Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Free Radical Scavenging Activity of Commelina Benghalensis Mediated Calcium Oxide Nanoparticles

Shreyas Loka[1], Meenakshi Rakesh M[2], Jayasree Anandan[1], Rajeshkumar Shanmugam[1*]

[1]Nanobiomedicine Lab, Centre for Global Health Research, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, India
[2]Department of Orthopaedics, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India
Email: rajeshkumars.smc@saveetha.com

Free radicals are molecules that consist of unpaired electrons. They cause cellular damage resulting in multiple diseases such as diabetes mellitus and diabetes insipidus, Alzheimer's, cancers and other autoimmune and neurological disorders. Free radical scavenging by drugs plays an important role in disease prevention and cure. The aim of this study was to evaluate the free radical scavenging activity of biosynthesized calcium oxide nanoparticles. Commelina benghalensis leaves were powdered, boiled and mixed with a precursor solution. The synthesized nanoparticles free radical scavenging and antioxidant activity were analyzed by DPPH (2,2-diphenyl-1-picrylhydrazyl), H2O2 (hydrogem peroxide), FRAP (Ferric reducing antioxidant power assay), ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) and Nitric oxide radical inhibition assays. Commelina benghalensis-mediated Calcium oxide nanoparticles showed a rise in free radical scavenging activity as concentrations increased from 10 µg /mL to 50 µg /mL. The study proves that the free radical scavenging activity of Commelina benghalensis-mediated Calcium oxide nanoparticles is relatively equal to the standard intervention indicating their potential usage in future drugs. Furthermore, biological trials could be conducted to evaluate their efficacy and potency.

Keywords: Calcium oxide, Commelina benghalensis, free radical scavenging, green synthesis.

1. Introduction

Nanotechnology, a field of biomedicine, includes everything from the manufacturing to characterization to evaluating properties of nanoparticles (NPs). This branch of science has been of growing importance over the last decade due to its vast potential applications in public interest. When some natural element particles are reduced to nanometer scale, their abilities and activities see a significant rise (Louwakul et al., 2016). Over the last few years, studying the antioxidant activity of nanoparticles has become an important part of nanotechnology (Das

et al., 2013). NPs exhibit unique physical and chemical properties courtesy of their high surface area and nanoscale size (Khan et al., 2019). Furthermore, when these NPs are synthesized through plant-based methods, they exhibit a wider range of activities. Metallic nanoparticles derived from Thymbra spicata leaf extract have high antioxidant activity while Moringa olifera leaf extracts allow the nanoparticles to showcase antibacterial and antioxidant activities (Marquis et al., 2016). Biosynthesis of multiple NPs like gold, silver, copper, selenium has been reported all over the world. Plant species of Alfalfa, Cissus quadrangularis, Cassia alata, Jathropha curcas etc are used to mediate their synthesis (Roy et al., 2013). Nanoparticles have proven to have a positive effect in preventing microbial growth in wounds and burn injuries (Harris et al., 2023).

Calcium oxide (CaO), a metal oxide, is easily available and is biocompatible with humans and animals. This property allows the CaO NPs to perforate cellular barriers making them potent drug delivery agents (Eram et al., 2021). The antibacterial and antifungal activity of CaO NPs has led to an increase in their demand which in turn has created a need for large scale synthesis. There are multiple synthesis approaches for CaO NPs with chemical synthesis being the easiest. But with the involvement of chemicals, there is always a risk of toxicity in organisms. Hence green synthesis has taken center stage in the production of nanoparticles due to its non-toxic, cost effective and easily accessible requirements (Kubmarawa et al., 2007). The green synthesis of NPs allows them to express a greater range of activities while the plant induces unique properties to them through its phytochemicals-tannins, flavonoids, lactones amongst many others (Pal et al., 2019). Green synthesis of nanoparticles is also proven to have helped overcome the adverse effects of chemical drugs (Ibrahim et al.,2010).

In the current study, we have synthesized the CaO NPs through Commelina benghalensis mediation. Commonly called the Benghal Dayflower or dew flower, this plant is native to tropical Africa and Asia. The plant has multiple medicinal applications including conjunctiva inflammation, epilepsy and scabies. Tannins and flavonoids found in the leaves and stems of the plant are strong free radical scavengers (Wu et al., 2021). Reactive oxygen species (ROS) such as superoxide and peroxide radicals have a dual role in organisms. At normal low levels, they act as signaling molecules for proliferation and differentiation of cells. But at higher levels they can lead to multiple diseases like cancer, autoimmune disorders and diabetes (Patil et al., 2022). The potential applications of nanoparticles as anti-tumor agents are being recognized and development of suitable methods and strategies will help in creating better therapeutic agents (Ahmad et al., 2024). This study aimed to evaluate the free radical scavenging or antioxidant property of Commelina benghalensis mediated calcium oxide nanoparticles. The applications of these biosynthesized nanoparticles will be vast and potent in the field of anti-cancer drugs.

2. Materials and Methods:

2.1 Preparation of plant extract:

Fresh Commelina benghalensis leaves, collected from the herbal garden of Saveetha medical college, were shade dried and powdered. 1 gram of Commelina benghalensis plant powder was added to 100 mL of distilled water. The plant extract was then boiled at 50-60°C using a

heating mantle for 10 minutes. Later, the boiled extract was filtered using a muslin cloth. 50 mL of the filtered plant extract was then added to 50 mL of precursor solution to form 100 mL of resultant solution.

2.2. Preparation of Nanoparticles:

C. benghalensis acts as the reducing and stabilizing agent. 30 millimolar calcium hydroxide [$Ca(OH)_2$] is mixed in 50 mL of distilled water. Calcium hydroxide in distilled water was the precursor solution. The resultant 100 mL solution was kept in a magnetic stirrer for 48 hours for synthesis of nanoparticles to occur. The magnetic stirrer was checked after the first 24 hours to confirm a change in color of the solution which is the preliminary confirmation for nanoparticle synthesis. After 48 hours, centrifugation of the nanoparticle solution was done at 8000 rpm(rotation per minute) for 10 minutes. The nanoparticles were deposited at the bottom and walls of the centrifuge tube. Following the completion of this procedure, the pellet was accumulated and preserved in the refrigerator for subsequent use.

2.3. Antioxidant activity

2.3.1. DPPH radical scavenging assay

A solution of 0.1 millimolar DPPH was created using methanol. For each assay conducted, a fresh working solution was generated by diluting the stock solution with methanol, resulting in a concentration of 20 µM. Various concentrations (10, 20, 30, 40, 50 µg/mL) of Commelina benghalensis-mediated calcium oxide nanoparticles were introduced to 200 µL of the DPPH working solution in a 96-well plate. The plate was then kept in the dark at room temperature for 10 minutes. Absorbance of the solution was measured at 517 nm using a spectrophotometer. Methanol was used as a blank. Ascorbic acid of the same different concentrations was used as standard.

2.3.2. Hydrogen peroxide radical scavenging assay

The scavenging efficiency of biosynthesized CaO NPs on H_2O_2 radicals was also evaluated. A 40 millimolar H_2O_2 solution was made in a 7.4 pH phosphate buffer. The test sample (CaO NPs) and a standard sample of ascorbic acid at different concentrations (all 5 test concentrations) were separately mixed with 0.6 mL of the H_2O_2 solution. The reaction solution was incubated in a dark place for 10 minutes and then its absorbance was observed spectrophotometrically at 230 nm. The standard taken for this procedure was vitamin C.

2.3.3. Ferric reducing antioxidant power assay:

A 300 millimolar acetate buffer having pH 3.6: 3.1g of $CH_3COONa.3H_2O$ (Sodium acetate trihydrate) was added to 16 ml of pure acetic acid. To get a total volume of 1 L, distilled water was added. 10 millimolar TPTZ (2, 4, 6-tripyridyl s-triazine) (molecular weight = 312.34) in 40 millimolar HCl (molecular weight = 36.46). 20 millimolar $FeCl_3.6\ H_2O$ (molecular weight = 270.30) - The combination of I, II, and III in a 10:1:1 ratio was prepared to form a FRAP reagent for the experiment just before testing. $FeSO_4.7H_2O$ in methanol ($\leq$ 1.5 millimolar) was selected as the reference standard. The FRAP reagent, measuring 2.3 mL, was combined with 0.7 mL of the aqueous extracts at all 5 test concentrations. Following this, the mixture was incubated in the dark at a temperature of 37ºC for duration of 30 minutes. A spectrophotometer was used at a wavelength of 593 nm to measure the absorbance and then compared with a

blank that contained all the reagents except for the sample. The rise in absorbance of the reaction mixture was due to enhancement of the reduction capability. The samples were measured in triplicates, with ascorbic acid being utilized as the standard.

2.3.4. ABTS assay

The ABTS radical cation was produced by mixing 7 millimolar ABTS with 2.45 millimolar potassium persulfate (in distilled water). The reagent was subsequently stored in a refrigerated environment for 24 hours. The reagent was prepared by diluting it with ethanol (50 mL ethanol mixed in 100 mL of water) until the absorbance value (at 734 nm) was 1.0. In 96-well microplates, 250 µL of ABTS radicals-containing solution and 20 µL of sample solution (of all 5 concentrations) were mixed in distilled water. Ascorbic acid served as the standard, while 20 µL of ethanol was used as the blank. Following a 10-minute reaction in darkness, a microplate photometer, set at 734 nm, was used to measure the absorbance.

2.3.5. Nitric oxide radical inhibition assay

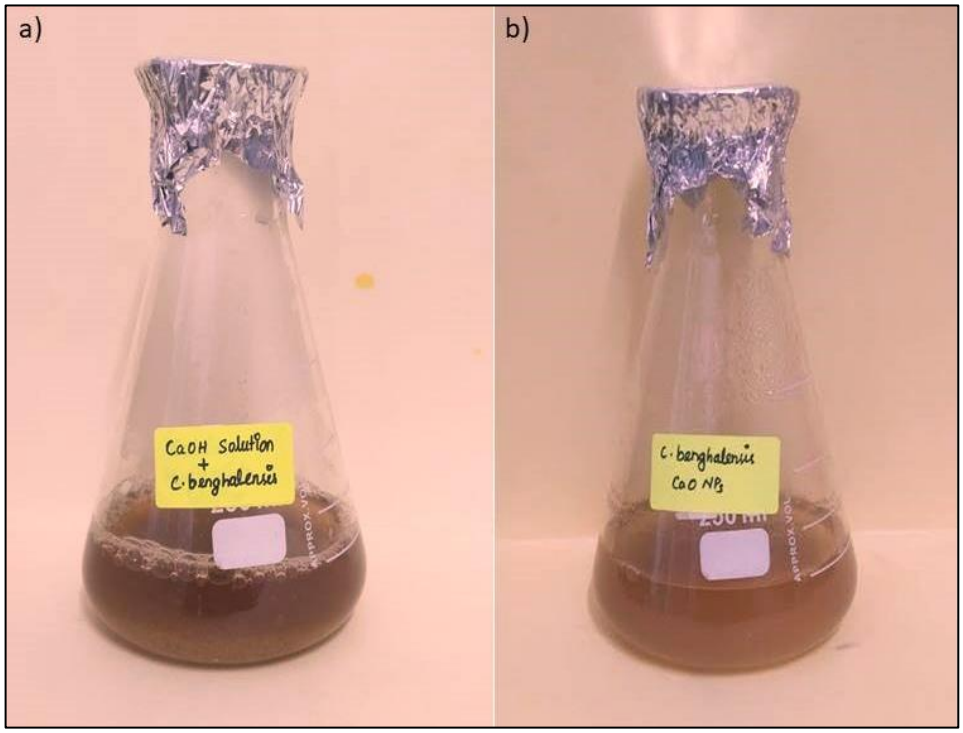
The inhibition of nitric oxide radicals can be assessed through the Griess Ilosvay reaction as described by Garrat in 1964. For this study, the Griess Ilosvay reagent was altered by substituting 5% 1-naphthylamine with 0.1% (w/v) naphthylethylene diamine dihydrochloride. A reaction mixture of 3 mL was prepared, consisting of 2 millimolar sodium nitroprusside (10 mm), 0.5 mL phosphate buffer saline and a standard solution (0.5 mL of ascorbic acid). After ensuring proper preparation, the combination was incubated at a temperature of 25°C for duration of 150 minutes. Upon completion of the incubation period, 0.5 mL of the reaction mixture was mixed with 1 mL of sulfanilic acid reagent and allowed to stand for duration of 5 minutes to ensure diazotization. Following this, 1 mL of $C_{12}H_{16}C_{l2}N_2$ (naphthyl ethylenediamine dihydrochloride) was added and thoroughly mixed. The sample was left undisturbed for 30 minutes at a temperature of 25°C. Diffused light analysis resulted in the formation of a chromophore exhibiting a pink hue. A spectrophotometer was used to evaluate the absorbance of the nanoparticles at 540 nm, relative to the corresponding blank solutions.

3. Results and Discussion

3.1. Visual observation

The color of the calcium oxide nanoparticles solution, synthesized using Commelina benghalensis plant extract, changed from dark brown to light brown after 24 hours as shown in figure 1. This color shift preliminarily confirms the successful formation of nanoparticles.

Figure 1 Visual observation of calcium oxide nanoparticles synthesized using Commelina benghalensis a) Initial colour change b) final colour change



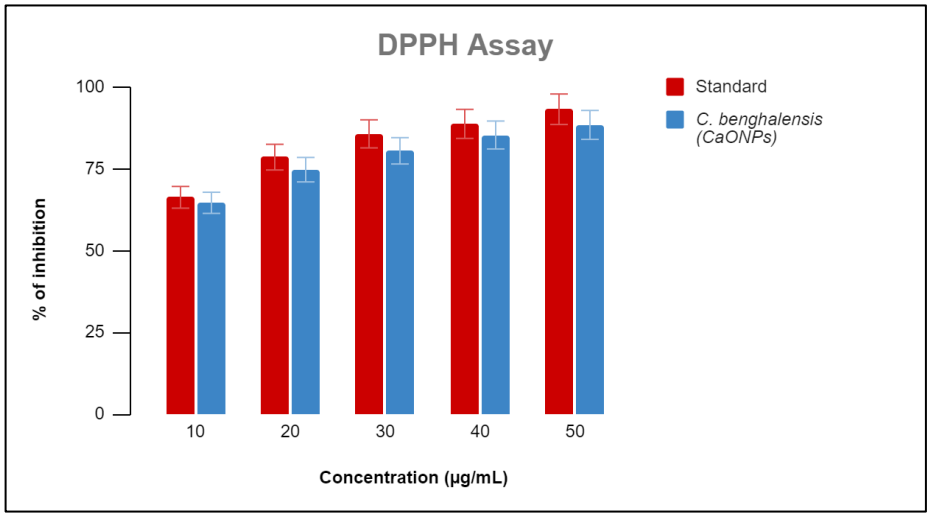
3.2. Antioxidant/Free radical scavenging activity

Evaluation of free radical scavenging properties of the nanoparticles was evaluated using DPPH, hydrogen peroxide, FRAP, ABTS and Nitric oxide assays. The antioxidant activity was evident and highly potent with the difference between the standards (DPPH assay - ascorbic acid; H_2O_2 assay - Vitamin C; FRAP assay - $FeSO_4$; ABTS assay - ascorbic acid; Nitric oxide assay - ascorbic acid) and Commelina benghalensis-mediated Calcium oxide nanoparticles being less than 5%.

3.2.1 DPPH assay

The results obtained from the assay indicates that the green synthesized calcium oxide nanoparticles had highly significant DPPH radical scavenging activity in comparison to the standard ascorbic acid as displayed in figure 2. The scavenging activity of the nanoparticles rose as the concentrations increased, mirroring the behavior of the standard. Highest activity was seen at 50 µg/ml while the closest difference to ascorbic acid was at 10 µg/ml.

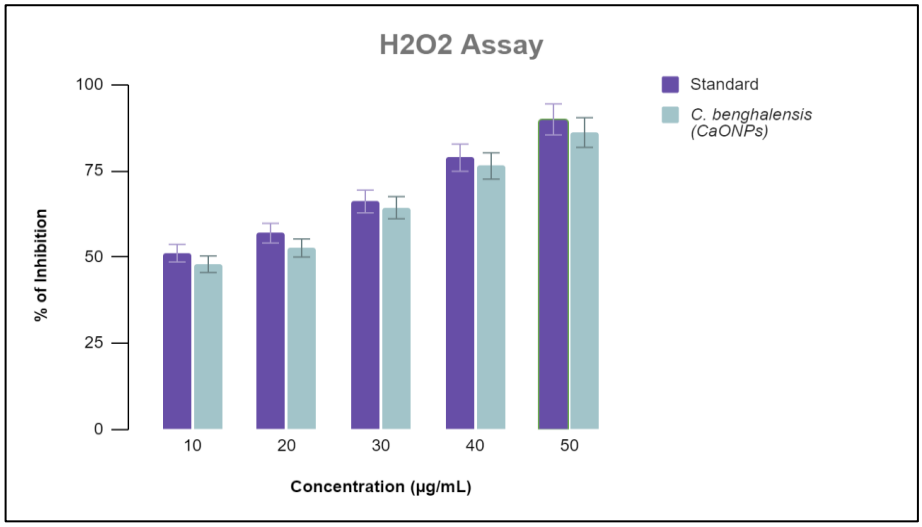
Figure 2: Graphical illustration of free radical scavenging activity of C. benghalensis-mediated Calcium oxide nanoparticles using the DPPH assay.



3.2.2 Hydrogen peroxide assay

This assay tested the free radical scavenging property of Commelina benghalensis-mediated Calcium oxide nanoparticles in comparison to Vitamin C as shown in figure 3. The results obtained showed that the free radical scavenging property of the nanoparticles increased as the concentration increased, a trend also seen in the standard. The difference of activity between the nanoparticles and the standard was considerably low. Least difference was seen at 30 and 40 µg/ml.

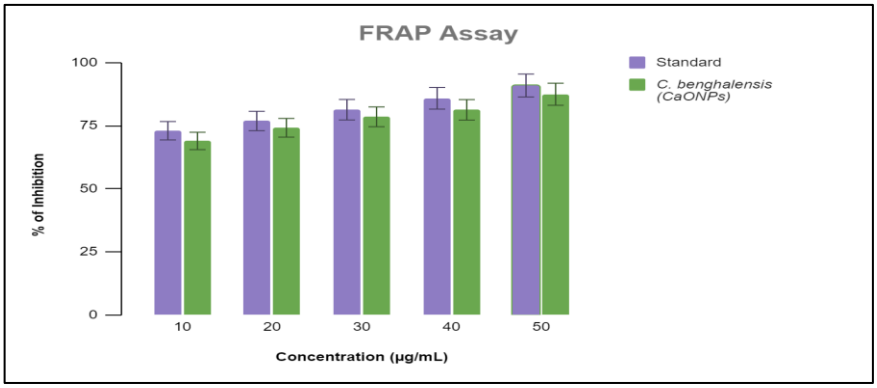
Figure 3: Graphical illustration of the antioxidant activity of Commelina benghalensis-mediated Calcium oxide nanoparticles using the hydrogen peroxide assay.



3.2.3 FRAP Assay

Green synthesized calcium oxide nanoparticles showed high free radical scavenging activity against the FRAP radicals as illustrated in figure 4. The results were compared to $FeSO_4$ which is the standard for this assay. The comparison showed that the difference in activity was low and would be reduced further at higher concentrations.

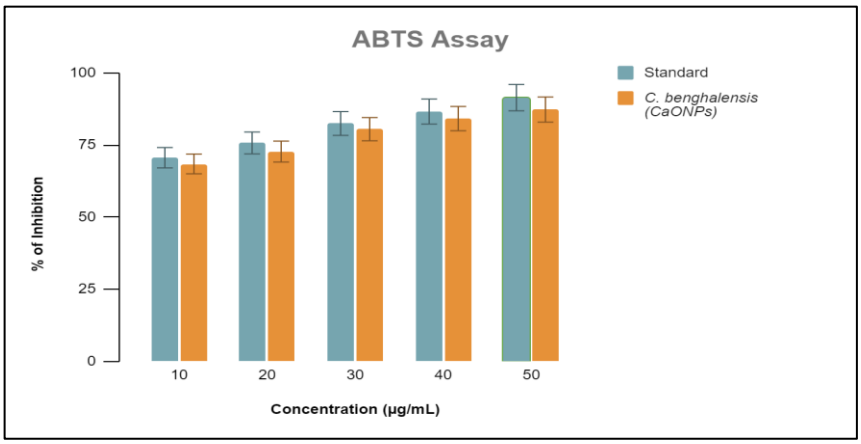
Figure 4 Graphical illustration of the antioxidant activity of Commelina benghalensis-mediated Calcium oxide nanoparticles using the ferric-reducing antioxidant powder (FRAP) assay.



3.2.4 ABTS Assay

Results of this assay showed that the Commelina benghalensis- mediated calcium oxide nanoparticles had highly significant free radical scavenging activity against ABTS radicals as shown in figure 5. On further comparison with ascorbic acid (standard), it was seen that the nanoparticles showed a similar activity trend as the standard and the difference between the activity levels was reduced with increase in concentration. Maximum ABTS radical scavenging efficiency was seen in the 50 µg/ml sample.

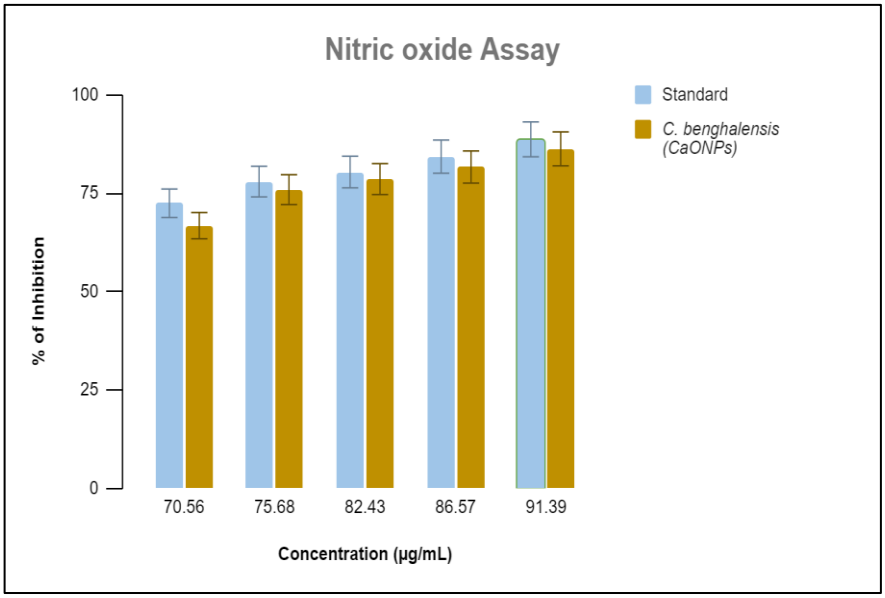
Figure 5: Graphical illustration of the antioxidant activity of Commelina benghalensis-mediated Calcium oxide nanoparticles using ABTS assay.



3.2.5. Nitric Oxide Assay:

Commelina benghalensis- mediated Calcium oxide nanoparticles exhibited high levels of free radical scavenging activity against nitric oxide radicals as shown in figure 6. The potency of the activity increased as the concentration increased. More than 80% inhibition was seen at 50 µg/ml. On comparing activity of the nanoparticles with activity of the standard ascorbic acid, negligible differences were observed.

Figure 6: Graphical illustration of the free radical scavenging activity of Commelina benghalensis- mediated Calcium oxide nanoparticles using the Nitric oxide (NO) assay.



4. Discussion:

In the past decade, there has been a surge in requirement for the implementation of nanotechnology within the medical field. Free radicals levels in humans are on the rise due to multiple reasons including dietary habits, pollution, exposure to radiating rays, metabolic disorders and health habits, hence raising the need for antioxidant interventions. Nanoparticles have been successful in providing a potent alternative. Commelina benghalensis, commonly called Benghal dayflower, has a rich phytochemical composition. These phytochemicals exhibit strong antioxidant, an antimicrobial, anti-inflammatory property amongst other medicinal attributes.

The mode of synthesis plays a major role in determining the properties of the synthesized compound. For this study, calcium oxide nanoparticles had been green synthesized through Commelina benghalensis mediation. This allows the plant extract to impart its beneficial properties to the nanoparticles while enhancing the natural attributes of the compound. As seen in a similar study where Azadirachta indica was proven to enhance the properties of metal oxide nanoparticles, the C. benghalensis extract of this study had yielded similar results

(Rajeshkumar et al., 2022).

Antioxidant activity of the nanoparticles was evaluated using a set of five standard assays - DPPH, H_2O_2, FRAP, ABTS, Nitric oxide. A study has revealed that CaO-NPs with high antioxidant and dispersion properties can be produced through green synthesis (Keerthiga et al., 2019). According to another study, where calcium oxide nanoparticles were combined with neem and clove along with melatonin, the nanoparticles showed significant antioxidant properties. Similar results were obtained in this study as the antioxidant activity levels displayed by the nanoparticles were comparable to existing standards (Mohapatra et al., 2020).

With further development and advanced integration, these nanoparticles can be potential non-toxic alternatives to existing standard antioxidant interventions. This study serves as a foundation for further research on the antioxidant properties of this unique plant-compound combination.

5. Conclusion:

Antioxidants are highly beneficial to living organisms. They neutralize the harmful free radicals and prevent damage to tissues. In this study, the free radical scavenging and antioxidant of property Commelina benghalensis- mediated Calcium oxide nanoparticles was evaluated. The plant is known to contain phytochemicals which have antioxidant and anti-inflammatory properties. The study incorporated these attributes of C. benghalensis to calcium oxide nanoparticles to enhance their existing properties. The antioxidant properties were evaluated using 5 standard free radical scavenging assays - DPPH, H_2O_2, FRAP, ABTS and Nitric Oxide. The results obtained indicated that the Commelina benghalensis- mediated CaO NPs had high antioxidant activity levels which were close to the existing standards. With further research, development and trials, these nanoparticles have a bright scope as a potential alternative to regularly used interventions. Ease of manufacturing, availability of plant, economic viability and non-toxicity of calcium oxide are some of the advantages these nanoparticles possess compared to other compounds.

Funding Details

No funding was acquired for carrying out this study.

Disclosure Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Ahmad, U., Khan, M. A., Zahid, M. U., Alarjani, K. M., Gawwad, M. R. A., Hussain, S. J., & Bokhari, S. a. I. (2024). Effect of surface capping on the biological properties of calcium oxide nanoparticles (CaO-NPs). Chemical Papers, 78(8), 4971–4990. https://doi.org/10.1007/s11696-024-03446-x
2. Das, D., Nath, B. C., Phukon, P., & Dolui, S. K. (2013). Synthesis and evaluation of antioxidant and antibacterial behavior of CuO nanoparticles. Colloids and Surfaces. B, Biointerfaces, 101,

430–433. https://doi.org/10.1016/j.colsurfb.2012.07.002

3. Eram, R., Kumari, P., Panda, P., Singh, S., Sarkar, B., Mallick, M., & Verma, S. (2021). Cellular Investigations on Mechanistic Biocompatibility of Green Synthesized Calcium Oxide Nanoparticles with Danio rerio. Journal of Nanotheranostics, 2(1), 51–62. https://doi.org/10.3390/jnt2010004
4. Harris, J., Malaiappan, S., & S, R. (2023). The Development and Evaluation of Melatonin-Loaded, Calcium Oxide Nanoparticle-Based Neem and Clove Extract: An In Vitro Study. Cureus. https://doi.org/10.7759/cureus.46293
5. Ibrahim, J., Ajaegbu, V. C., & Egharevba, H. O. (n.d.). Pharmacognostic and Phytochemical Analysis of Commelina benghalensis L. OpenSIUC. https://opensiuc.lib.siu.edu/ebl/vol2010/iss5/7/
6. Keerthiga, N., Anitha, R., Rajeshkumar, R. S., & Lakshmi, T. (2019). Antioxidant Activity of Cumin Oil Mediated Silver Nanoparticles. Pharmacognosy Journal, 11(4), 787–789. https://doi.org/10.5530/pj.2019.11.125
7. Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry, 12(7), 908–931. https://doi.org/10.1016/j.arabjc.2017.05.011
8. Kubmarawa, D., Ajoku, G., Enwerem, N., & Okorie, D. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. https://www.ajol.info/index.php/ajb/article/view/57755
9. Louwakul, P., Saelo, A., & Khemaleelakul, S. (2016). Efficacy of calcium oxide and calcium hydroxide nanoparticles on the elimination of Enterococcus faecalis in human root dentin. Clinical Oral Investigations, 21(3), 865–871. https://doi.org/10.1007/s00784-016-1836-x
10. Andriushchenko, Kateryna, et al. "Intelligent enterprise capital control based on Markov chain." Acta Innovations 45 (2022): 18-30.
11. Mohapatra, S., Leelavathi, L., Rajeshkumar, S., Sakthi, D. S., & Jayashri, P. (2020, June 22). Assessment of Cytotoxicity, Anti-Inflammatory and Antioxidant Activity of Zinc Oxide Nanoparticles Synthesized Using Clove and Cinnamon Formulation--An In-Vitro Study. Document - Gale Academic OneFile. https://go.gale.com/ps/i.do?id=GALE%7CA630993246&sid=googleScholar&v=2.1&it=r&linkaccess=abs&issn=22784748&p=AONE&sw=w&userGroupName=anon%7Efa992986&aty=open-web-entry
12. Pal, G., Rai, P., & Pandey, A. (2019). Green synthesis of nanoparticles: A greener approach for a cleaner future. In Elsevier eBooks (pp. 1–26). https://doi.org/10.1016/b978-0-08-102579-6.00001-0
13. Patil, S. P., Chaudhari, R. Y., & Nemade, M. S. (2022). Azadirachta indica leaves mediated green synthesis of metal oxide nanoparticles: A review. Talanta Open, 5, 100083. https://doi.org/10.1016/j.talo.2022.100083
14. Adewuyi, Reuben, et al. "Prediction of Impact Strength of TIG Welded Cr-Mo Steel Using Artificial Neural Networks." Metallurgical and Materials Engineering 30.1 (2024): 61-69.
15. Roy, A., Gauri, S. S., Bhattacharya, M., & Bhattacharya, J. (2013). Antimicrobial Activity of CaO Nanoparticles. Journal of Biomedical Nanotechnology, 9(9), 1570–1578. https://doi.org/10.1166/jbn.2013.1681
16. Wu, L., Wu, Y., Che, X., Luo, D., Lu, J., Zhao, R., Iqbal, M. Z., Zhang, Q., Wang, X., & Kong, X. (2021a). Characterization, antioxidant activity, and biocompatibility of selenium nanoparticle-loaded thermosensitive chitosan hydrogels. Journal of Biomaterials Science. Polymer Ed., 32(10), 1370–1385. https://doi.org/10.1080/09205063.2021.1917813