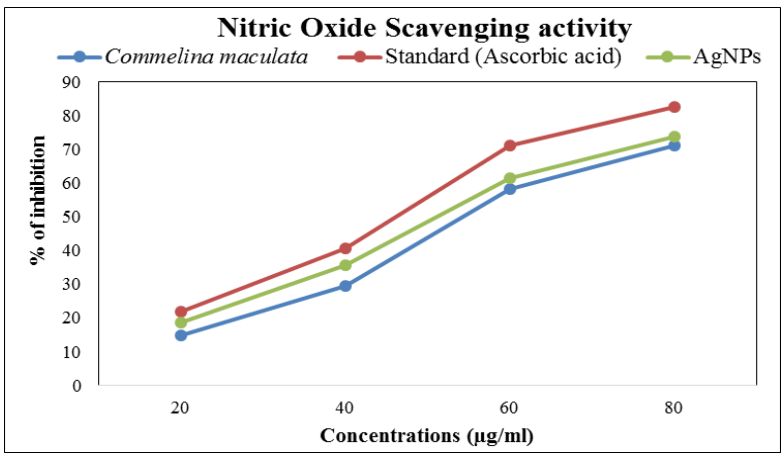

Fig 4: Nitric oxide scavenging activity of *Commelina maculata* leaves extract, AgNPs and Ascorbic acid at different concentrations

Reducing power activity

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

Table 5: Reducing power activity of *Commelina maculata* leaves extract, AgNPs and Ascorbic acid at different concentrations

Concentration	20 µg/ml	40µg/ml	60µg/ml	80µg/ml
Commelina maculate	0.27±0.01	0.45±0.03	0.71±0.04	0.75±0.05
AgNPs	0.31±0.02	0.48±0.03	0.79±0.05	0.81±0.05
Standard ascorbic acid	0.37±0.02	0.55±0.03	0.85±0.05	0.91±0.06

Values are expressed as Mean± SD for triplicates

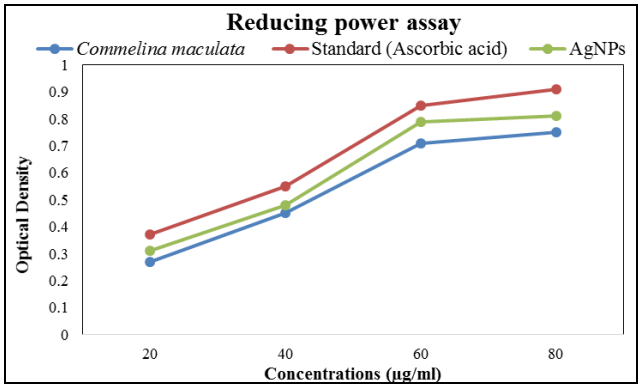

Fig 5: Reducing power activity of *Commelina maculata* leaves extract, AgNPs and Ascorbic acid at different concentrations

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) [11]. DPPH radical scavenging activity of *Commelina maculata* leaf extract, AgNPs and standard as ascorbic acid were investigated. The half inhibition concentration (IC_{50}) of *Commelina maculata* 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 as compared to *Commelina maculata* leaf extract. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentrations. AgNPs has potential antioxidant activity than *Commelina maculata* extract and near to standard**.** Similar results observed in Soumya menon *et al*. (2017) [12] studies.

Total antioxidant activity

Total antioxidant capacity of AgNPs and *Commelina maculata* 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) [9]. 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 *Commelina maculata* leaf extract, AgNPs and ascorbic acid was dose dependent manner. The half inhibition concentration (IC_{50}) of *Commelina maculata* leaf extract, AgNPs and ascorbic acid were 48.11, 38.49 and 42.38µg/ml respectively. The AgNPs exhibited a significant dose dependent antioxidant activity. AgNPs has potential antioxidant activity than *Commelina maculata* 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) [13]. The superoxide scavenging activity of *Commelina maculata* leaf extract, AgNPs and ascorbic acid was increased markedly with the increase of concentrations. The half inhibition concentration (IC_{50}) of *Commelina maculata* leaf extract and AgNPs were 49.59, 41.52µg/ml and ascorbic acid was 31.60µg/ml respectively. AgNPs has potential superoxide anion scavenging activity than *Commelina maculata* extract and near to standard.

Nitric oxide scavenging activity

The nitric oxide scavenging activity of the nanoparticles was detected by its ability to inhibit the formation of nitrite through direct competition with oxygen and oxides of nitrogen in reaction mixture (Tylor *et al* 1997) [14]. Excess production of NO is associated with several diseases Sun and Ho (2005) [15]. Present study shows the silver nanoparticles had nitric oxide scavenging activity. The scavenging activity of the nanoparticles was nearest to the standard ascorbic acid.

Reducing Power Assay

The electron donating capacity (reducing power) of compound is associated with antioxidant activity (Yen *et al* 1993) [16].

The reducing power of silver nanoparticles increases in dose dependent manner. The obtained results are shows nearly same efficacy as that of standard ascorbic acid surprisingly with lower concentration silver nanoparticles shown more reducing power than standard. Reducing power is evaluated by the transformation of Fe^{3+} to Fe^{2+} in presence of compound (Gulcin *et al* 2003) [17]. The reducing capacity of silver nanoparticles may serve as significant indicator of its potential antioxidant activity.

Present results agreement with Abdul-Rehman Phull *et al*. (2016) [18] studies who reported that Antioxidant, cytotoxic and antimicrobial activities of green synthesized silver nanoparticles from crude extract of *Bergenia ciliate*. A similar results were also found with silver nanoparticles synthesized by using plant leaves *Excoecaria agallocha* Arun and Saraswathi (2015) [19].

Conclusion

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

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