Assessment of in vitro Antioxidative and Anti-Inflammatory Effect of Caesalpinia Bonducella Seed Stabilized Silver Nanoparticles

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**ABSTRACT**
Membrane lipid peroxidation and DNA, protein damage is mediated by free radicals, which form the basis of chronic pathological complications. AgNPs are an important class of nanomaterials for a wide range of biomedical applications. The present study endeavors in vitro antioxidant and anti-inflammatory activity of green synthesized silver nanoparticles (AgNPs) using medicinal plant extract from Caesalpinia bonducella seeds. Total flavonoid and phenolic contents were determined. The antioxidant potential of capped AgNPs was assessed using DPPH assay, Phosphomolybdenum assay, FRAP assay, metal chelating, hydrogen peroxide, and hydroxyl radical scavenging methods. In vitro anti-inflammatory assay of CB seed AgNPs were performed against the standard drug. CB seed AgNPs possessed high flavonoid and phenol compared to aqueous CB seed extract. The antioxidant methods confirmed that the silver nanoparticles have more antioxidant activity as compared to vitamin C. The synthesized silver nanoparticles exhibited potential anti-inflammatory activity with the IC50 71.3 µg/ml. Hence, this work clearly demonstrated that the coated AgNPs with CB seeds act as a potent free radical scavenger and could be considered as a potential source for anti-inflammatory drugs.

**INTRODUCTION**
Nanotechnology is a progressing field imparting wide application in biomedical science and biotechnology research area. Though a number of approaches are convenient for the synthesis of the silver nanoparticles, environment-friendly technology holds great importance (Natarajan et al., 2010; Klueh et al., 2000). Existing literature reports that usage of medicinal plants is valuable as a result of their medicinal properties would pass over to the nanoparticles (Santhoshkumar et al., 2011; Zargar et al., 2011). The active molecules in the plant extract such as flavonoid, tannins, amines, aldehyde/ketone groups, and polyols and proteins act as reducing and capping agents transfer useful properties to the green synthesized nanoparticle by controlling morphology, size and protect the surface preventing aggregation (Verma and Mehata, 2016).

**Caesalpinia bonducella** belongs to the Family Caesalpiniaceae found mainly in the southern amino acids, fatty acids alkaloids, terpenoids, phytosterols, and carbohydrates (Kannur, 2011). In tradi-
tional Indian medicine, the usage of Caesalpinia bonducella seeds has created a great impact in ameliorating various diseases. It possesses various medicinal effects such as anti-hyperlipidemic, anti-hyperglycemic effect, antipyretic, anti-inflammatory, and antioxidant activity (Kannur et al., 2006; Archana et al., 2005; Shukla et al., 2009).

Natural antioxidants, mainly the flavonoids in the plants, provide protection against harmful free radicals countering the oxidative damage and are firmly associated with diminished risk of chronic diseases (Krishnaiah et al., 2007; Kim et al., 2003) have recorded that phytocompounds of the medicinal plants provide antioxidant properties to the nanoparticles. (Zima et al., 2001) have reported that in biological systems, free radicals are produced during metabolism and other physiological activities within the limits of free radical scavenging mechanism of the system. The various forms of ROS that include superoxide anion free radicals, hydroxyl free radicals, H$_2$O$_2$, less stable excited oxygen, which is generated during inflammation and its associated diseases intensifies the cellular damage and age declining process (Halliwell, 2001; Venkataraman et al., 2013). These free radical production and antioxidant defenses are correlated with adversity to a broad spectrum of molecular species that include lipids, proteins, and nucleic acids. The intricate relationship between oxidative stress and inflammation were emphasized by several studies. They are involved in multiple chronic inflammatories and deteriorating disorders imposing poor health status and enhancing the probability of persistent diseases such as cancer, atherosclerosis, and Alzheimer’s disease (Birben et al., 2012; Mittal et al., 2014). The redox properties of phenolic and flavonoid compounds in CB seeds enable them to be potent antioxidants (Sachan et al., 2010).

Therefore, the present investigation was undertaken to predict the antioxidant and anti-inflammatory activities of green synthesized C. bonducella seed nanoparticles (CB seed AgNPs) in vitro. This study also attempts to explore the relationship between total phenol, flavonoid content of CB seed AgNPS, and its effective antioxidant property.

**MATERIALS AND METHODS**

**Plant material and preparation of the extract**

The synthesis of the silver nanoparticle is accomplished using Caesalpinia bonducella seeds purchased from the Madurai local market, and the nanoparticles synthesis process was carried out as described in our previous publication (Shyam et al., 2018). The synthesized silver nanoparticles were used for the screening of antioxidant activity and anti-inflammatory activity.

Total phenolic contents were determined by the Folin-Ciocalteau reagent with slight modifications of (Singleton et al., 1999). 1mL(1g) of CB seed AgNPs was diluted to 46 ml using distilled H$_2$O, added 1mL Folin–Ciocalteau reagent with intense mixing. 3 minutes later, 3mL of 2% sodium carbonate was added and left for 3 h with periodic shaking. The absorbance of the blue color that developed was read at 760 nm.

**Estimation of total Flavonoids by AlCl$_3$ method**

The total flavonoid was determined by the colorimetric method in which AlCl$_3$ forms complex with flavones and flavonols. To 1ml(100μg) of Caesalpinia bonducella, seed extract, and seed AgNPs added 0.5ml of 10% Aluminium chloride, 0.5 ml of 1M potassium acetate and 1ml of distilled H$_2$O. After 30 mins. Of incubation, read the absorbance at 415nm in Spectronic 20.

**Antioxidant Activity Determination**

**DPPH radical scavenging assay**

The antioxidant activity of CB seed AgNPs was measured as described by (Brand-Williams et al., 1995). Absorbance was measured at 517nm after incubation for 30 minutes in the dark using a UV-Visible spectrophotometer. The following formula was applied for calculating inhibition percentage,

\[
\% \text{Inhibition} = \left( \frac{\text{Control} - \text{Sample}}{\text{Control}} \right) \times 100
\]

**Phosphomolybdenum reduction assay**

The assay was performed according to the procedure described by (Prieto et al., 1999). Below pH 7 molybdenum is reduced by the CB seed AgNPs and results in the development of green phosphomolybdenum complex. The absorbance was measured at 695nm. Ascorbic acid was used as the standard. The antioxidant potential of CB seed AgNPs was indicated as an ascorbic acid equivalent.

**Ferric (Fe$^{3+}$) reducing power assay**

The assay using CB seed AgNPs was performed with slight modifications of the method described (Barros et al., 2007). The absorbance was measured at 700nm along with ascorbic acid, which is the standard reference.

**Metal chelating assay**

250 μL (100μg) of CB seed AgNPs was added to a solution of 2 mmol/L FeCl$_2$ (0.05 ml). The reaction
was initiated by the addition of 5 mmol/l ferrozine (0.2 mL), and the mixture was shaken vigorously and left for 10 minutes at room temperature (Dinis et al., 1994). The absorbance of the solution was then measured at 562 nm. Metal chelating activity by the nanoparticle was calculated by comparing it with standard EDTA. The results were mentioned as 1 mg EDTA proportionate to 1 g CB seed AgNPs.

\[
\text{Percentage inhibition} = 100 \times \left( 1 - \frac{V_t}{V_c} \right)
\]

Hydroxyl Radical Scavenging Activity Assay (HRSA)

To various concentrations of silver nanoparticle of Caesalpinia bonducella seed AgNPs 1 mL Fe · EDTA solution, 0.5 mL EDTA solution, 1 mL 10% DMSO and 0.5 mL of 0.22% Ascorbic acid were added (Klein et al., 1981). The solution was placed for 15 minutes in a boiling water bath of 80 to 90°C. 1 mL of ice-cold TCA and 3 mL of Nash reagent were then added, and the reaction mixture was left at room temperature for 15 min. The absorbance was read at 412 nm. The HRSA was calculated as follows:

\[
\% \text{HRSA} = \left( 1 - \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100
\]

Scavenging of Hydrogen peroxide

H₂O₂ scavenging assay by CB AgNPs was determined using the method described by (Jayaprakasha et al., 2004). To different concentrations of silver nanoparticles of Caesalpinia bonducella, seed extract added 2 mL of hydrogen peroxide solution in phosphate-buffered saline (pH 7.4). The absorbance was read at 230 nm after an incubation of 10 minutes. Compared with standard Ascorbic acid.

Anti-inflammatory bioassay in vitro

To varying concentrations of CB seed, AgNPs extract added 0.2 mL of egg albumin, 2.8 mL of phosphate-buffered saline (pH 6.4). After incubation at 37°C in a BOD incubator for 15 minutes, the mixture was heated to 70°C for 5 minutes (Chandra et al., 2012). Cooled and the absorbance was read 660 nm against blank (Double distilled water with reagents) was used as The experiment was carried out similarly using the reference drug diclofenac.

RESULTS AND DISCUSSION

In recent years medicinal plants have gained importance for their limited toxicity and for their efficient antioxidant property. In vitro techniques provide the tool for the detection of antioxidants present in the silver nanoparticle, which are established on the basis of the phyto compounds’ ability to eliminate or scavenge free radicals (Mohanta et al., 2016). Antioxidant efficiency of medicinal plants is due to the redox potential of phytochemicals, which can play an efficient role in abolishing singlet and triplet oxygen, thereby resulting in peroxide decomposition or free radicals neutralization. This antioxidant efficacy from the extract adsorbs onto the surface of the nanoparticles, enhancing its redox potential (Patil and Kumbhar, 2017). Antioxidants hold the capacity to minimize the oxidative damage precisely via counteracting with free radicals or indirectly by impeding the enzymes that produce free radicals. Free radicals may lead to inflammation, which as an early protective homeostatic immune response to tissue damage. Thus, the production of ROS plays a key role in many inflammatory diseases (Mittal et al., 2014). In our study, phytoconstituents mainly flavonoid and phenol of Caesalpinia bonducella seeds silver nanoparticles were quantified and screening of their antioxidant, anti-inflammatory potential performed.

Total phenolic and flavonoid content

The total phenol components of the aqueous and CB seed AgNp extracts using Folin-Ciocalteau’s reagent were detected to be 115.08 ± 0.64 and 120.15 ± 0.30 mg GAE/g, and it is the measure that corresponds to gallic acid. The total flavonoids estimation of each extract carried out using aluminum chloride reagent and quercetin as standard, and the results revealed that the CB seed AgNPs possessed 168.86 ± 0.33 while aqueous extract contains 154.28 ± 0.21 mg QE/g respectively, the total flavonoid content of the extracts were calculated in terms of quercetin equivalent. Results of phytoconstituents quantification proves the fact that besides acting as good reductants and stabilizers for Ag ions they play an important role in scavenging free radicals or ROS and modulating the extent of the inflammatory response. Similar relationship was also observed by (El-Hela et al., 2017) between the phytochemicals and antioxidant activity in Crataegus sinaica leaves. Flavonoids are the classes of polyphenols that protect nucleic acids, proteins, and lipids from oxidative destruction. There are many reports in the literature which shows the relation between flavonoids content and antioxidant activity (Firoozi et al., 2016; Shukla and Mehta, 2017). The high...
flavonoid content in CB seed AgNPs attributes to the higher antioxidant ability Table 1.

DPPH radical scavenging assay

The DPPH radical scavenging potency of different concentrations of CB seed AgNPs is summarized in Fig 1a. Among the five different concentrations used in the study (25-125 μg/ml), test solution of 125 μg/ml exhibited the highest scavenging activity 93.25% same as ascorbic acid at the same concentration showed 93.55%, which were very close to each other (Figure 1a). The IC₅₀ value of CB seed AgNPs and Ascorbic acid was 19.7 and 17.15μg. The variation in the antioxidant activity was due to the reason that AgNPs were enriched with the presence of different extent of flavonoids and phenols. The antioxidant potential, as evidenced by the DPPH assay of AgNPs (Figure 1a), could be due to the functional groups adhered to them, which were acquired from the CB seeds. Normally, the higher free radical scavenging activity and lower IC₅₀ values demonstrate a higher antioxidant activity. The observed less IC₅₀ value for DPPH assay supports the significance of CB seed AgNPs as a promising source of antioxidants. Our results are in agreement with the investigation of (Shukla and Mehta, 2017), who reported similarly that aqueous extract of CB seeds exhibited increased DPPH radical scavenging potency in a dose-dependent manner.

Phosphomolybdenum reduction assay

Figure 1b presents the total antioxidant capacity obtained through the phosphomolybdenum assay of CB seed AgNPs in comparison with that of Ascorbic acid. The CB seed AgNPs exhibited powerful antioxidant activity with the absorbance of 0.35 at 400 μg nearing to that of the standard ascorbic acid, which showed the absorbance of 0.35 at the same concentration of AgNPs. CB seed AgNPs revealed a satisfying total antioxidant activity (Figure 2a), better than that of the standard reference ascorbic acid. It has been anticipated that the bioactivity of CB seed AgNPs is due to their respective phenolics and flavonoid contents. A similar result was also observed in Alpinia katsumadai seed AgNPs (He et al., 2017).

Ferric (Fe³⁺) reducing power assay

From the results of FRAP (Figure 1c), the antioxidants in the CB seed AgNPs would bring about the reduction of Fe³⁺ to Fe²⁺, which therefore correlates with the decrease in the generation of reactive hydroxyl species. CB seed AgNPs revealed a good reducing capacity with the absorbance of 0.977 at 100 μg/ml, which is bordering to the value of standard ascorbic acid. The promising antioxidant activity of a compound is evidenced by its reducing power. The reduction of Fe³⁺ was increased consistently with increasing concentration of AgNPs. Notably, the CB seed AgNPs exhibited better reducing power than the standard Ascorbic acid as the extract was enriched with phytoconstituents. Still, the phytoconstituents like phenols also possess electron-donating antioxidant capacity (Qidwai et al., 2018). This result is in agreement with the attempt of (Dipankar et al., 2012), who reported similarly in Caesalpinia bonducella leaves AgNPs.

Metal Chelating assay

The metal ion chelating efficacy of the CB seed AgNPs was assayed and reported in Figure 2a. The decolorization of the color (red) relies on the reduction of ferrous ions by the CB seed nanoparticle. The results were indicated as mg EDTA eq./g. Compared to ascorbic acid, the nanoparticle showed a little lower activity of 88.36 mg EDTA/μg extract. Evidence state that ROS formation is accelerated by metals such as iron and copper. The origination of ROS associated with redox-active metal catalysis could be circumvented by chelating the metal ions (Kurutas, 2016). Antioxidant potential transferred to the silver nanoparticles by the aqueous CB seed extract forms an integrated complex with the metal ions that best describes its chelating activity and hinders the electron transfer. Thus oxidation of cellular metabolic reaction is seized, resulting in the absence of free radicals production.

Hydroxyl Radical Scavenging Activity Assay

CB seed silver nanoparticles exhibited dynamic inhibition against hydroxyl radical (Figure 2b), which was similar to Ascorbic acid. This result proved that silver nanoparticles synthesized from seed extract of CB are highly influential in neutralizing hydroxyl radicals. The majority of the hydroxyl radicals were scavenged by the CB seed AgNPs. IC₅₀ determined for Ascorbic acid and CB AgNPs were 249.92 and 261.45μg/ml. Result outcomes were found statistically satisfactory (P < 0.05). The hydroxyl radical is presumably the ultimate dangerous element governing the free radical-induced tissue damage. Hydroxyl radical reacts with most of the molecules found in living cells, such as phospholipids and nucleotides (Hochstein and Atallah, 1988). The hydroxyl radical scavenging activity of CB seed AgNPs predicted in comparison with that of vitamin C (Figure 2b) showed that the synthesized nanoparticles are remarkably potent in neutralizing hydroxyl radicals.

Scavenging of Hydrogen peroxide

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Table 1: Total phenol (mg GAE/ g), flavonoid (mg QE/ g) content of aqueous CB seed, and silver nano particle extract of Caesalpinia bonducella seeds.

| Extract            | Total phenol | Total flavonoid |
|--------------------|--------------|-----------------|
| CB seed            | 115.08 ± 0.64| 154.28 ± 0.21   |
| CB seed AgNPs      | 120.15 ± 0.30| 168.86 ± 0.33   |

*Data represented by means ± standard error of triplicate experiments.

The results of H$_2$O$_2$ scavenging reported that the synthesized CB seed AgNPs possessed reliable hydrogen peroxide scavenging potential. H$_2$O$_2$ scavenging of CB seed AgNPs was examined to be in the range of 38% to 80%, which was closely related to that Ascorbic acid (Figure 2c). The IC$_{50}$ values for CB seed AgNPs and Ascorbic acid were recorded to be 167.94 and 157.76. The data also suggests that the CB seeds AgNPs were efficient in scavenging H$_2$O$_2$ correlative with the standard Vitamin C.

The formation of hydrogen peroxide in vivo occurs by the reactions catalysed by superoxide dismutase. H$_2$O$_2$ possesses the potential of crossing the membrane and can damage a number of compounds as a result of its oxidizing ability. Hydrogen peroxide targets many cellular energy-producing systems. Excess H$_2$O$_2$ leads to metabolic perturbations (Hyslop et al., 1988). Thus, Dose-dependent hydrogen peroxide scavenging activity reveals that CB seed AgNPs are effectual free radical scavengers, acting probably as an essential antioxidant.

**Inhibition effect of AgNPs on egg albumin denaturation**

The potent anti-inflammatory strategy is the ability of any drug to prevent the denaturation of proteins during inflammatory disease conditions. The results of this assay indicated that the synthesized CB seed AgNPs retained significant anti-
Figure 2: Free radical scavenging activity. (a) Metal Chelating assay (b) Hydroxyl Radical Scavenging Activity Assay (c) Scavenging of Hydrogen peroxide

Table 2: Influence of CB seed silver nano particles and Diclofenac against protein denaturation

| Sample    | Concentration (µg/mL) | % inhibition | IC50 (mg/mL) |
|-----------|-----------------------|--------------|--------------|
| Control   | -                     | -            | -            |
| CBS AgNps | 50                    | 49.06        | 71.3         |
| CBS AgNps | 100                   | 52.09        |              |
| CBS AgNps | 150                   | 61.33        |              |
| CBS AgNps | 200                   | 68.29        |              |
| CBS AgNps | 250                   | 78.56        |              |
| DF        | 50                    | 50.23        | 51.87        |
| DF        | 100                   | 55.21        |              |
| DF        | 150                   | 68.25        |              |
| DF        | 200                   | 77.26        |              |
| DF        | 250                   | 80.69        |              |
inflammatory potential. The highest inhibition was observed to be in the range of 78.56% and 80.69% for nanoparticle and Diclofenac, respectively (Table 2). Also, the IC50 values for the CB AgNPs and Diclofenac were recorded to be 71.3 and 51.87. This evidence also suggests that the CB seed AgNPs were efficacious in inhibiting albumin denaturation and was much equally comparative with Diclofenac. ROS plays a crucial role in inflammatory disease progression. Inflammation is a defensive immune response against pathogens that may cause denaturation of tissue proteins, thus producing autoantigens in certain arthritic disease (Nasri and Rafieian-Kopaei, 2013). Targeting protein denaturation with the natural plant-derived nanoparticles, therefore, would serve as a potent anti-inflammatory agent. CB seed AgNPs exhibited a marked in vitro anti-inflammatory effect by inhibiting the protein denaturation, and our results were evidenced by previous studies (Gnanasundaram and Balakrishnan, 2017; Kedi et al., 2018).

**CONCLUSION**

Thus, the green synthesized silver nanoparticles from *C. bonducella* seed extract act as a potent free radical scavenger and exhibited excellent anti-inflammatory potential, thereby establishing their therapeutic significance. It is a promising and great finding that green bio-synthesized AgNPs capped with CB seed exhibited significant potential, which was much more remarkable than using this seed extract alone. This can be a promising future therapeutic agent for ROS and inflammation-mediated diseases such as cancer, Alzheimer’s, ischemic injury, and rheumatoid arthritis.

**Conflict of interest statement**

The authors declare that they have no conflict of interest.

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