Antioxidant Activity of *Ocimum gratissimum* Stem Extract Mediated Silver Nanoparticles- An in Vitro Study

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Abstract: Silver nanoparticles made from plant extracts have drawn a lot of interest because of the potential uses for them in nanotechnology and medicine. *Ocimum gratissimum* was used since the ancient past for its antioxidant, antibacterial, anti-inflammatory, and immunomodulatory properties and it has long been employed in herbal therapy. *Ocimum gratissimum*'s stem extract was used in several research to synthesize silver nanoparticles and assess their antioxidant properties. The antioxidant capacity of the stem extract is influenced by the phytochemicals found in it, including phenolic substances, flavonoids, and tannins. These phytochemicals have the ability to stabilize the synthesized nanoparticles and serve as reducing agents. The DPPH radical scavenging activity and Hydroxyl radical scavenging activity was used to evaluate the antioxidant activity of the synthesized silver nanoparticles. The results of the conducted assays shows that the *Ocimum gratissimum* silver nanoparticles has shown to possess significant antioxidant activity and can be used as a therapeutic alternative to the commercial antioxidant drugs.

Keywords: *Ocimum gratissimum*, silver nanoparticles, antioxidant activity.

1. INTRODUCTION

Nanotechnology is a rapidly growing research field (T Singh et al 2017). Nanotechnology has the potential to detect and treat various diseases. Animals have been actively studied for the treatment and prevention of various infections using nanotechnology. It can improve the oral bioavailability of many antimicrobial drugs (Rajeshkumar S, Ezhilarasan D., 2022). Green synthesis method is an eco-friendly process which does not use toxic chemicals (S Rajeshkumar et al 2021). Large energy consumption, environmental pollution, health problems are some disadvantageous effects caused by chemical methods. Green synthesis uses plant extracts to reduce metal ions. Metal nanoparticles produced using green technologies are of even higher quality than those produced using chemical processes (Z Guan et al 2022)

Silver nanoparticles contribute to various applications such as ointment, nanomedicine, data storage, cell biology, cosmetics, textiles, etc,. Silver nanoparticles play a major role in disinfectants, antimicrobial agents (S Ahmad et al 2019). Lycopene with silver nanoparticles showed a better percentage of inhibition in which they analyzed antioxidant and anti-inflammatory assays (MV Chaithanya et al 2021). *Ocimum gratissimum* is also known as african basil, clove basil. *Ocimum gratissimum* belongs to the family of Lamiaceae. It helps in acne and infections (P Eshwar et al 2016). *Ocimum gratissimum* is used as an ingredient in traditional medicine preparations. After meals, the leaves of *Ocimum*

gratissimum were chewed to aid digestion. This plant is used in the treatment of fever, diarrhea, and infections. *Ocimum gratissimum* acts as flavoring agent (N Ontao et al 2021).

2. MATERIALS AND METHODS

2.1. COLLECTION OF STEM EXTRACT

The plant *Ocimum gratissimum* is grown and harvested at Saveetha Herbal Garden. At that time, stems were collected and kept under shadow for 10 days for drying. And it is stored for further research. Then the dried stem is crushed using a mixer grinder. Later, the stem extract is collected.

2.2. PREPARATION OF STEM EXTRACT

1g of *Ocimum gratissimum* stem extract was used in the study. 1g of *Ocimum gratissimum* stem extract was dissolved in 100mL of distilled water. Using a heating mantle, boil the *Ocimum gratissimum* stem extract solution for 15 to 20 minutes at 60°C to 70°C. The plant extract was filtered using filtering cloth and used for the synthesis of silver nanoparticles.

2.3. SYNTHESIS OF SILVER NANOPARTICLES

1 mM of silver nitrate was dissolved in 80mL of distilled water in the conical flask to make a silver nitrate solution. 20 mL of *O. gratissimum* extract was added to the silver nitrate solution.. Then the conical flask containing the silver nitrate solution is covered with foil and kept in an orbital shaker for 1 hour at 110 rpm. After, add 20mL of filtered stem extract solution to the 80 mL silver nitrate solution present in the conical flask. After 48 hours, the silver nitrate solution was centrifuged at 8000 RPM for 10 minutes. The pellet was collected and kept in the centrifuge for further use.

2.4. Antioxidant activity

2.4.1. DPPH assay

A stock solution of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared in methanol. For each assay, a fresh working solution was prepared by diluting the stock solution to a final concentration of 20 µM in methanol. Different concentrations (10,20,30,40,50 µg/mL) of the *O. gratissimum* mediated silver nanoparticles was added to 200µL of the DPPH working solution in a 96-well plate. The plate was incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm using a microplate reader. Methanol was used as a blank. The percentage of DPPH scavenging activity was calculated using the following formula:

% DPPH Scavenging Activity = [(Acontrol – Asample) / Acontrol] × 100

Where Acontrol is the absorbance of the control (DPPH solution without the sample), and Asample is the absorbance of the sample (DPPH solution with the green synthesized silver nanoparticles).The positive control group consisted of ascorbic acid (1 mg/mL).

2.4.2. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging assay was used in this study to evaluate the antioxidant activity using the method proposed by Halliwell et al. 1 mL of reaction mixture with 100 µL of 28mM of 2-deoxy-2-ribose was prepared. To that, various concentrations of *O. gratissimum* mediated silver nanoparticles (10-50 µg/mL) were added. Along with that, 200 µL of 200 µm ferric chloride, 200 µL of EDTA, 100 µL ascorbic acid was added. Then it was incubated for 1 h at 37 °C and the optical density was measured at 532 nm against the blank solution. Vitamin E was used as a positive control.

Hydroxyl radical scavenging activity (%) = [(ABlank − Asample)/Ablank] × 100

Where Ablank is the absorbance of the control reaction (without sample), and Asample is the absorbance of the reaction with the sample.

3. RESULTS

Figure 1: Boiling of *Ocimum gratissimum* stem extract using heating mantle.

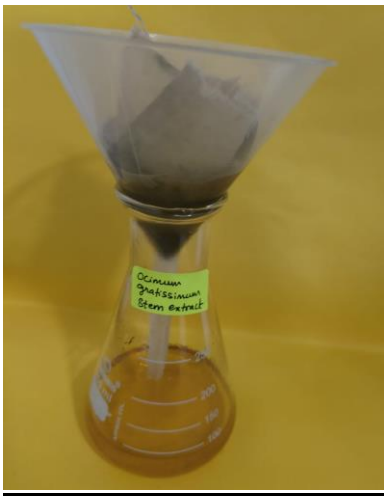


Figure 2: Filtering of *Ocimum gratissimum* stem extract using funnel and filtering cloth

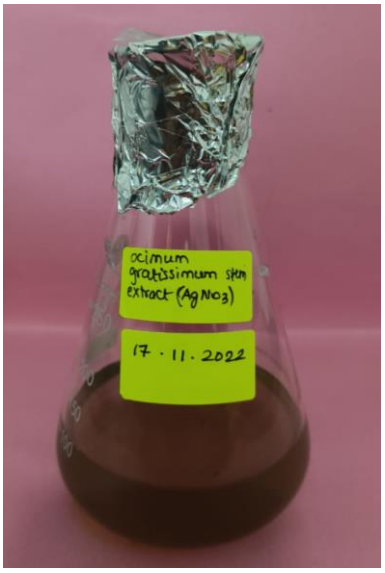
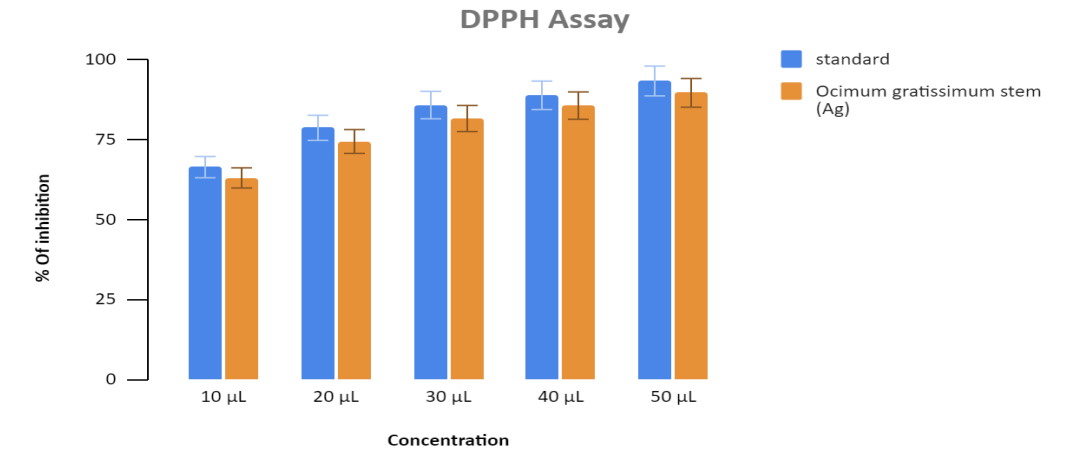
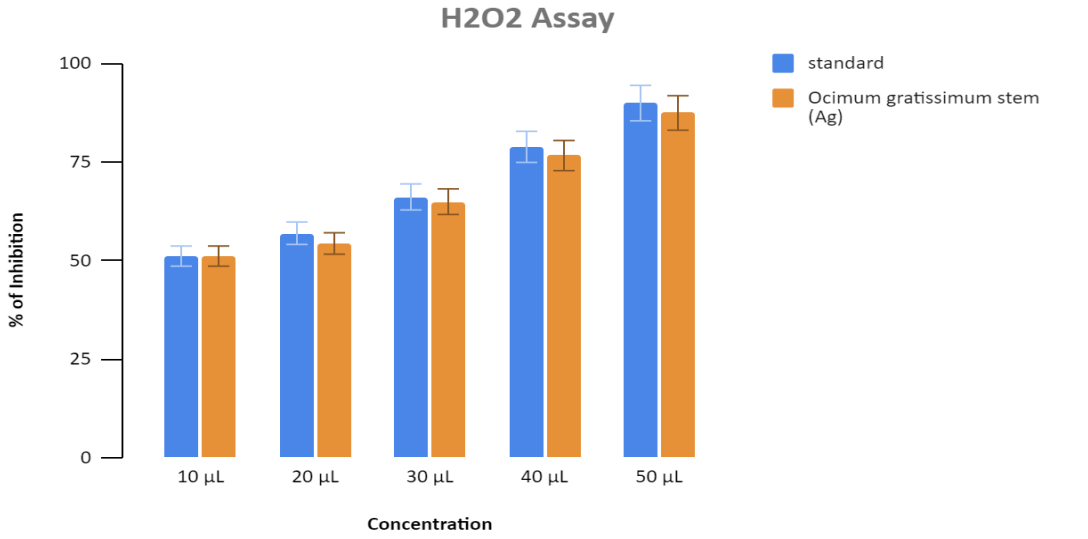


Figure 3: Silver nanoparticles synthesized using *Ocimum gratissimum* stem extract.



(a)



(b)

Figure 4: Antioxidant activity of the *O. gratissimum* mediated silver nanoparticles (a) DPPH radical scavenging assay (b) Hydroxyl radical scavenging assay.

The DPPH and Hydroxyl radical scavenging assay was used to evaluate the free radical scavenging activity of *O. grattisimum* mediated silver nanoparticles. In a DPPH assay, stem extract of *O. grattisimum* mediated silver nanoparticles shows 85% and 60% inhibition at 50µL and 10µL concentration. In a H2O2 assay, stem extract of *O. grattisimum* shows 80% and 50% inhibition at 50µL and 10µL concentration. So when compared to Hydroxyl radical scavenging assay, DPPH assay shows more scavenging activity of stem extract of *O. grattisimum* mediated silver nanoparticles. Previous study that looked at the *O. grattisimum* methanol extract's antioxidant properties discovered that it was capable of considerably lowering oxidative stress in rats (Oyem JC et al., 2021). Similarly, a study conducted using ethanol extract of *O. grattisimum* has strong antioxidant activity in vitro, as it could scavenge free radicals and reduce lipid peroxidation (Ugbogu OC et al., 2021).

Understanding the precise mechanisms of action might offer useful insights into their prospective uses and aid in the creation of specialized antioxidant treatments. To assess the antioxidant activity of AgNPs mediated by *Ocimum gratissimum*, it was planned to carry out thorough in vitro and in vivo experiments in near future. In the previous study, the antioxidant efficacy of lycopene infused with silver nanoparticles was about more than 90%. Spectrophotometry analysis of antioxidant assay revealed that 50 µL of the herbal formulation with silver nanoparticles had the highest

percentage of inhibition about 93.15% (Chaithanya MV et al., 2021). In this study, the silver nanoparticles display comparatively lower antioxidant activity (90%).

4. CONCLUSION

The present study shows that the antioxidant activity of the silver nanoparticles synthesized using *Ocimum grattisimum* has shown to possess potential antioxidant activity. But when compared to the standard, it shows slightly lower antioxidant activity. These findings show that the green synthesized silver nanoparticles can be used as an alternative to the commercial synthetic antioxidant drugs which might possess many side effects to the body.

5. REFERENCES

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DOI: https://doi.org/10.15379/ijmst.v10i5.3419