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Anti-Inflammatory Activity of Root Assisted Synthesis of Copper Oxide Nanoparticle Using *Gloriosa superba*

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ABSTRACT
Introduction: The quest for natural, plant-based compounds in nanotechnology has heightened interest in medicinal plants with bioactive properties. *Gloriosa superba*, known as the flame lily, is valued in traditional medicine for its anti-inflammatory, analgesic, and antimicrobial effects, attributed to compounds like colchicine and gloriosine. This study evaluates *G. superba* root extracts as natural reducing agents for the green synthesis of copper oxide nanoparticles (CuO NPs) and investigates their anti-inflammatory properties.
Materials and Methods: *G. superba* roots were collected, washed, and air-dried before being ground into powder. Preparation of Gloriosa superba Root Extract and Synthesis of Copper Oxide Nanoparticles Done. Anti-inflammatory activity was assessed using the Egg Albumin (EA) assay and Membrane Stabilization assay, measuring the inhibition of inflammation at varying concentrations of CuO NPs (10, 25, and 50 µg/mL).
Results: The results indicated significant dose-dependent anti-inflammatory activity of *G. superba*-synthesized CuO NPs. All assays demonstrated increased inhibition with higher concentrations, achieving levels comparable to standard treatments.
Conclusion: This study highlights the potential of *G. superba* extracts for eco-friendly synthesis of CuO NPs with promising anti-inflammatory properties. These findings support the use of plant-based methods in developing effective therapeutic agents, warranting further exploration of their biomedical applications.

Keywords: *Gloriosa superba*, copper oxide nanoparticles, green synthesis, anti-inflammatory activity, medicinal plants.

INTRODUCTION:

The search for natural, plant-based compounds as biocompatible agents in nanotechnology has led to significant interest in medicinal plants, particularly those with known bioactive properties. *Gloriosa superba*, a perennial herbaceous climber widely recognized for its striking flame-like flowers, has long held a prominent place in traditional medicine due to its potent anti-inflammatory, analgesic, and antimicrobial properties (1). This plant, commonly known as the flame lily, is indigenous to tropical regions of Africa and Asia, where it has been used in folk medicine to treat various ailments(2). Its pharmacological activities are primarily attributed to bioactive compounds such as colchicine and gloriosine, which have demonstrated significant anti-inflammatory potential(3).

Recent advancements in green synthesis have explored the use of plant-derived compounds for the eco-friendly synthesis of nanoparticles, with *G. superba* emerging as a promising candidate for this approach(4). The plant's roots, in particular, contain high concentrations of phenolic and flavonoid compounds, which not only contribute to its therapeutic properties but also serve as reducing and stabilizing agents in nanoparticle synthesis(5).

Copper oxide nanoparticles (CuO NPs) have attracted significant interest in recent years due to their unique chemical, physical, and biological properties(6). As versatile nanomaterials, CuO NPs exhibit potent anti-inflammatory, antimicrobial, and antioxidant activities, making them valuable in various biomedical applications(7,8). Their small size and large surface area-to-volume ratio enhance their reactivity, which is beneficial for targeted drug delivery, cancer therapy, wound healing, and anti-inflammatory treatments. These nanoparticles have demonstrated strong bioactivity against a range of pathogens and inflammatory processes, highlighting their potential as therapeutic agents in both clinical and environmental applications(9,10).

Traditional synthesis methods for CuO NPs often involve the use of chemical agents that can be toxic or environmentally hazardous. As a response, green synthesis methods have gained momentum, utilizing biological resources particularly medicinal plants as natural reducing and stabilizing agents(11). Such eco-friendly approaches not only reduce the environmental impact but also incorporate plant-based bioactive compounds that may enhance the therapeutic properties of the nanoparticles(12,13). In this context, *Gloriosa superba*, a medicinal plant known for its anti-inflammatory constituents, offers a promising alternative for the biosynthesis of CuO NPs. The plant's root extracts are rich in phenolic and flavonoid compounds, which can serve as natural reducing agents, thereby facilitating nanoparticle synthesis while potentially enhancing their anti-inflammatory activity.

Inflammation is a complex biological response to harmful stimuli, such as pathogens, damaged cells, or irritants, and plays a critical role in the body's immune defense. However, chronic inflammation is associated with numerous health conditions, including arthritis, cardiovascular diseases, and neurodegenerative disorders(14,15). While conventional anti-inflammatory drugs are widely used, they can often lead to side effects, driving a need for safer and more effective alternatives(16). Nanotechnology, specifically the use of nanoparticles, has shown promise in creating targeted and efficient anti-inflammatory treatments. Among these, copper oxide nanoparticles (CuO NPs) have gained attention due to their inherent anti-inflammatory, antimicrobial, and antioxidant properties, which offer potential for a range of biomedical applications(16,17).

This study investigates the potential of *Gloriosa superba* root extracts as a natural reducing agent in the green synthesis of copper oxide nanoparticles. By examining the anti-inflammatory properties of CuO NPs synthesized via *G. superba*, this work aims to explore novel, sustainable approaches to nanoparticle synthesis with potential therapeutic applications. The environmentally friendly synthesis method and the incorporation of natural plant-based compounds hold promise for advancing anti-inflammatory therapies and reducing the environmental impact associated with conventional nanoparticle production methods.

MATERIALS AND METHOD:

Preparation of *Gloriosa superba* Root Extract

Gloriosa superba roots were collected, thoroughly washed, and air-dried for 7-10 days. The dried roots were then ground into a fine powder (figure 1). To prepare the extract, 20 g of this powder was mixed with distilled water in a 1:10 (w/v) ratio and heated at 60-70°C for 1 hour with continuous stirring. After cooling, the mixture was filtered through Whatman No. 1 paper to remove residues. The resulting extract was stored at 4°C for use in synthesizing copper oxide nanoparticles.

Figure 1: Preparation of Gloriosa superba Root Extract

Synthesis of Copper Oxide Nanoparticles (CuO NPs)

A 0.1 M copper sulfate solution was prepared and mixed with *Gloriosa superba* root extract in a 1:1 (v/v) ratio, with continuous stirring at room temperature for 2-3 hours. The mixture was then heated to 70°C to promote nanoparticle formation. Afterward, the solution was centrifuged at 10,000 rpm for 15 minutes to collect the CuO

NPs, which were washed with distilled water and ethanol, dried at 60°C, and stored for further analysis (figure 2).

Figure 2: Synthesis of Copper Oxide Nanoparticles (CuO NPs)

Preparation of Bovine Serum Albumin (BSA) Solution

Bovine Serum Albumin (BSA) powder was dissolved in phosphate-buffered saline (PBS) to prepare a 1% (w/v) stock solution. The solution was gently mixed until fully dissolved, then filtered through a 0.22 µm syringe filter for sterility. The filtered BSA solution was aliquoted and stored at 4°C for use in anti-inflammatory activity assays and characterization of the synthesized CuO NPs.

Evaluation of Anti-Inflammatory Activity Using the Egg Albumin (EA) Assay

Fresh egg albumin was prepared by mixing egg whites to create a uniform suspension, which was then diluted with phosphate-buffered saline (PBS) to a final concentration of 1-2% (w/v). Experimental groups included a control group (PBS only) 50 µg/mL and copper oxide nanoparticles (CuO NPs) at concentrations of 10, 25, and

50 µg/mL. Each group received 1 mL of the egg albumin suspension and the respective treatments, followed by incubation at room temperature for 30 minutes. Samples were subjected to thermal stress at 55°C for 30 minutes to induce inflammation, then cooled and allowed to stand for 1 hour. Turbidity was measured at 660 nm using a spectrophotometer.

Membrane Stabilization Assay

The membrane stabilization assay evaluated the anti-inflammatory activity of synthesized copper oxide nanoparticles (CuO NPs) by measuring their ability to stabilize lysosomal membranes. Fresh goat or sheep blood was centrifuged to obtain a 10% (v/v) erythrocyte suspension in phosphate-buffered saline (PBS). Experimental groups included a control (PBS), and CuO NPs at concentrations of 10, 25, and 50 µg/mL. Each group received 1 mL of erythrocyte suspension and the respective treatments, followed by incubation at 37°C for 30 minutes and thermal stress at 56°C for 30 minutes.

RESULTS:

The BSA assay graph (figure 3) shows dose-dependent anti-inflammatory activity of *Gloriosa superba*-synthesized CuO nanoparticles, with inhibition increasing as concentration rises. The nanoparticles perform comparably or better than the standard at higher concentrations.

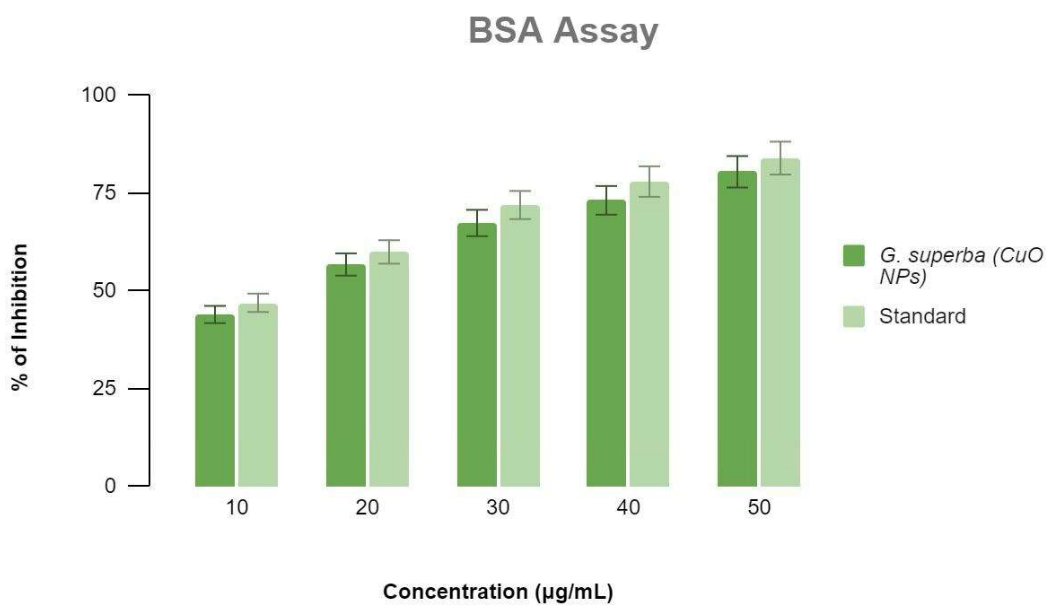

Figure 3: BSA ASSAY

The EA (Egg Albumin) assay graph (figure 4) shows the anti-inflammatory activity of *Gloriosa superba*-synthesized CuO nanoparticles compared to the standard. The percentage of inhibition increases with concentration, indicating a dose-dependent effect. The CuO nanoparticles perform similarly to the standard, achieving high inhibition levels at higher concentrations, showing their effectiveness in reducing inflammation.

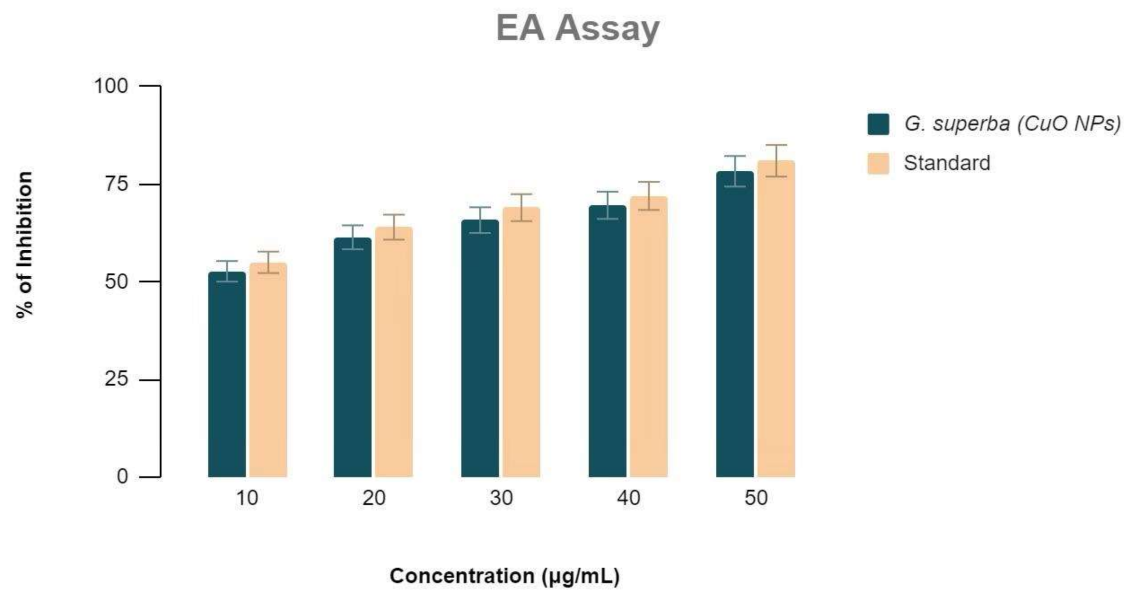

Figure 4: EA ASSAY

The Membrane Stabilization Assay graph (figure 5) indicates the anti-inflammatory potential of *Gloriosa superba*-synthesized CuO nanoparticles. With increasing concentration, the percentage of inhibition rises, showing a dose-dependent effect. The nanoparticles achieve inhibition levels similar to the standard, suggesting their effectiveness in stabilizing cell membranes and reducing inflammation.

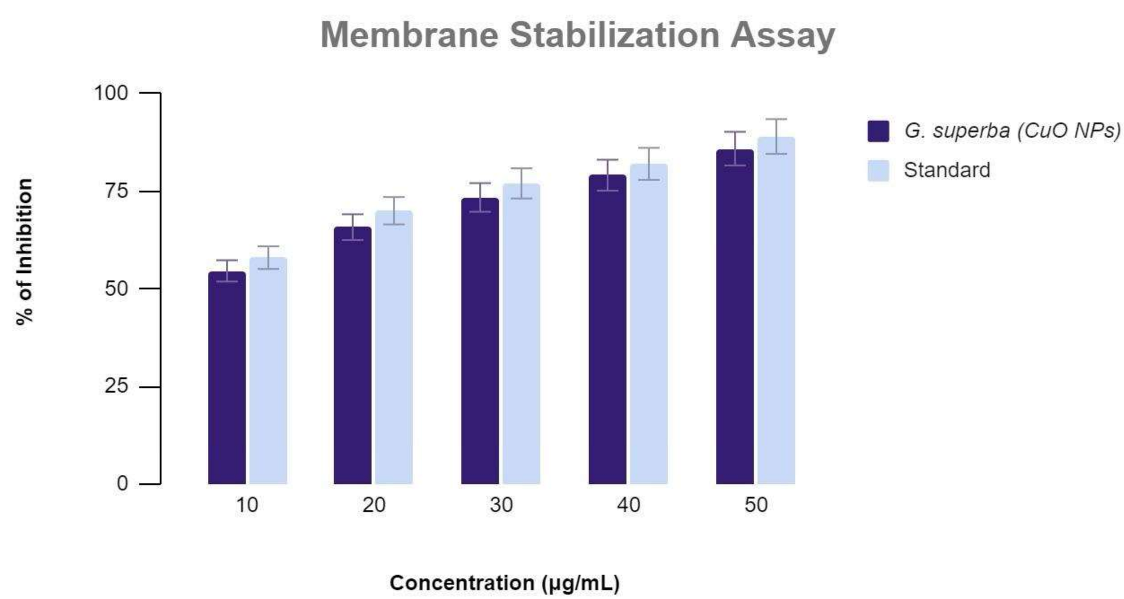

Figure 5: MSA

The anti-inflammatory assays—BSA, EA, and Membrane Stabilization, demonstrate the effectiveness of *Gloriosa superba*-synthesized copper oxide (CuO) nanoparticles across concentrations (10–50 µg/mL). In all assays, the nanoparticles show a dose-dependent increase in inhibition, achieving levels comparable to or exceeding the standard, especially at higher concentrations. These results suggest that *G. superba* CuO nanoparticles possess significant anti-inflammatory activity, stabilizing cell membranes and inhibiting proteins involved in inflammation, thus providing a potential natural alternative for anti-inflammatory applications.

DISCUSSION:

A similar study demonstrates that ginger and lemongrass-synthesized copper nanoparticles effectively inhibit lung cancer cell proliferation, with an IC_{50} of 20 µg/mL, showing statistically significant differences ($p < 0.05$) between the control and treatment groups. Post-treatment, a reduction in cell number was observed, alongside morphological changes such as cell shrinkage and cytoplasmic membrane blebbing. DAPI staining further revealed chromatin condensation and nuclear fragmentation in treated cells, indicating apoptosis. These findings suggest that, with further development, ginger and lemongrass copper nanoparticles could serve as an alternative cancer treatment with potentially minimal side effects. (18)

Similarly copper nanoparticles synthesized from *Piper longum* and *Piper betle*, revealing potent antibacterial, anti-inflammatory, and antioxidant activities. The nanoparticles, with diameters between 30 and 90 nm, showed significant antibacterial effects against *E. faecalis*, *S. aureus*, *C. albicans*, and *S. mutans*, comparable to standard treatments. They also exhibited anti-inflammatory activity similar to standard values. In antioxidant assays, the nanoparticles achieved up to 85.16% inhibition at 50 µg/mL and 50.62% at 10 µg/mL. These findings indicate that *Piper*-mediated copper nanoparticles have strong potential for applications requiring antibacterial, anti-inflammatory, and antioxidant properties. (19)

The successful biosynthesis of copper oxide (CuO) nanoparticles using *Moringa oleifera* extract and copper sulfate. Characterization by TEM and UV-Vis spectroscopy confirmed spherical nanoparticles with a size range of 2–100 nm, peaking at 300 nm in UV-Vis analysis. The synthesized CuO nanoparticles exhibit excellent biocompatibility, with smaller particles showing potential to enhance immunity. These findings suggest that *Moringa oleifera*-mediated CuO nanoparticles could be promising candidates for biomedical applications, particularly in drug delivery and immune support. (19,20)

The synthesized copper nanoparticles from turmeric and Tulsi exhibited significant anti-inflammatory properties, as indicated by the UV-spectrophotometric analysis. The combination of Tulsi and turmeric with copper extract demonstrates enhanced anti-inflammatory activity, suggesting a promising avenue for developing natural therapeutic agents.The study highlights the potential of utilizing traditional medicinal plants in conjunction with nanotechnology to create effective anti-inflammatory agents. Future research should investigate the mechanisms behind these effects and their applicability in clinical settings. (21)

Potential of green-synthesized copper nanoparticles mediated by *Cardiospermum halicacabum*as were effective anti-inflammatory and antioxidant agents. The observed increase in activity with higher concentrations underscores the dose-dependent efficacy of these nanoparticles. The use of plant-based methods for nanoparticle synthesis is not only cost-effective but also environmentally friendly, minimizing toxicity concerns associated with conventional synthesis techniques. The promising results indicate that *C. halicacabum* mediated copper nanoparticles could play a significant role in various applications, including biomedical fields, where their enhanced properties may lead to novel therapeutic strategies. Continued exploration of their mechanisms and broader applications will further establish their potential in drug discovery and development. (22)

CONCLUSION:

This study successfully demonstrates the potential of *Gloriosa superba* root extracts for the green synthesis of copper oxide nanoparticles (CuO NPs) and their significant anti-inflammatory and antioxidant properties. The biosynthesis approach utilizing this medicinal plant not only provides an environmentally friendly alternative to conventional methods but also harnesses the bioactive compounds inherent in *G. superba*, enhancing the therapeutic efficacy of the nanoparticles. The dose-dependent increase in anti-inflammatory activity across various assays suggests that these CuO NPs may serve as effective agents in treating inflammatory conditions. Given their promising performance, further research is warranted to explore their mechanisms of action and potential applications in biomedical fields, positioning *G. superba*-mediated CuO NPs as viable candidates for future therapeutic development. Overall, this study contributes to the growing body of evidence supporting the integration of nanotechnology and natural products in advancing healthcare solutions.

REFERENCE:

1. Critical review on medicinally potent plant species: Gloriosa superba. Fitoterapia. 2011 Apr 1;82(3):293–301.

2. Ade R, Rai MK. Review: Current Advances in Gloriosa superba L. Biodiversitas [Internet]. 2009 Jul 7 [cited 2024 Oct 30];10(4). Available from: https://smujo.id/biodiv/article/view/293

3. Website [Internet]. Available from: https://www.researchgate.net/profile/Kaliyaperumal-Ashokkumar/publication/287294241_Gloriosa_superba_L_A_Brief_Review_of_its_Phytochemical_Properties_and_Pharmacology/links/5d5ace99a6fdcc55e8194211/Gloriosa-superba-L-A-Brief-Review-of-its-Phytochemical-Properties-and-Pharmacology.pdf

4. Green synthesis of CuO nanoparticles using Gloriosa superba L. extract and their antibacterial activity. Journal of Taibah University for Science. 2015 Jan 1;9(1):7–12.

5. Clewer HWB, Green SJ, Tutin F. XCI.—The constituents of Gloriosa superba. J Chem Soc, Trans. 1915 Jan 1;107(0):835–46.

6. Website [Internet]. Available from: https://www.researchgate.net/profile/Abdelkader-Bouaziz/post/Method_for_the_synthesis_of_colloidal_copper_oxide_nanoparticles/attachment/631a4c1b0c295f1f9ad54b16/AS%3A11431281083622667%401662667803254/download/CuO+1.pdf

7. Characterisation of copper oxide nanoparticles for antimicrobial applications. International Journal of Antimicrobial Agents. 2009 Jun 1;33(6):587–90.

8. Chun-Kwei Wu †., Ming Yin ‡., Stephen O'Brien ‡. And, Jeffrey T. Koberstein* †. Quantitative Analysis of Copper Oxide Nanoparticle Composition and Structure by X-ray Photoelectron Spectroscopy. 2006 Nov 15 [cited 2024 Oct 30]; Available from: https://pubs.acs.org/doi/abs/10.1021/cm061596d

9. Verma N, Kumar N. Synthesis and Biomedical Applications of Copper Oxide Nanoparticles: An Expanding Horizon. ACS Biomaterials Science & Engineering [Internet]. 2019 Feb 13 [cited 2024 Oct 30]; Available from: https://pubs.acs.org/doi/abs/10.1021/acsbiomaterials.8b01092

10. Siddiqi KS, Husen A. Current status of plant metabolite-based fabrication of copper/copper oxide nanoparticles and their applications: a review. Biomaterials Research [Internet]. 2020 Jun 3 [cited 2024 Oct 30]; Available from: https://spj.science.org/doi/10.1186/s40824-020-00188-1

11. Saif Hasan S, Singh S, Parikh RY, Dharne MS, Patole MS, Prasad BLV, et al. Bacterial Synthesis of Copper/Copper Oxide Nanoparticles. 2008 Jun [cited 2024 Oct 30]; Available from: http://dx.doi.org/10.1166/jnn.2008.095

12. Chakraborty N, Banerjee J, Chakraborty P, Banerjee A, Chanda S, Ray K, et al. Green synthesis of copper/copper oxide nanoparticles and their applications: a review. Green Chemistry Letters and Reviews [Internet]. 2022 Jan 2 [cited 2024 Oct 30]; Available from: https://www.tandfonline.com/doi/abs/10.1080/17518253.2022.2025916

13. Akintelu SA, Folorunso AS, Folorunso FA, Oyebamiji AK. Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation. Heliyon [Internet]. 2020 Jul 1 [cited 2024 Oct 30];6(7). Available from: http://www.cell.com/article/S2405844020313529/abstract

14. Website [Internet]. Available from: https://www.annualreviews.org/content/journals/10.1146/annurev.bioeng.8.061505.095708

15. Libby P. Inflammatory Mechanisms: the Molecular Basis of Inflammation and Disease. Nutr Rev. 2007 Dec 1;65(suppl_3):S140–6.

16. Vane J, Botting R. Inflammation and the mechanism of action of anti-inflammatory drugs. The FASEB Journal. 1987 Aug 1;1(2):89–96.

17. Faisal S, Jan H, Abdullah, Alam I, Rizwan M, Hussain Z, et al. In Vivo Analgesic, Anti-Inflammatory, and Anti-Diabetic Screening of Bacopa monnieri-Synthesized Copper Oxide Nanoparticles. ACS Omega [Internet]. 2022 Jan 27 [cited 2024 Oct 30]; Available from: https://pubs.acs.org/doi/full/10.1021/acsomega.1c05410

18. Krishnakumar S, Thangavelu L, Shanmugam R, Perumal E. Copper nanoparticles synthesize using ginger and lemongrass and its anti-cancer activity against lung cancer cell line. SFS. 2023 Mar 8;10(1S):176–85.

19. Ambati T, Nizampuram V, Selvaganesh S, Rajeshkumar S, Nesappan T. Efficacy of copper oxide nanoparticles using Piper longum and Piper betle. Bioinformation. 2023 Sep 30;19(9):964.

20. Website [Internet]. Available from: https://www.researchgate.net/publication/335206475_Biosynthesis_of_copper_oxide_nanoparticles_using_herbal_formulation_and_its_characterisation

21. Rajkumar KV, Jothipriya A, Arivarasu L, Kumar R, Devi G. ANTI-INFLAMMATORY ACTIVITY OF TULASI AND TURMERIC MEDIATED COPPER NANOPARTICLES. PLANT CELL BIOTECHNOLOGY AND MOLECULAR BIOLOGY. 2020 Oct 30;21(47-48):39–42.

22. Chandran T, Rajeshkumar S, Arivarasu L. GREEN SYNTHESIS, ANTI INFLAMMATORY AND ANTIOXIDANT PROPERTY OF Cardiospermum halicacabum MEDIATED COPPER NANOPARTICLE. PLANT CELL BIOTECHNOLOGY AND MOLECULAR BIOLOGY. 2020 Nov 28;21(57-58):17–21.