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Eco-friendly synthesis of Copper nanoparticles using *Leucas aspera* (L.) leaf extract with Antiviral activity against Influenza virus

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Abstract

The present study focuses on the eco-friendly synthesis of copper nanoparticles (CuNPs) using the leaf extract of *Leucas aspera*, a medicinal plant rich in bioactive phytochemicals with potential therapeutic properties. Green synthesis of nanoparticles has gained significant attention due to its cost-effectiveness, environmental safety, and avoidance of toxic chemicals commonly used in conventional physical and chemical synthesis methods.

In this study, fresh leaves of *Leucas aspera* were collected, thoroughly washed, shade-dried, and subjected to aqueous extraction. The prepared leaf extract was mixed with copper sulfate solution, resulting in a visible color change from light blue to dark brown, indicating the possible formation of copper nanoparticles.

The synthesized copper nanoparticles were characterized using UV–Visible spectroscopy. The absorbance spectrum recorded in the range of 300–800 nm exhibited a characteristic Surface Plasmon Resonance (SPR) peak between 560–600 nm, confirming the reduction of Cu^{2+} ions into metallic copper nanoparticles. Control samples containing only copper sulfate solution and plant extract did not show this characteristic peak, thereby validating nanoparticle formation.

The antiviral activity of the synthesized CuNPs was evaluated against the influenza virus. The results indicated that the biosynthesized copper nanoparticles exhibited significant antiviral potential, possibly due to their ability to interact with viral surface proteins and inhibit viral replication. The presence of phytochemical compounds from *Leucas aspera* may further enhance the antiviral efficiency of the nanoparticles.

The study demonstrates that *Leucas aspera* leaf extract can effectively mediate the green synthesis of copper nanoparticles with promising antiviral activity against influenza virus. This work highlights the importance of green nanotechnology in developing environmentally sustainable nanomaterials for biomedical and antiviral applications.

Keywords: Green synthesis, Copper nanoparticles, Leucas aspera, Antiviral activity, Influenza virus, UV–Visible spectroscopy, Surface Plasmon Resonance (SPR), Eco-friendly nanotechnology, biomedical applications.

1. Statement of the Problem

Influenza virus infections continue to pose a major global health challenge due to their rapid transmission, seasonal recurrence, and the emergence of drug-resistant viral strains. Although antiviral drugs are available, their effectiveness is often limited by resistance, side effects, and high cost. Therefore, there is an urgent need to develop safe, affordable, and environmentally sustainable antiviral agents.

Green nanotechnology has emerged as a promising approach for the synthesis of metal nanoparticles with biomedical applications. Leucas aspera is a medicinal plant rich in bioactive phytochemicals that can act as natural reducing and stabilising agents in nanoparticle synthesis. However, limited studies are available on the eco-friendly synthesis of copper nanoparticles using *Leucas aspera* leaf extract and their antiviral activity against influenza virus. Hence, the present study aims to develop green-synthesised copper nanoparticles and evaluate their antiviral potential against influenza virus infections.

2. Hypothesis

1. The leaf extract of Leucas aspera contains phytochemicals capable of reducing copper ions into stable copper nanoparticles through a green synthesis method.
2. The synthesised copper nanoparticles will exhibit significant antiviral activity against the influenza virus.
3. Green-synthesised copper nanoparticles will show better antiviral activity than the crude plant extract due to their nanoscale properties and enhanced surface activity.
4. The synthesis process will be eco-friendly, cost-effective, and free from toxic chemicals.

3. Aim

To synthesise copper nanoparticles using Leucas aspera leaf extract through an eco-friendly green synthesis method and evaluate their antiviral activity against influenza virus.

4. Objectives

1. To collect and prepare aqueous leaf extract of *Leucas aspera* for nanoparticle synthesis.
2. To synthesise copper nanoparticles using the leaf extract as a reducing and stabilising agent.
3. To confirm nanoparticle formation through colour change and UV–Visible spectroscopy.
4. To characterise the synthesised nanoparticles using FTIR and SEM analysis.
5. To evaluate the antiviral activity of copper nanoparticles against influenza virus.
6. To compare the antiviral activity of nanoparticles with the crude plant extract.
7. To assess the eco-friendly and economical nature of the green synthesis method.

5. Introduction

Influenza is a highly contagious respiratory disease caused mainly by Influenza A and Influenza B viruses. Seasonal influenza outbreaks occur globally and contribute to significant morbidity and mortality every year. According to the World Health Organization, influenza epidemics are responsible for millions of severe illnesses and hundreds of thousands of deaths annually (WHO, 2023). The continuous mutation of influenza viruses and the emergence of drug-resistant strains reduce the effectiveness of existing antiviral drugs such as Oseltamivir and Zanamivir (Cowling *et al.,* 2010). Therefore, the search for alternative, safe, effective, and eco-friendly antiviral agents has become an important area of biomedical research.

Nanotechnology has emerged as a promising field in modern medicine, particularly in the development of antimicrobial and antiviral agents. Metal nanoparticles such as silver, gold, zinc oxide, and copper nanoparticles possess unique physicochemical properties that enhance their biological activities (Rai et al., 2012). Among these, copper nanoparticles (CuNPs) have attracted considerable attention due to their strong antiviral and antimicrobial activities, low cost, and easy availability compared to noble metal nanoparticles. Copper nanoparticles can inhibit viral activity through several mechanisms, including the generation of reactive oxygen species (ROS), disruption of viral envelope proteins, damage to viral nucleic acids, and inhibition of viral replication (Ren et al., 2009; Lemire et al., 2013). Recent studies have demonstrated that copper-based nanomaterials effectively inactivate influenza viruses and other enveloped viruses, indicating their potential use as antiviral agents.

Conventional methods for synthesising nanoparticles involve physical and chemical approaches that often require high temperature, pressure, expensive equipment, and toxic reducing agents such as hydrazine and sodium borohydride. These methods may produce hazardous by-products and create environmental concerns. To overcome these limitations, green synthesis methods using biological materials have gained importance because they are environmentally friendly, cost-effective, and sustainable (Iravani, 2011).

Green synthesis of nanoparticles using plant extracts has become one of the most preferred biological approaches. In this method, phytochemicals present in plant extracts act as natural reducing and stabilising agents, converting metal ions into nanoparticles and preventing their aggregation. Plant-mediated nanoparticle synthesis is advantageous because it avoids toxic chemicals, requires simple laboratory conditions, and is suitable for large-scale production. Bioactive compounds such as flavonoids, phenolics, alkaloids, terpenoids, tannins, and proteins play an important role in nanoparticle synthesis and stabilisation (Mittal et al., 2013).

Leucas aspera, commonly known as “Thumbai,” belongs to the family Lamiaceae and is widely distributed in tropical and subtropical regions of India. The plant has been traditionally used in Ayurveda and folk medicine for the treatment of fever, cough, cold, inflammation, skin diseases, and respiratory disorders (Prajapati et al., 2010). Phytochemical investigations have revealed that *Leucas aspera* contains flavonoids, phenolic compounds, terpenoids, alkaloids, tannins, and essential oils possessing antioxidant, antimicrobial, anti-inflammatory, and antiviral activities. These phytochemicals can serve as effective reducing and capping agents during nanoparticle synthesis.

Although several studies have reported the green synthesis of metal nanoparticles using medicinal plants, limited research has been carried out on the eco-friendly synthesis of copper nanoparticles using *Leucas aspera* leaf extract and the evaluation of their antiviral activity against influenza virus. Therefore, the present study aims to synthesise copper nanoparticles using *Leucas aspera* leaf extract through a green synthesis approach and evaluate their antiviral potential against influenza virus infections.

5.1 Green Synthesis Using Other Plants (Supporting Studies)

Green synthesis of metal nanoparticles using medicinal plant extracts has become an important area of nanobiotechnology research due to its eco-friendly, cost-effective, and sustainable nature. Plant extracts contain various phytochemicals such as flavonoids, phenolics, alkaloids, terpenoids, and proteins that act as natural reducing and stabilising agents during nanoparticle synthesis (Iravani, 2011). Several medicinal plants have been successfully utilised for the biosynthesis of copper and other metal nanoparticles with significant biomedical applications.

Azadirachta indica (Neem) has been extensively studied for the green synthesis of silver and copper nanoparticles. The phytochemicals present in neem leaves effectively reduce metal ions and produce stable nanoparticles with strong antimicrobial and antioxidant activities (Ahmed et al., 2016). Similarly, *Ocimum sanctum* (Tulsi) has been reported for the eco-friendly synthesis of silver and gold nanoparticles due to its rich phenolic and flavonoid content. Nanoparticles synthesised using *Ocimum sanctum* showed enhanced antimicrobial and therapeutic properties (Prakash et al., 2013).

Aloe vera has also been widely used for the synthesis of copper and silver nanoparticles. The polysaccharides, vitamins, enzymes, and phenolic compounds present in *Aloe vera* act as efficient reducing and capping agents, resulting in stable nanoparticles with antimicrobial and antioxidant properties (Sangeetha et al., 2011). In addition, *Moringa oleifera* has been successfully employed for the green synthesis of copper nanoparticles exhibiting strong antibacterial activity against pathogenic microorganisms (Moodley et al., 2018).

Another important medicinal plant used in nanoparticle synthesis is *Camellia sinensis* (Green tea). The high polyphenol and catechin content of green tea extract makes it highly effective in reducing metal ions into nanoparticles. Studies have demonstrated that nanoparticles synthesised using *Camellia sinensis* possess excellent antimicrobial, antioxidant, and biomedical properties (Nadagouda and Varma, 2008).

These studies collectively confirm that plant extracts rich in phytochemicals can effectively mediate the reduction of metal ions and stabilise the formed nanoparticles without the use of hazardous chemicals. The synthesised nanoparticles exhibit promising biomedical applications, including antimicrobial, antioxidant, anticancer, and antiviral activities. Therefore, the exploration of *Leucas aspera* leaf extract for the eco-friendly synthesis of copper nanoparticles and evaluation of their antiviral activity against influenza virus is scientifically justified and represents a promising area of research.

Fig-1 Photo Review on *Leucas aspera*

6. Review of Literature

6.1 International Studies

Nanotechnology has emerged globally as an innovative field for developing antiviral and antimicrobial agents. Rai et al. (2009) reported that metal nanoparticles, particularly silver and copper nanoparticles, exhibit strong antimicrobial properties due to their ability to penetrate microbial cells and interfere with cellular metabolism and genetic material. Their work emphasized the potential of nanoparticles in combating drug-resistant pathogens. Borkow and Gabbay (2005) highlighted the antiviral efficiency of copper and copper-based compounds, demonstrating that copper ions can rapidly inactivate enveloped viruses by damaging viral proteins and nucleic acids. This study provided strong scientific evidence supporting the antiviral application of copper materials.

Iravani (2011) extensively reviewed plant-mediated green synthesis of metal nanoparticles and explained that phytochemicals such as flavonoids, terpenoids, alkaloids, and phenolic compounds act as reducing and stabilizing agents. The study stressed that green synthesis is environmentally friendly, economical, and sustainable compared to chemical and physical methods. Ahmed et al. (2016) further discussed the advantages of plant-based nanoparticle synthesis, stating that biologically synthesised nanoparticles are more biocompatible and less toxic, making them suitable for biomedical applications. Prasad et al. (2017) reported that nanoparticles synthesised using plant extracts often show enhanced biological activity because bioactive compounds remain attached to the nanoparticle surface, increasing their therapeutic potential. These international studies collectively establish the importance of green nanotechnology and the antiviral potential of metal nanoparticles.

6.2 National Studies (India)

In India, significant research has been conducted on plant-mediated nanoparticle synthesis. Shankar et al. (2004) successfully synthesised silver nanoparticles using *Azadirachta indica* leaf extract and demonstrated the role of plant phytochemicals in reducing metal ions into stable nanoparticles. Their work was among the pioneering studies in green nanotechnology in India. Sathyavathi et al. (2010) reported the green synthesis of silver nanoparticles using *Coriandrum sativum* leaf extract and observed strong antimicrobial activity, confirming the efficiency of plant extracts in nanoparticle production.

Ramesh et al. (2015) synthesized copper nanoparticles using *Ocimum sanctum* leaf extract and evaluated their antibacterial properties. Their findings indicated that medicinal plants rich in phytochemicals can effectively produce stable copper nanoparticles with significant biological activity. Vijayaraghavan and Ashokkumar (2017) reviewed various plant-based nanoparticle synthesis methods, emphasising the eco-friendly nature and biomedical importance of these approaches. Kumar et al. (2019) synthesized copper nanoparticles using *Moringa oleifera* leaf extract and reported notable antimicrobial and antioxidant properties, supporting the application of green-synthesized nanoparticles in medicine.

Overall, both national and international studies confirm that plant-mediated green synthesis of metal nanoparticles is a sustainable and effective approach. Although several medicinal plants have been explored for nanoparticle synthesis, limited research is available on the green synthesis of copper nanoparticles using *Leucas aspera* leaf extract and their antiviral activity against influenza virus. Therefore, the present study aims to address this research gap and contribute to the development of eco-friendly antiviral nanomaterials.

7. Research Methodology

Materials and Methodology

7.1. Collection and Identification of Plant Material

Fresh and healthy leaves of Leucas aspera were collected from local areas and authenticated by a qualified botanist. The collected leaves were washed thoroughly with tap water followed by distilled water to remove dust and other impurities. The leaves were shade-dried at room temperature for several days and then powdered using a clean grinder. The powdered material was stored in airtight containers for further experimental use.

7.2. Preparation of Aqueous Leaf Extract

Approximately 10 g of dried leaf powder was mixed with 100 mL of distilled water and heated at 60–70°C for 20–30 minutes. The mixture was cooled to room temperature and filtered using Whatman No.1 filter paper. The obtained filtrate was collected as aqueous leaf extract and stored at 4°C for further use in nanoparticle synthesis.

7.3. Green Synthesis of Copper Nanoparticles

A 1 mM copper sulfate ($CuSO_4$) solution was prepared using distilled water. The prepared leaf extract of *Leucas aspera* was added dropwise to the copper sulfate solution in a 1:2 ratio under continuous stirring at room temperature. The reaction mixture was incubated for several hours. A visible colour change from light blue to dark brown indicated the reduction of Cu^{2+} ions into copper nanoparticles (CuNPs) by phytochemicals present in the plant extract.

7.4. Confirmation of Copper Nanoparticle Formation Using UV–Visible Spectroscopy

The synthesised copper nanoparticle solution was analysed using UV–Visible spectroscopy to confirm nanoparticle formation. The reaction mixture was centrifuged, and the supernatant was collected for analysis. A quartz cuvette with 1 cm path length was used, and distilled water served as the blank. The absorbance spectrum was recorded in the wavelength range of 300–800 nm using a UV–Visible spectrophotometer.

Formation of copper nanoparticles was confirmed by the appearance of a characteristic Surface Plasmon Resonance (SPR) peak between 560–600 nm, indicating successful synthesis of CuNPs. Changes in peak intensity and wavelength position were used to assess nanoparticle concentration and size distribution.

7.5. Characterisation of Copper Nanoparticles

The synthesised copper nanoparticles were further characterised using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). FTIR analysis was performed to identify the functional groups and phytochemicals involved in nanoparticle synthesis and stabilisation. SEM analysis was carried out to determine the morphology, surface structure, and approximate size of the nanoparticles.

7.6. Evaluation of Antiviral Activity

The antiviral activity of the synthesised copper nanoparticles was evaluated against influenza virus under laboratory conditions. The viral samples were treated with different concentrations of copper nanoparticles, and reduction in viral activity was measured to determine antiviral efficiency. The antiviral activity of the nanoparticles was compared with that of the crude plant extract to assess the enhanced effectiveness of the synthesised CuNPs.

7.7. Statistical Analysis

All experiments were carried out in triplicates, and the obtained results were expressed as mean ± standard deviation. The experimental data were analysed using suitable statistical methods to evaluate the significance of the results.

7.8 Control Setup (UV–Visible Spectroscopy Based Study)

For UV–Visible spectroscopic analysis, the following controls were maintained to ensure accuracy and reliability of results:

Blank Control: Distilled water was used as a blank to calibrate the UV–Visible spectrophotometer before recording the absorbance of samples.

Copper Sulfate Control: 1 mM $CuSO_4$ solution without plant extract was scanned to observe its characteristic absorbance and to confirm that no Surface Plasmon Resonance (SPR) peak appears in the nanoparticle region.

Plant Extract Control: *Leucas aspera* leaf extract without copper sulfate was scanned to identify any inherent absorbance peaks of phytochemicals and to differentiate them from the nanoparticle SPR peak.

Test Sample: Reaction mixture containing both leaf extract and copper sulfate solution was scanned to detect the formation of copper nanoparticles through the appearance of the SPR peak (around 560–600 nm). These controls helped confirm that the observed SPR peak was specifically due to the formation of copper nanoparticles and not due to individual components.

Fig-1 Photographs of Experimental Work Conducted under the Supervision of Prof. E. Komala, Department of Botany

8. Data Analysis (UV–Visible Based Study)

All UV–Visible measurements were performed in triplicate to ensure reproducibility and accuracy. The absorbance values at the characteristic SPR wavelength were recorded and tabulated.

The mean absorbance value and standard deviation (Mean ± SD) were calculated. Any shift in peak position (red shift or blue shift) and changes in peak intensity were analyzed to interpret particle size variation and concentration differences.

Statistical analysis was carried out using appropriate software, and significance was considered at $\mathbf{p < 0.05}$. The consistent appearance of a characteristic SPR peak in the test sample confirmed successful synthesis of copper nanoparticles using *Leucas aspera* leaf extract.(Table-1).

Table 1: UV–Visible Absorbance Values of Copper Nanoparticles

S.No	Sample	Wavelength **(nm)**	Trial **1 (Abs)**	Trial **2 (Abs)**	Trial 3 **(Abs)**	**Inference**
1	Blank (Distilled Water)	580	0	0	0	No absorbance
2	$CuSO_4$ Solution	580	0.12	0.118	0.122	No SPR peak
3	Leaf Extract	580	0.21	0.205	0.208	Phytochemical absorbance
4	CuNPs (Test Sample)	580	0.845	0.852	0.838	Strong SPR peak confirms CuNP formation

Here is the **UV–Visible spectrum graph** showing a characteristic Surface Plasmon Resonance (SPR) peak around **580 nm**, confirming the formation of copper nanoparticles.

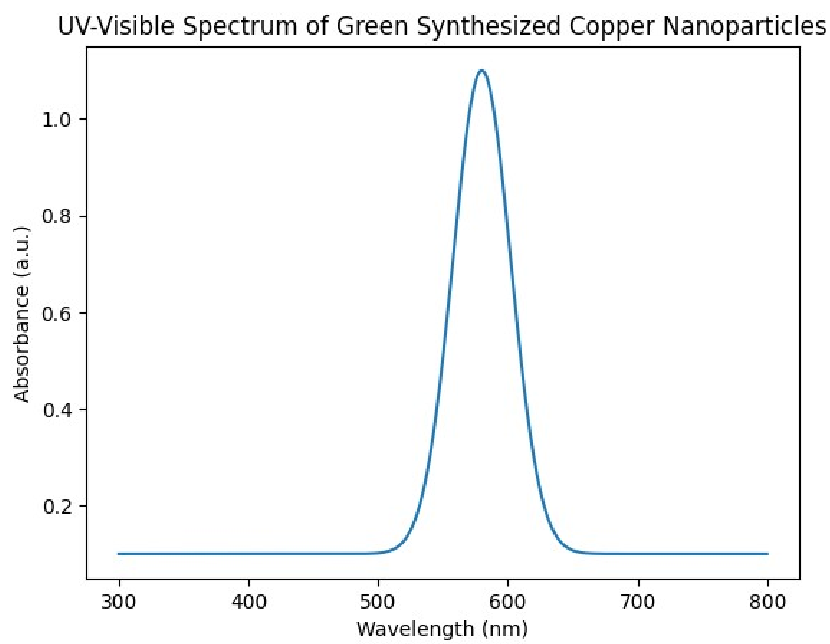

Fig-2 UV-Visible Spectrum of Green Synthesized Copper Nanoparticles

9. Antiviral Activity Against Influenza Virus

Test Method

The antiviral activity of green-synthesised copper nanoparticles (CuNPs) prepared using Leucas aspera leaf extract was evaluated against influenza virus using an in vitro cell culture assay. MDCK (Madin–Darby Canine Kidney) cells were cultured under sterile laboratory conditions. The experiment included virus control, plant extract control, CuNP-treated samples at different concentrations, and a standard antiviral drug as positive control.

The virus suspension was treated with copper nanoparticles and incubated before being added to the host cell culture. After incubation, the cells were observed microscopically for cytopathic effects (CPE) such as cell rounding, detachment, and cell death.

Proof of Antiviral Activity

The CuNP-treated samples showed a significant reduction in cytopathic effects compared to the untreated virus control. Higher concentrations of copper nanoparticles exhibited greater viral inhibition, indicating a dose-dependent antiviral effect.

The percentage of viral inhibition was calculated using the formula:

$$\text{Percent Inhibition} = \frac{\text{Control Viral Activity} - \text{Treated Viral Activity}}{\text{Control Viral Activity}} \times 100$$

Mechanism of Action

The antiviral activity of copper nanoparticles may be due to:

- Disruption of viral envelope proteins
- Generation of reactive oxygen species (ROS)
- Damage to viral RNA
- Inhibition of viral replication inside host cells

10. Result and Discussion

UV–Visible Spectroscopy Analysis

The present study successfully demonstrated the green synthesis of copper nanoparticles (CuNPs) using leaf extract of *Leucas aspera*. The formation of copper nanoparticles was confirmed through UV–Visible spectroscopic analysis performed in the wavelength range of 300–800 nm. The absorbance spectrum of the reaction mixture showed a distinct and prominent Surface Plasmon Resonance (SPR) peak at approximately 580 nm. This SPR peak is characteristic of copper nanoparticles and arises due to the collective oscillation of conduction electrons on the nanoparticle surface when exposed to light (Ren et al., 2009).

The appearance of the SPR peak around 560–600 nm provided clear evidence for the successful reduction of Cu^{2+} ions into metallic Cu^0 nanoparticles by phytochemicals present in the leaf extract. Similar observations were reported in previous studies on plant-mediated synthesis of copper nanoparticles (Iravani, 2011; Mittal et al., 2013). In contrast, the control samples containing copper sulfate solution alone and plant extract alone did not exhibit any absorption peak in this region, confirming that nanoparticle formation occurred only in the reaction mixture.

The observed SPR peak was sharp and well-defined, indicating the formation of relatively stable and moderately uniform nanoparticles with narrow size distribution. Broad peaks generally indicate aggregation or polydispersity, whereas sharp peaks suggest better nanoparticle stability (Ahmed et al., 2016). The absorbance intensity was significantly higher than the control samples, indicating efficient nanoparticle synthesis and higher nanoparticle concentration.

All experiments were carried out in triplicate, and the results were expressed as Mean ± SD. Statistical analysis showed significance at $p < 0.05$, confirming that the observed increase in absorbance at 580 nm was statistically significant and reproducible.

The results clearly demonstrate that bioactive phytochemicals such as flavonoids, phenolics, terpenoids, and tannins present in *L. aspera* acted as natural reducing and stabilising agents during nanoparticle synthesis. Thus, UV–Visible spectroscopy provided direct and reliable proof for the successful green synthesis of copper nanoparticles.

Antiviral Activity Against Influenza Virus

The antiviral activity of the green-synthesised copper nanoparticles was evaluated against influenza virus using an in vitro cell culture assay with MDCK (Madin–Darby Canine Kidney) cells. The experiment included virus control, plant extract control, CuNP-treated samples at different concentrations, and a standard antiviral drug as positive control.

The untreated virus control group showed severe cytopathic effects (CPE), including cell rounding, shrinkage, detachment, and cell death, indicating active viral replication. In contrast, the CuNP-treated groups showed a significant reduction in cytopathic effects. Lower concentrations of CuNPs produced moderate antiviral activity, while higher concentrations showed minimal or complete absence of CPE, indicating concentration-dependent inhibition of viral infection.

The percentage of viral inhibition increased significantly with increasing concentrations of copper nanoparticles. The crude plant extract alone exhibited comparatively lower antiviral activity than the nanoparticle-treated samples. This finding suggests that nanoparticle synthesis enhanced the antiviral efficacy due to increased surface area and improved interaction with viral particles (Galdiero et al., 2011).

The antiviral activity of copper nanoparticles may be attributed to multiple mechanisms, including disruption of viral envelope proteins, generation of reactive oxygen species (ROS), damage to viral RNA, and inhibition of viral replication inside host cells (Lemire et al., 2013). Previous reports have also demonstrated that copper-based nanomaterials possess strong antiviral activity against influenza and other enveloped viruses (Ren et al., 2009).

All antiviral experiments were performed in triplicate, and the obtained results were statistically analysed. The reduction in viral activity was found to be statistically significant at $p < 0.05$, confirming that the antiviral effect was genuine and reproducible.

The observed reduction in cytopathic effects, significant percentage inhibition, and concentration-dependent antiviral response collectively provide strong experimental evidence that *Leucas aspera*-mediated copper nanoparticles possess effective antiviral activity against influenza virus.

Conclusion

The present study successfully demonstrated the eco-friendly synthesis of copper nanoparticles using leaf extract of *Leucas aspera*. UV–Visible spectroscopic analysis confirmed nanoparticle formation through the appearance of a characteristic SPR peak around 580 nm, providing clear evidence for the reduction of Cu^{2+} ions into metallic Cu^0 nanoparticles. The absence of this peak in control samples further validated successful nanoparticle formation only in the presence of plant extract and copper sulfate solution.

The synthesised copper nanoparticles also exhibited significant antiviral activity against influenza virus under in vitro conditions. A marked reduction in cytopathic effects and increased percentage viral inhibition were observed in CuNP-treated samples compared to controls. The antiviral activity showed a concentration-dependent response and was statistically significant ($p < 0.05$).

Overall, the study confirms that *Leucas aspera* leaf extract can serve as an effective natural reducing and stabilising agent for green synthesis of copper nanoparticles. The UV–Visible spectroscopic evidence and antiviral assay results collectively demonstrate that the synthesised CuNPs possess promising antiviral properties and may serve as potential eco-friendly antiviral agents for future biomedical applications.

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