



## ***In vitro* antioxidant, total phenolic, total flavonoid content and biosynthesis of silver nanoparticles using *Cassia auriculata* leaves extracts**

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### **Abstract**

Biosynthesis of nanoparticles is an emerging field of nanotechnology, select over physical, chemical and biological synthesis due to their protection, cost-effectiveness nature, bio-compatibility, environmentally and scalable properties. In the present study was carry out biosynthesis of silver nanoparticles using leaves extract of *Cassia auriculata* and also antioxidant activity, total phenolic, total flavonoids content were analyzed. AgNP and leaf extracts prepared individually in different solvents such as ethanol, ethyl acetate, hexane and aqueous were evaluated for their bio-efficacies. The free radical scavenging activity, total antioxidant, total phenols and flavonoids in AgNPs containing leaves extract were 82.96 (Scavenging activity %), 240.68( $\mu\text{g}$  AA/mL), 250.14 ( $\mu\text{g}$  GAE/g extract), and 890.56 ( $\mu\text{g}$  QE/g extract) respectively. Biosynthesized AgNPs showed a more activity for increased concentration of leaves extract. It was concluded that *C. auriculata* leaves extracts can be extensively used in the production of capacity antioxidant activity.

**Keywords:** *C. auriculata*, AgNPs, total phenolic, total flavonoids, antioxidant

### **Introduction**

Nanotechnology is one of the most important and attracting in this era, growing day by day, influencing various aspects of human life (Roco and Bainbridge, 2005) [1]. Past few decades, nano scale particles have been attracting more and more attention owing to their compatibility in the field of pharmaceuticals, biology, materials science and electronics at nanoscale level (Yokoyama & Welchons, 2007) [2]. Even though accessible in many ways, the synthesis of nanoparticles is important with a biological approach (Kaushik *et al.*, 2010) [3]. In the recent situation, the biosynthesis of nanoparticles mediated plant extracts is much simpler than the chemical synthesis method and can be used for many ameliorate treatments (Mittal *et al.*, 2012) [4]. Moreover, the synthesis of nanoparticles using plant extract reduction and covering agents is more beneficial than the microbial synthesis. Recently, the collection of NPs using living agents (fungicides, bacteria and plant extracts) is preferred over conventional methods because of their simpler process, stability, rapidness, high drug loading capacity, non-pathogenic, low cost and eco-friendliness (Boomi *et al.*, 2019) [5].

Presently, the plant-mediated green synthesis of silver nanoparticles has grown into a new and key branch of nanotechnology. Since it is environmentally friendly and cost effective, it is less toxic when associated with chemical hazards, so it has emerged and gained prominence. In the physical and chemical nanoparticles synthesis, the green synthesis has many benefits (Chanel *et al.*, 2017) [6]. In the physical and chemical nanoparticles synthesis, the green synthesis has many benefits. For example (i) low toxic and hazardous substances and environmentally friendly solvents, (ii) simple, fast and inexpensive, (iii) uses low energy and operates under moderate operating conditions, (iv) combines the energy of silver nanoparticles and plant active ingredients. In this respect, plant-mediated silver nanoparticles are reported to contain higher biological

functions than chemically synthesized nanoparticles (Choudhury *et al.*, 2016) [7].

Previous studies have suggested the use of various plant parts such as leaf, stem, bark, fruit, bud, wood, and root, for the synthesis of silver nanoparticles. Plant-mediated synthesis of silver nanoparticles is preferred over microbe-mediated synthesis. The latter is not feasible and requires more aseptic conditions, a time-consuming process and a longer incubation period. Furthermore, the reduction properties of secondary metabolites of plants are attributed to the more ability of plant extracts to synthesize nanoparticles with enhanced properties (Kharissova *et al.*, 2013) [8].

Free radical plays an important role in oxygen based (aerobic) living systems. They are part of cell respiration and other key cellular processes, but are also involved in aging and disease development (Harrman, 1992; Bagchi and Puris, 1994) [9-10]. Free radicals are unstable molecules with free unconnected electrons. They are very reactive because free electrons constantly bind to other electrons to form covalent pairs. In this activity, free radicals eliminate the electrons from other molecules. Beyond affecting the regulation of cells, it can also injury molecules known as carbohydrates, fats, proteins and nucleic acids (Young and Woodside, 2001; Valko *et al.*, 2007) [11-12]. There are various sources of free radicals within the cell and its environment. In aerobic organisms, free radicals are developed during normal metabolic processes (Mohammed and Ibrahim, 2004) [13]. Key sources include electron transfer in the plasma membrane and cell respiration in the mitochondrial membrane. Mitochondria are the main source of antioxidant damage because free radicals such as superoxide can run away from the electron transport chain (Shigenaga *et al.*, 1994; Liu *et al.*, 1999) [14-15]. To exempt intracellular damage by free radicals, cells have formed an endogenous antioxidant system. This system converts free electrons into non-reactive form by proteins.

Antioxidants are very important for the protection of cells and biological macromolecules from degenerative reactions produced by free radicals and reactive oxygen species (Hermans *et al.*, 2007) [16]. Antioxidant properties of different plant products, i.e. polyphenolic substances (e.g. flavonoids and tannins) derived from various plant and herbal extracts (Agati *et al.*, 2012; Dajas, 2012) [17-18] and Elumbotti, Sweet Potato, *Datura*, *Sesuvium portulacastrum*, Green tea, Tuber extract (sweet potato), (Sivakumar and Gajalakshmi, 2013; Sivakumar *et al.*, 2010; Sivakumar and Panneerselvam, 2011; Anburaj *et al.*, 2012; Senthilkumar and Sivakumar, 2014; Sivakumar *et al.*, 2015) [19-24].

*Cassia auriculata* comes under the family Leguminosae and subfamily of Fabaceae and is broadly used as conventional medicine in India and Southeast Asia. It is a vital herb that is widely distributed in India, Vietnam, Sri Lanka, Indonesia, Malaysia, Australia, and Japan tropical Africa and the southwestern parts of the People's Republic of China. *C. auriculata* is a key medicinal plant and is used in traditional medicine, Ayurvedic, homeopathic remedies and also used in the cure of various diseases such as eye complaints, stomach problems, heart disease, respiratory disorders, constipation, dyspepsia, hemorrhoids. *C. auriculata* plant extract has been reported to have antibacterial and antidiabetic activity (Parkas 2006; Thirumal Sivakumar, 2021) [25-26]. In the present research work, an attempt has been made to synthesize silver nanoparticles and characterization of silver nanoparticles using the leaf extract of *Cassia auriculata*. The synthesized silver nanoparticles were analyzed for their *in vitro* antioxidant, total phenolic, total flavonoids content of leaves extracts of *Cassia auriculata* (L).

## Materials and Methods

### Collection and synthesis of silver nanoparticles

*Cassia auriculata* (L.). leaves were collected from Elusempon, Villupuram District, Tamil Nadu, India. The collected leaves were separated and thoroughly washed with sterile double distilled water and dried under room temperature then grind with the help of mortar and pestle. The leaves were grind until it reaches to a paste form and then the leaves extracts was filtered with Whatmann no.1 filter paper and filtrate saved. The silver nanoparticles were synthesized by adding 90ml of 0.1mM aqueous AgNO<sub>3</sub> silver nitrate solution and mixed with 10ml of *Cassia auriculata* leaves extracts. The synthesis was carried out in a dark condition to minimize the photo activation of silver nitrate. The development of silver nanoparticles was indicated by the color change. After the synthesis, the reaction mixture was shaken thoroughly and kept into the oven for one week. The pellets containing silver nanoparticles was collected and dried at room temperature and also dried powder was used for further experiments.

### Antioxidant assay

#### DPPH radical scavenging assay

The antioxidant activity, the free radical scavenging of DPPH (2,2-diphenyl 1-picrylhydrazyl) was analyzed using a modified method followed by MacDonald-Wicks *et al.*, (2006) [27]. The reaction mixture was prepared by adding 50µL of various concentrations of leaves solvents extract, combining AgNPs with 75µL of methanol and then adding 100µL of 0.1mm concentration to the DPPH solution. The mixture was stirred for 10 min and then incubated for 25

min at room temperature. Absorption was then recorded using a spectrophotometer at 517nm and taken as standard compared to vitamin C (ascorbic acid). The experiments were repeated three times. The antioxidant contents of leaves extracts prepared in various solvents and AgNPs was calculated as the percentage reduction of DPPH discoloration using the following equation:

$$\text{Percent Inhibition} = 100 \times 1 - (A_{\text{sample}} / A_{\text{blank}}),$$

where,  $A_{\text{blank}}$  is absorbance of the solution when nothing was added;  $A_{\text{sample}}$  is absorbance of the solution when extract or AgNPs was added

### Total antioxidant activity

Total antioxidant capacity (TAC), phosphomolybdenum was analyzed according to the technique followed by Eddine *et al.*, (2016) [28]. One mg of various solvents was dissolved in leaves extracts AgNPs in 10µL DMSO (dimethyl sulfoxide) and volume was made up to 1mL using methanol. 1mg/mL ascorbic acid solution was used as positive control. A stock solution of ascorbic acid (1mg/mL) was prepared using methanol. In the reaction mixture, 100µL test samples was added to the test tube containing 1mL of freshly prepared Phosphomolybdate reagent solution (28mM sodium dihydrogen orthophosphate, 0.6mM sulphuric acid, and 4mM ammonium molybdate tetrahydrate) and shaken vigorously and test tubes were covered with an aluminum foil and incubated in a water bath at 90°C for 2h. After incubation, the test tubes were allowed to cool to room temperature and reading was measured at 695nm using a UV-Vis spectrophotometer and methanol was taken as a negative control.

### Total phenolic content determination

Total phenolic content (TPC), the Folin-Ciocalteu evaluation was followed by slight modification protocol as suggested by Slinkard and Singleton, (1977) [29]. Test samples of 100µL aliquots were taken and total volumes were made up to 3mL using distilled water. Under dark conditions, 0.5 mL of Folin Ciocalteu reagent was mixed to the reactive sample, mixed well and allowed to stand for 3 min. 2mL of 20% (w/v) Na<sub>2</sub>CO<sub>3</sub> (anhydrous) solution was added and well mixed. The reaction sample was later incubated in the dark for 60min. For the blank, the reaction compound was taken with methanol instead of the test samples. Absorbance was recorded at 650nm using a UV-Vis spectrophotometer and total phenolic content was expressed as µg of gallic acid (GA)/mg of dry extract (µgGA/mg).

### Total flavonoid content determination

Total flavonoid content determination (TFC) of each leaves extract prepared separately in different solvents (ethanol, ethyl acetate, hexane and methanol) compared to AgNPs was assessed using aluminum chloride using colorimetric method and Quercetin was used as standard solution. In a reaction mixture, 125µL of each test sample was added to a test tube containing 37.5µL of NaNO<sub>2</sub> (5% w / v) solution. The mixture was then stirred well and incubated at room temperature for 7 min. After incubation, 75µL of AlCl<sub>3</sub>(10% w/v) mixture was added, followed by 250µL of NaOH(1N). The total volume of the reaction mixture was added to 2.5 mL of distilled water. Absorption of the reaction compound was recorded at 510nm using a UV-Vis spectrophotometer

and the results were expressed as quercetin equivalent (QE) /mg fresh extract ( $\mu\text{gQE} / \text{mg}$ ).

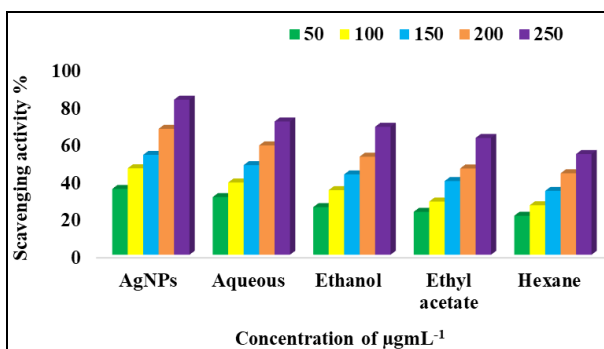
### Statistical analysis

Statistical analysis was performed using SPSS/20 software. The experimental data were expressed as mean $\pm$ standard deviation and were obtained from biological and technical three replicates of each experiment.

## Results and Discussion

### Antioxidant potential

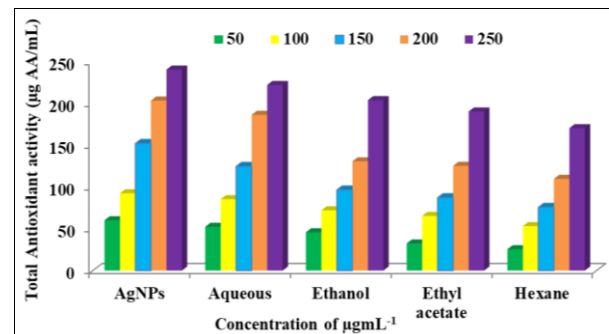
Present research DPPH and phosphomolybdenum studies were carried out to study the antioxidant capacity of AgNPs compared to crude extract prepared separately in various solvents. In DPPH methods, the nitrogen atom of DPPH contains the odd electron, which is reduced by accepting hydrogen ion and free electrons from the antioxidant compounds before converting the purple colored DPPH into the yellow colored hydrazine molecule. The DPPH free radical scavenging activities relative to the size of the test samples expressed in percentage were recorded as depict in (Figure. 1). The results exhibited a higher in scavenging activity by increasing the concentration of the samples. In various treatments, AgNPs exhibited the increased antioxidant activity (82.96%), followed by aqueous, ethanol, ethyl acetate and hexane extract 71.29, 68.45, 62.49 and 53.86% scavenging activity at 1mg/mL, dose of test samples, respectively. Present results combined the intense scavenging activity of AgNPs synthesized using *C. auriculata* leaves extract. The powerful active scavenging activity of AgNPs may be due to the presence of significant amounts of phenols, flavonoids and alkaloids in the leaves extract, which may play an vital role as capping and stabilizing agents. The same results, (Abel-Aziz *et al.*, 2014; Senthilkumar *et al.*, 2017; Jothi *et al.*, 2019; Sivakumar, 2019; Angelin *et al.*, 2020; Thiyagarajan and Sivakumar, 2020; Sivakumar, 2021) [30-36], showed higher antioxidant activity of leaves extract containing AgNPs.



**Fig 1:** Free radical Scavenging capacity of different *C. auriculata* leaves extracts and AgNPs according to phosphomolybdate assay, expressed as  $\mu\text{g/mL}$  of ascorbic acid (AA).

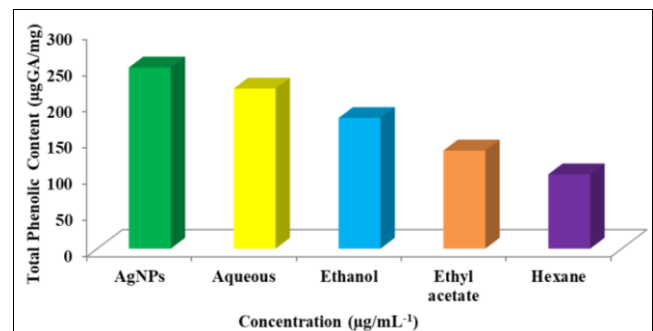
Furthermore, AgNPs and plant extracts produced in various solvents were assessed for total antioxidant capacity (TAC) using a phosphomolybdate evaluation. In this evaluation, molybdenum (VI) is decrease to molybdenum (V) when the incident of antioxidants (reducing agents). The results revealed improved antioxidant capacity of AgNPs ( $240.68\mu\text{g} / \text{mL}$ ) compared to plant crude extracts, followed by aqueous extract, ethanol, ethyl acetate and hexane extract of 222.32, 204.12, 190.53 and  $170.59 \mu\text{g/mL}$ , of AA at 1mg/mL concentrations of samples, respectively. Figure. 2.

depicted to the dose-dependent higher in the total antioxidant activity of the exposed samples. Related to our results, Nakala and Sadras (2016) [37], exhibited higher antioxidant activity of AgNPs synthesized using *P. longum* fruit extract. Present reports indicate that the combination of phenols, alkaloids, and flavonoid compounds may have enhanced antioxidant activity of AgNPs. Present reports show that phenols, alkaloids and flavonoid phytochemicals may have increased antioxidant activity of AgNPs due to the diffusion of plant extracts into the metal (Phull *et al.*, 2016) [38]. In this regard, the total phenolic and flavonoid content of AgNPs and plant extracts equipped into different solvents were evaluated.



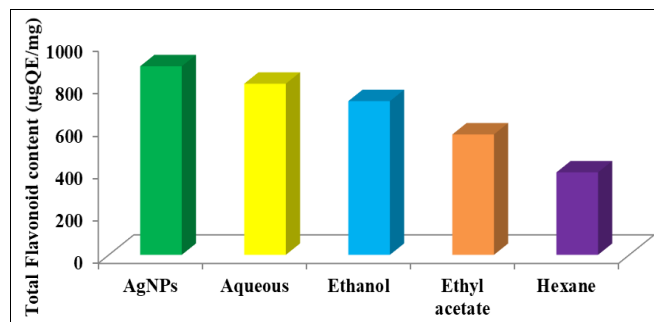
**Fig 2:** Total antioxidant capacity of various *C. auriculata* leaves extracts and AgNPs according to phosphomolybdate assay, expressed as  $\mu\text{g/mL}$  of ascorbic acid (AA).

The TPC ( $250.14\mu\text{g GA/mg}$  fresh extract) of the synthesized AgNPs was found to be the increased, followed by aqueous extract, ethanol, ethyl acetate and hexane extract 221.03, 180.36, 135.45 and  $102.59\mu\text{g GA/mg}$  respectively. The total phenolic content of AgNPs and the leaves extract formulated in different solvents are shown in Figure 3.



**Fig 3:** Total phenolic content of *C. auriculata* leaves extract prepared in various solvents and AgNPs expressed as  $\mu\text{g}$  of gallic acid equivalent (GA)/mg of fresh extract.

The total flavonoid content (TFC) of AgNPs  $890.56 \mu\text{gQE/mg}$  of fresh extract was compared with the leaves extracts prepared separately in aqueous, ethanol extract, ethyl acetate and hexane extract containing 808.21, 725.89, 568.75 and  $389.54\mu\text{g QE/mg}$  of leaves extract, respectively, as showed in Figure.4. Same to our results (Pull *et al.*, 2016) [38] showed increased TPC and TFC values of AgNPs synthesized using *Bergenia ciliata* compared to crude extracts. From the above results, a strong correlation was found between TFC, TPC, TAC and DPPH values, resulting in higher phenolics and flavonoid content observed for their concluded that the presence of synthesized AgNPs was responsible for their notable antioxidant activity.



**Fig 4:** Total flavonoid content of extracts prepared in various solvents and AgNPs of *C. auriculata* leaves extract expressed as µg of quercetin equivalent (QE)/mg of fresh extract.

## Conclusions

The recent study examined that silver nanoparticles can be synthesized in a simple method using *C. auriculata* leaves extract. Total phenolic, total flavonoids, total antioxidant, antioxidant activity was higher in plant-AgNPs compared to the other solvents extracts. Plant biosynthesized nanoparticles exhibited a more *in vitro* antioxidant activity.

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