Free Radical Scavenging and Anti-Inflammatory Activity of Ormocarpum Cochinchinense Mediated Calcium Oxide Nanoparticles

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**Abstract:** The increased interest in nanotechnology has created new opportunities to improve the therapeutic potential of medicinal plants. Ormocarpum cochinchinense, a plant with traditional medicinal applications, has shown promise in modern medicine due to its antioxidant and anti-inflammatory characteristics. Recent research has focused on the creation of calcium oxide nanoparticles (CaO NPs) mediated Ormocarpum cochinchinense extracts in order to improve these bioactivities. This article looks at the free radical scavenging and anti-inflammatory properties of Ormocarpum cochinchinense-mediated CaO NPs, emphasizing their importance in oxidative stress and inflammation-related illnesses.

**Keywords**: Ormocarpum cochinchinense, antioxidant, oxidative stress, anti-inflammatory, free radical scavenging.

# Introduction

Ormocarpum cochinchinense is a medicinal plant known for its anti-inflammatory effects and prospective use in osteogenic applications.[[(NIIR Board of Consultants and Engineers, 2006)](https://paperpile.com/c/nGyI9K/LQVm)] Recent studies show its efficacy in encouraging bone healing, while it has traditionally been employed in bone fracture therapies. Its extracts promote osteoblastic proliferation, speed bone matrix synthesis, and help to create calluses, all of which are necessary for bone healing. The plant's success is related to its strong antioxidant and anti-inflammatory phytochemicals, making it a good choice for natural treatments in bone health and inflammation.[(Mahdi, 2017)](https://paperpile.com/c/nGyI9K/eX9T) [(Harsha & Subramanian, 2022)](https://paperpile.com/c/nGyI9K/KP5pC)[(Deepika et al., 2022)](https://paperpile.com/c/nGyI9K/co1CB)[(Solanki et al., 2022)](https://paperpile.com/c/nGyI9K/EdsQN) The plant's bioactive components, particularly flavonoids and polyphenols, have been shown to have strong anti-inflammatory properties by blocking pro-inflammatory cytokines and lowering oxidative stress. Furthermore, it increases osteoblastic activity, boosts bone matrix formation, and hastens fracture healing.[(Markham, 1862)](https://paperpile.com/c/nGyI9K/exGE)[(Chidambaram et al., 2022)](https://paperpile.com/c/nGyI9K/EyV4L).[(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/nGyI9K/WuOzE) Animal studies have demonstrated that its extracts can promote bone regeneration while decreasing inflammation, making it a good candidate for treating bone fractures and inflammatory illnesses.​[(Naqbi, 2017)](https://paperpile.com/c/nGyI9K/kw4W)[(Ajay, Rakshagan, et al., 2022)](https://paperpile.com/c/nGyI9K/egOgu) Ormocarpum cochinchinense has strong free radical scavenging activities, owing to its high concentration of phenolic compounds, flavonoids, and other antioxidants. These chemicals neutralize free radicals, shielding cells from oxidative stress and the resulting damage. [(Tringali, 2011)](https://paperpile.com/c/nGyI9K/gEcK), [(Jadhav, 2008; Shukla & Iravani, 2018; Tringali, 2011)](https://paperpile.com/c/nGyI9K/gEcK+nXbY+tbNX)[(Jabin et al., 2021)](https://paperpile.com/c/nGyI9K/tkJ3s)[(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/nGyI9K/KqYOz) [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/nGyI9K/VVAHh) Studies have shown that various extracts of this plant may effectively scavenge free radicals such as DPPH and ABTS, indicating its high antioxidant potential. This activity is critical for alleviating oxidative stress-related disorders, highlighting the therapeutic usefulness of Ormocarpum cochinchinense. [[(Shukla & Iravani, 2018)](https://paperpile.com/c/nGyI9K/tbNX)][(Ajay, Suma, et al., 2022)](https://paperpile.com/c/nGyI9K/1JUTN) [(Katyal et al., 2021)](https://paperpile.com/c/nGyI9K/j1ZXV)

# Materials and Methods

Ormocarpum cochinchinense leaves, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Calcium Oxide. One gram of leaf powder was combined with one hundred milliliters of deionized water, brought to a boil at sixty degrees Celsius for five minutes, and then allowed to sit in a rotating apparatus for three days.[[(Ratajczak, 2020)](https://paperpile.com/c/nGyI9K/YCFx)] Whatman filter paper (no. 1) was used to filter the supernatant. Following another centrifugation of the extract, the pellets were collected.

## Preparation of Calcium oxide nanoparticles

About 50 mL of Ormocarpum cochinchinense plant extract was added to 50 mL of an aqueous solution containing 0.37 g of calcium hydroxide in order to create the calcium oxide nanoparticles. These flasks were then placed in an orbital shaker and allowed to remain at room temperature for a while. Color changes were detected in the nanoparticle solutions. The color shifts showed nanoparticle formation and UV readings over several hours.[[(Jideani & Anyasi, 2020; Shukla & Iravani, 2018)](https://paperpile.com/c/nGyI9K/tbNX+8WGr)] The produced nanoparticles were centrifuged for 10 minutes at 8000 rpm, and the pellets have been extracted for future research.

## Antioxidant Activity Assessment

### DPPH Radical Scavenging Assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) was produced as a 0.1 mM stock solution in methanol. A workable solution (20 µM) was made from scratch using the stock for the test. 200 µL of the DPPH working solution was then added to Ormocarpum cochinchinense mediated Calcium Oxide nanoparticles at varying concentrations (10, 20, 30, 40, and 50 µg/mL) on a 96-well plate. For ten minutes, the mixture was incubated at room temperature in the dark. Using methanol as the blank, absorbance was measured at 517 nm using a spectrophotometer. Using the following formula, the proportion of DPPH scavenging activity was determined.[[(Mohedano & Mingo, 2021)](https://paperpile.com/c/nGyI9K/ZJl0)]

% DPPH Scavenging Activity = [(A Control – A Sample) / Acontrol] × 100 (1)

### Hydrogen Peroxide (H₂O₂) Radical Scavenging Assay

The H₂O₂ scavenging activity of biosynthesized Ormocarpum cochinchinense mediated Calcium Oxide nanoparticles was determined using a 40 mM H₂O₂ solution prepared in phosphate buffer (pH 7.4).[[(Ozturk & Hakeem, 2018)](https://paperpile.com/c/nGyI9K/ZIcw)] Different concentrations (10, 20, 30, 40, 50 µg/mL) of CaO NPs and ascorbic acid (standard) were added to 0.6 mL of H₂O₂ solution. Following 10 minutes of incubation in the dark, the absorbance was measured at 230 nm using a spectrophotometer. The percentage of H₂O₂ scavenging activity was calculated using the formula:

% inhibition= ((Absorbance of control- Absorbance of sample)×100)/ Absorbance of control

### Ferric Reducing Antioxidant Power (FRAP) Assay

* Acetate buffer (300 mM, pH 3.6) was prepared by dissolving 3.1 g sodium acetate trihydrate in 16 mL glacial acetic acid, then diluting to 1 L with distilled water.
* TPTZ(2,4,6-tripyridyl-s-triazine) Prepared as a 10 mM solution in 40 mM HCl.
* FeCl₃·6H₂O was Prepared as a 20 mM solution.
* Working FRAP reagent was Prepared by mixing the above solutions in a 10:1:1 ratio immediately before use.[[(Gašparović, 2020)](https://paperpile.com/c/nGyI9K/gvj8)]

In the assay, 2.3 mL of the FRAP reagent was mixed with 0.7 mL of the sample solution Ormocarpum cochinchinense mediated Calcium Oxide nano particle at various concentrations (10, 20, 30, 40, 50 µg/mL). The mixture was incubated at 37°C for 30 minutes in the dark, and the absorbance was read at 593 nm using a spectrophotometer. An increased absorbance indicated a higher reduction capability. The assay was conducted in triplicate, with ascorbic acid as the standard.

### ABTS Radical Cation Decolorization Assay

The ABTS radical cation (ABTS⁺) was generated by reacting 7.0 mM ABTS in 50% ethanol with 2.45 mM potassium persulfate in distilled water,[[(Chauhan et al., 2020)](https://paperpile.com/c/nGyI9K/FA62)] followed by storage at 4°C for 24 hours. Prior to use, the reagent was diluted with 50% ethanol to achieve an absorbance of 1.0 (±0.02) at 734 nm. In 96-well microplates, 250 µL of ABTS⁺ solution was added to 20 µL of the sample solution of O.cochinchinense CaO NP at different concentrations (10, 20, 30, 40, 50 µg/mL). The reaction was incubated for 10 minutes in the dark, followed by absorbance measurement at 734 nm. The radical scavenging activity was calculated using:

I (%) = [(Abs0−Abs1)/Abs0] × 100, where Abs0 is the absorbance of the blank and Abs1 is the absorbance in the presence of the test compound at different concentrations.

### Nitric Oxide (NO) Radical Inhibition Assay

The nitric oxide radical inhibition was evaluated using the Griess Illosvoy reaction. The reaction mixture contained 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate-buffered saline, and 0.5 mL of the O.cochinchinense CaO NP extract or standard (ascorbic acid) at various concentrations (10, 20, 30, 40, 50 µg/mL). Following incubation at 25°C for 150 minutes, 0.5 mL of the reaction mixture was added to 1 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 minutes. Subsequently, 1 mL of naphthyl ethylenediamine dihydrochloride was added and mixed. The reaction was incubated for 30 minutes at 25°C, resulting in the formation of a pink chromophore. Absorbance was measured at 540 nm, and the percentage of NO radical inhibition was calculated as follows:

% scavenging/Reduction = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100 (2)

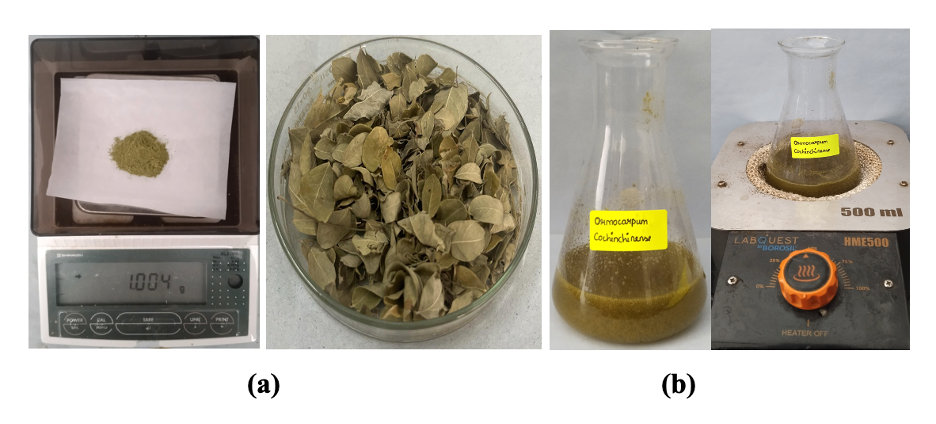
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Figure 1: (a) O.cochinchinense leaf powder (b) O.cochinchinense aqueous extract

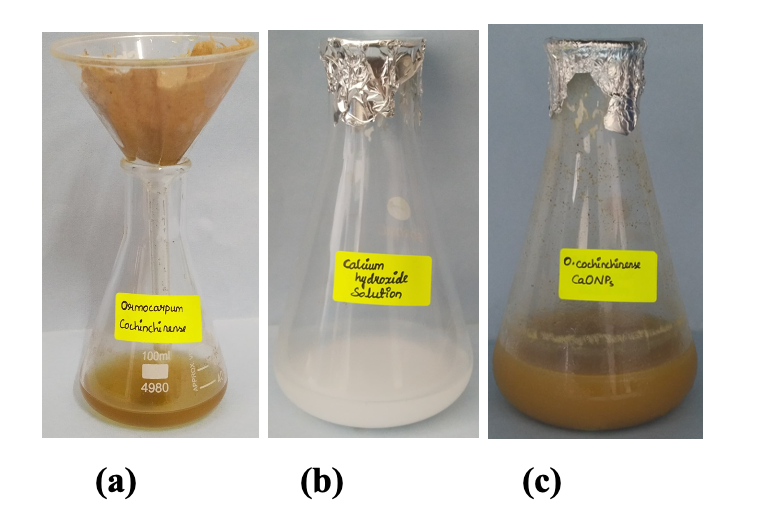
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Figure 2: (a) O.cochinchinense extract filtrate (b) Calcium Hydroxide solution (c) O.cochinchinense mediated Calcium Oxide Nanoparticle.

## Anti-inflammatory Activity

Three common tests were used to assess the anti-inflammatory properties of the green-synthesized calcium oxide nanoparticles mediated by Ormocarpum cochinchinense the denaturation assay for bovine serum albumin (BSA), the denaturation assay for egg albumin, and the membrane stabilization assay[(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/nGyI9K/xNMl2)[(Sushanthi et al., 2021)](https://paperpile.com/c/nGyI9K/IVpv6)[(Harsha et al., 2022)](https://paperpile.com/c/nGyI9K/JN64I)

The BSA denaturation experiment was performed to evaluate the prevention of protein denaturation.[(Prakash, 2020)](https://paperpile.com/c/nGyI9K/5UiW)[(Tiwari & Jain, 2023)](https://paperpile.com/c/nGyI9K/87mye)[(Graf et al., 2023)](https://paperpile.com/c/nGyI9K/PjaCi)In this experiment, different quantities of O.cochinchinense mediated CaO NP (10, 20, 30, 40, and 50 µg/mL) were combined with 0.45 mL of bovine serum albumin(Chehelgerdi et al., 2023). The mixture's pH was raised to 6.3. After being left at room temperature for ten minutes, the samples were incubated for thirty minutes at 55°C in a water bath. Dimethyl sulfoxide (DMSO) was the control, and diclofenac sodium was the standard reference. At 660 nm, the absorbance was determined using spectrophotometry.

The percentage of protein denaturation inhibition was calculated using the formula:

% inhibition= (Absorbance of control- Absorbance of sample)×100)/ Absorbance of control (3)

Egg Albumin Denaturation Assay, the anti-inflammatory effect was assessed further using the egg albumin denaturation assay. 2.8 mL of 1X phosphate buffer and 0.2 mL of fresh egg albumin were combined to create a reaction mixture.[[(Dinda, 2019)](https://paperpile.com/c/nGyI9K/j2DW)] Then, various quantities of O. cochinchinense Calcium Oxide nanoparticles (10, 20, 30, 40, and 50 µg/mL) were applied. After adjusting the pH to 6.3, the mixture was incubated for 10 minutes at ambient temperature and then for 30 minutes at 55°C in a water bath (Saadh et al., 2024). The control was DMSO, while the standard was Diclofenac sodium. At 660 nm, the absorbance was measured. Using the following formula, the percentage of inhibition of protein denaturation was determined:

% inhibition= (Absorbance of control- Absorbance of sample)×100)/Absorbance of control (4)

### Membrane stabilization assay

The produced silver nanoparticles' ability to stabilize membranes was assessed using the membrane stabilization assay. This assay assesses a compound's capacity to maintain cell membrane stability by averting disruption.[[(Selvaraj et al., 2022)](https://paperpile.com/c/nGyI9K/Eazr)]

### Preparation of RBC suspension

To separate red blood cells (RBCs) from other components, fresh human blood was drawn into a sterile tube with an anticoagulant. The blood was centrifuged at 3000 RPM for 10 minutes at room temperature. After removing the supernatant, the RBCs underwent three rounds of washing in phosphate-buffered saline (PBS)[(Neha et al., 2021)](https://paperpile.com/c/nGyI9K/Svrdi)[(Maliael et al., 2021)](https://paperpile.com/c/nGyI9K/3KHqu)[(Lakshmi, 2021)](https://paperpile.com/c/nGyI9K/uRrWP). After being cleaned, the red blood cells were again suspended in 50 mM Tris-HCl buffer (pH 7.4), resulting in a 10% (v/v) RBC suspension.

Each centrifuge tube had 1 mL of the RBC suspension added to it. Next, various quantities of calcium oxide nanoparticles (10, 20, 30, 40, and 50 µg/mL) were introduced into each tube. After carefully combining the tubes, they were incubated for 30 minutes at 37°C. To remove the RBCs, the tubes were centrifuged for five minutes at room temperature at 2500 RPM after incubation. On a UV-Vis spectrophotometer, the absorbance of the supernatant was measured at 560 nm.

The % inhibition of hemolysis were calculated using formula:

% inhibition = [(OD control – OD sample) / OD control] x 100 (5)

# RESULTS AND DISCUSSION

## Antioxidant activity

The antioxidant activity of Ormocarpum cochinchinense calcium oxide nanoparticles (CaO NPs) was determined using five different assays: DPPH, H2O2, FRAP, ABTS, and Nitric Oxide. Results were compared to conventional antioxidants at doses ranging from 10 to 50 µg/mL.The DPPH radical scavenging activity demonstrates that both conventional and CaO NPs display increasing antioxidant activity at greater doses. The standard showed 93.15% inhibition at 50 µg/mL, while CaO NPs showed 91.43%, indicating a minor difference in efficacy. Table 1, Graph 1.

TABLE :1 Antioxidant activity OF Ormocarpum cochinchinense mediated CaO NP using DPPH radical scavenging assay:

|  |  |  |
| --- | --- | --- |
| DPPH | **Standard** | Ormocarpum cochinchinense (CaO NPs) |
| 10 | 66.25 | 62.82 |
| 20 | 78.52 | 75.72 |
| 30 | 85.63 | 83.37 |
| 40 | 88.68 | 86.14 |
| 50 | 93.15 | 91.43 |

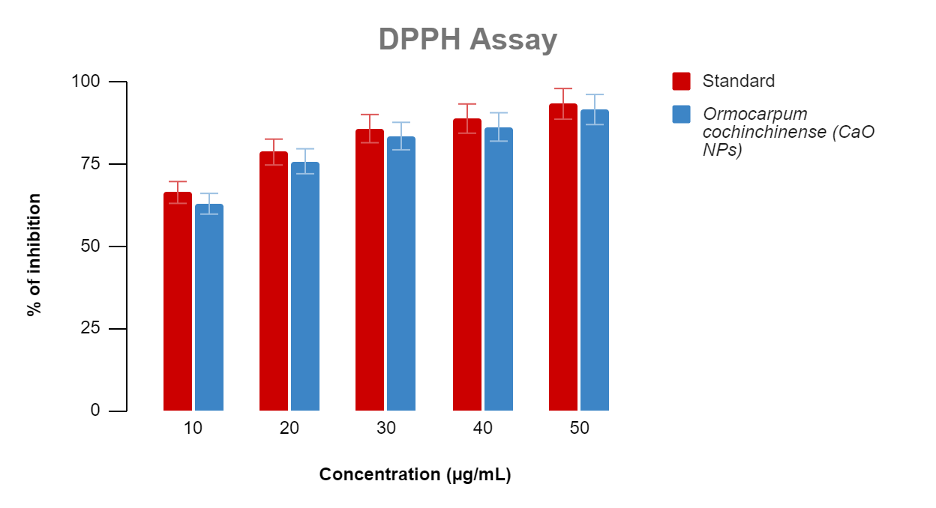
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Figure 1: Antioxidant activity of O.cochinchinense mediated CaO NP- DPPH radical scavenging assay.

At the greatest concentration, the standard inhibited hydrogen peroxide (H2O2) by 89.9%, while CaO NPs inhibited it by 87.8%. The findings show that Ormocarpum cochinchinense CaO NPs exhibit slightly lower but equivalent activity to the norm.

TABLE 2: Antioxidant activity OF Ormocarpum cochinchinense mediated CaO NP using H2O2 radical scavenging assay

|  |  |  |
| --- | --- | --- |
| H2O2 | **Standard** | Ormocarpum cochinchinense (CaO NPs) |
| 10 | 51.1 | 48.3 |
| 20 | 56.9 | 52.7 |
| 30 | 66.1 | 64.1 |
| 40 | 78.8 | 74.2 |
| 50 | 89.9 | 87.8 |

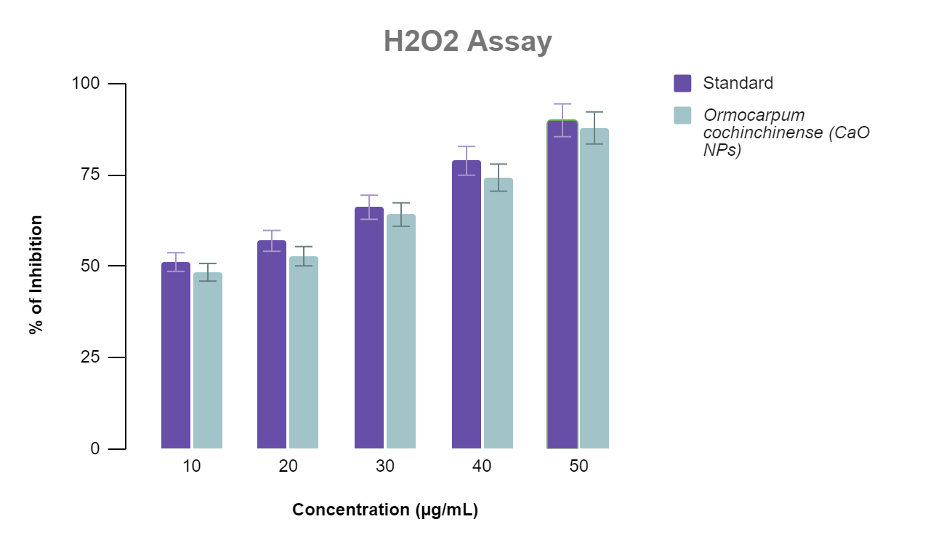
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Figure 2: Antioxidant activity of O.cochinchinense mediated CaO NP- H2O2 radical scavenging assay.

The Ferric Reducing Antioxidant Power (FRAP) experiment showed that the standard reached 90.89% at 50 µg/mL, while CaO NPs achieved 87.61%. The results consistently show that CaO NPs have high antioxidant capabilities at all concentrations.

TABLE 3: Antioxidant activity OF Ormocarpum cochinchinense mediated CaO NP using H2O2 radical scavenging assay

|  |  |  |
| --- | --- | --- |
| FRAP |  |  |
| conc | standard | Ormocarpum cochinchinense (CaO NPs) |
| 10 | 72.98 | 70.42 |
| 20 | 76.84 | 74.58 |
| 30 | 81.31 | 78.63 |
| 40 | 85.84 | 81.49 |
| 50 | 90.89 | 87.61 |

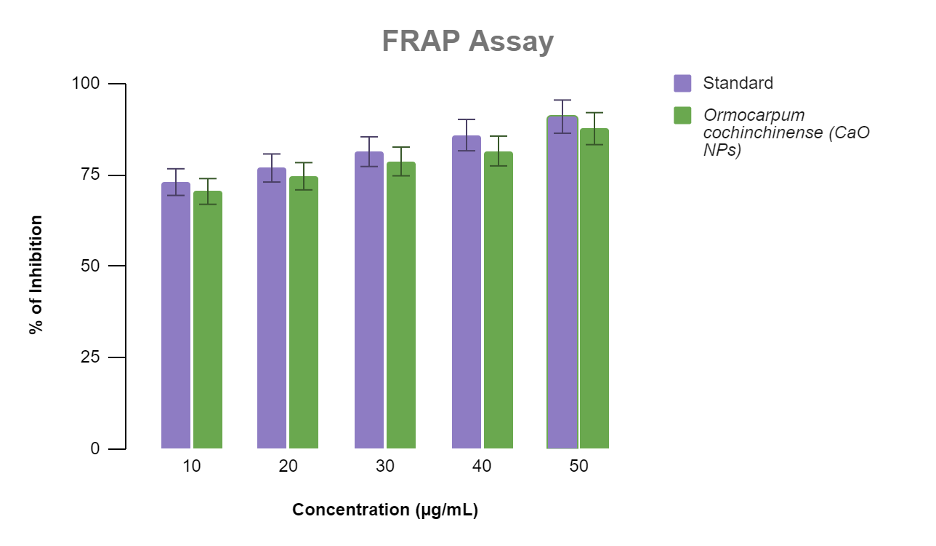
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Figure 3: Antioxidant activity of O.cochinchinense mediated CaO NP- FRAP assay.

In the ABTS radical scavenging experiment, both the standard and CaO NPs demonstrated concentration-dependent action. The standard achieved 91.39% inhibition at the maximum concentration, while CaO NPs reached 88.32%.

TABLE 4: Antioxidant activity OF Ormocarpum cochinchinense mediated CaO NP using ABT radical scavenging assay

|  |  |  |
| --- | --- | --- |
| ABTS |  |  |
| conc | standard | Ormocarpum cochinchinense (CaO NPs) |
| 10 | 70.56 | 66.84 |
| 20 | 75.68 | 72.91 |
| 30 | 82.43 | 78.45 |
| 40 | 86.57 | 84.79 |
| 50 | 91.39 | 88.32 |

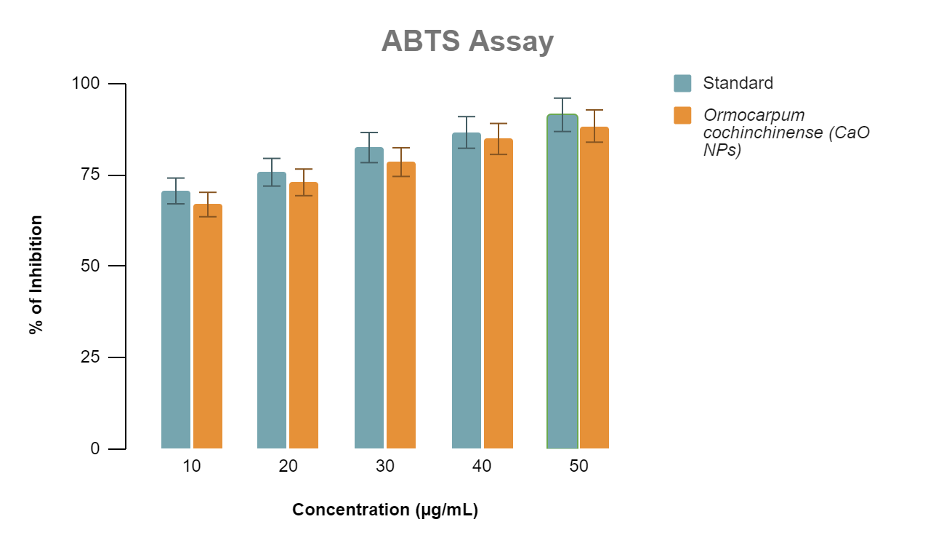
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Figure 4: Antioxidant activity of O.cochinchinense mediated CaO NP- ABTS assay.

## NITRIC OXIDE SCAVENGING ASSAY

Nitric oxide scavenging activity increases with concentration. At 50 µg/mL, the standard demonstrated 88.67% inhibition, followed by CaO NPs with 86.16%. These findings provide additional support for the CaO NPs' antioxidant activity.

TABLE 5: Antioxidant activity OF Ormocarpum cochinchinense mediated CaO NP using Nitric Oxide radical scavenging assay

|  |  |  |
| --- | --- | --- |
| Nitric oxide |  |  |
| conc | standard | Ormocarpum cochinchinense (CaO NPs) |
| 10 | 72.43 | 68.67 |
| 20 | 77.94 | 73.39 |
| 30 | 80.37 | 77.45 |
| 40 | 84.28 | 82.71 |
| 50 | 88.67 | 86.16 |

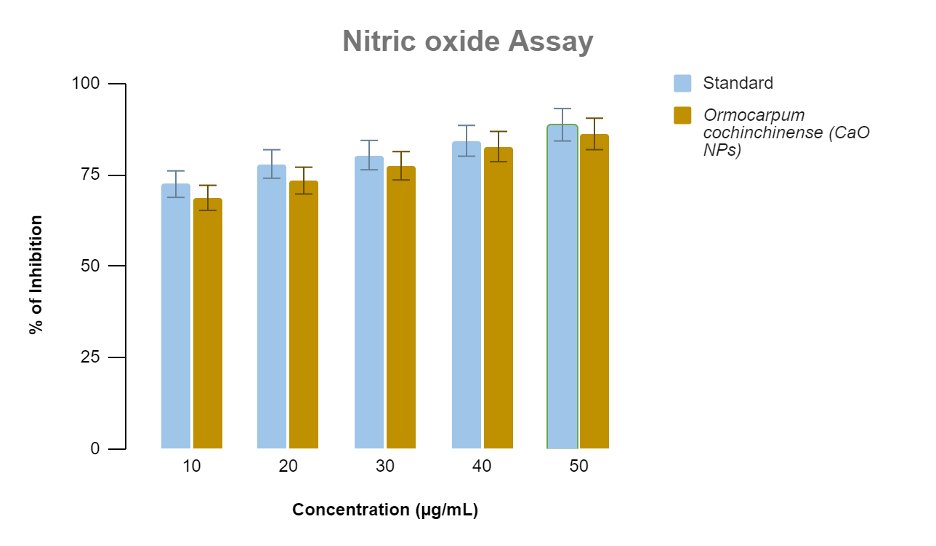
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Figure 5: Antioxidant activity of O.cochinchinense mediated CaO NP- Nitric Oxide assay.

Overall, the antioxidant activity data show that Ormocarpum cochinchinense CaO NPs have a high potential as an antioxidant agent, with results nearly identical to those of traditional antioxidants across all assays.

# Anti-inflammatory activity

The anti-inflammatory effect of Ormocarpum cochinchinense CaO NPs was tested using three distinct assays: BSA denaturation, egg albumin denaturation (EA), and membrane stabilization assay (MSA).

TABLE 6: Antiinflammatory activity OF Ormocarpum cochinchinense mediated CaO NP using BSA assay

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| BSA | 10 | 20 | 30 | 40 | 50 |
| Ormocarpum cochinchinense (CaO NPs) | 44 | 55 | 67 | 74 | 79 |
| Standard | 47 | 60 | 72 | 78 | 84 |

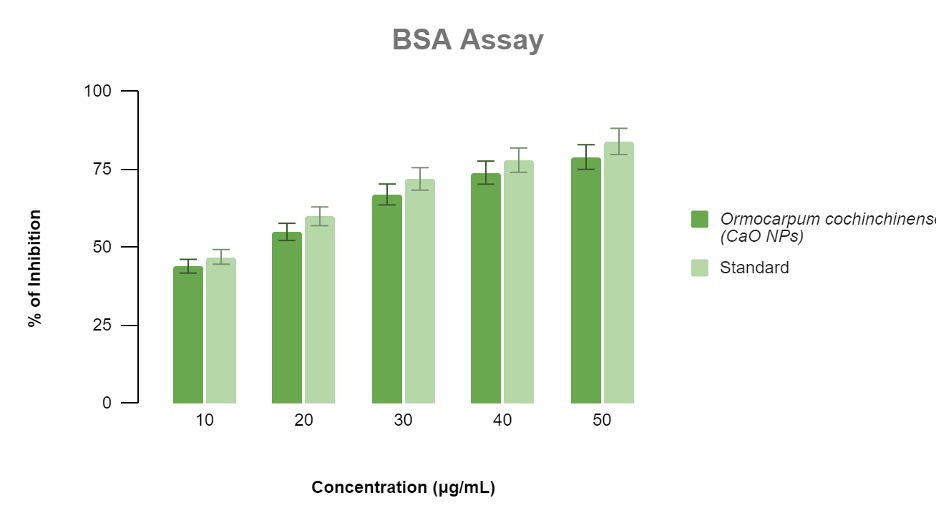
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Figure 6: Anti-inflammatory activity of O. cochinchinense mediated CaO NP- BSA assay.

CaO NPs effectively inhibited protein denaturation, with a maximum inhibition of 79% at 50 µg/mL, surpassing the standard's 84%. This shows that CaO NPs are almost as effective as the standard in preventing protein denaturation, which is an important sign of anti-inflammatory effectiveness.

TABLE 7: Anti-inflammatory activity OF Ormocarpum cochinchinense mediated CaO NP using EA assay:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| EA | 10 | 20 | 30 | 40 | 50 |
| Ormocarpum cochinchinense (CaO NPs) | 51 | 59 | 64 | 68 | 77 |
| Standard | 55 | 64 | 69 | 72 | 81 |

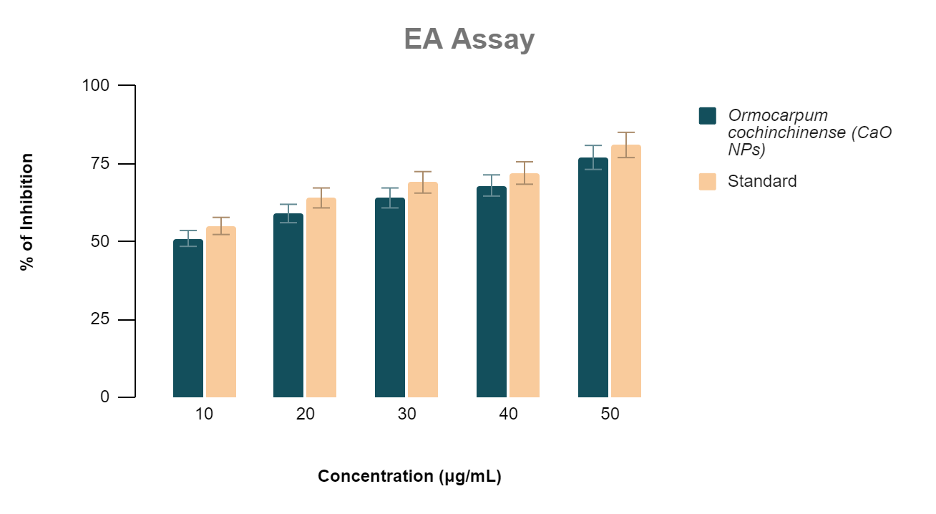
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Figure 7: Anti-inflammatory activity of O.cochinchinense mediated CaO NP- EA assay.

Denaturation of Egg Albumin (EA): CaO NPs demonstrated inhibition levels ranging from 51% at 10 µg/mL to 77% at 50 µg/mL, compared to the typical range of 55% to 81%. The close inhibition results show that CaO NPs are nearly as effective as the standard in suppressing protein denaturation.

TABLE 8: Antiinflammatory activity OF Ormocarpum cochinchinense mediated CaO NP using MSA assay:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| MSA | 10 | 20 | 30 | 40 | 50 |
| Ormocarpum cochinchinense (CaO NPs) | 52 | 64 | 72 | 77 | 83 |
| Standard | 58 | 70 | 77 | 82 | 89 |

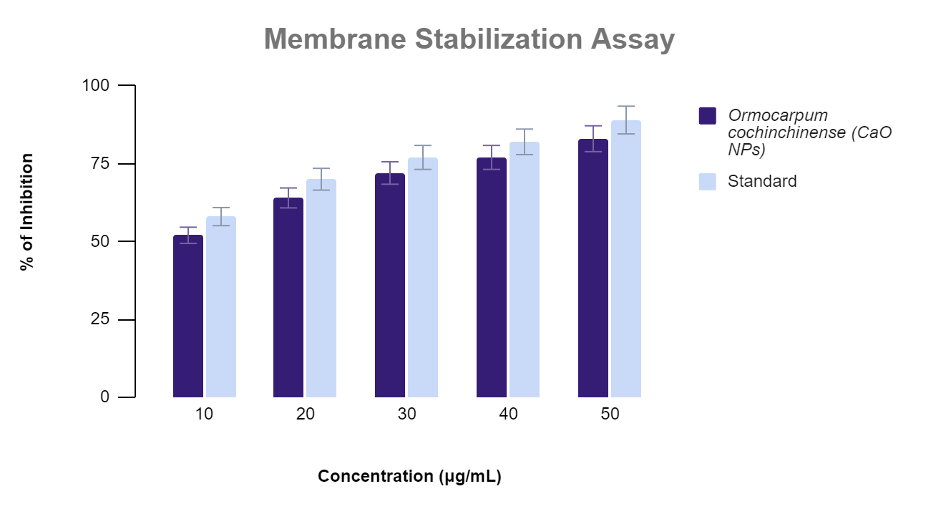
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Figure 8: Antiinflammatory activity OF Ormocarpum cochinchinense mediated CaO NP using MSA assay:

Membrane Stabilization Assay (MSA): The MSA results showed that CaO NPs successfully prevent hemolysis, with 83% inhibition at the maximum concentration versus 89% for the standard. The findings indicate that CaO NPs offer significant protection against cell membrane damage, supporting their anti-inflammatory properties.

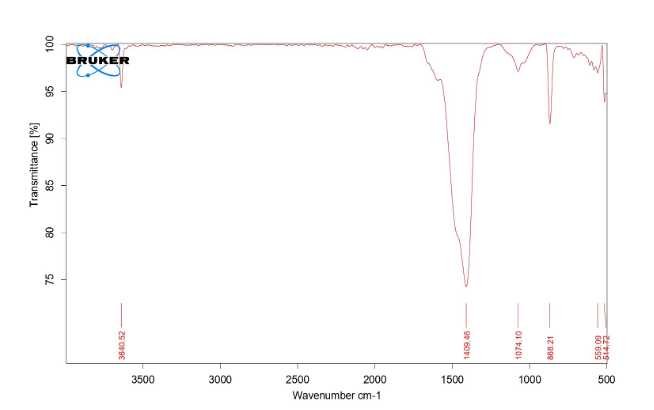
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Figure 9: FTIR analysis of O.cochinchinense CaO nanoparticles.

The FTIR spectrum offers insights into the functional groups found in the Ormocarpum cochinchinense calcium oxide nanoparticle material. The large signal at 3640 cm⁻¹ indicates the presence of hydroxyl groups, either from water adsorbed onto nanoparticles or residual plant-based organic molecules. The peaks in the lower wavenumber area (about 559 and 541 cm⁻¹) are likely calcium oxide, indicating successful production of CaO nanoparticles. Peaks at 1409, 1074, and 868 cm⁻¹ indicate organic functional groups interacting with the nanoparticle surface. This analysis highlights the characteristic vibrational frequencies of both organic and inorganic components in the sample, suggesting a composite nature with calcium oxide nanoparticles [(Dharman 2021)](https://paperpile.com/c/nGyI9K/CJY5q). The sharp and prominent peak at 3640.52 cm⁻¹: O-H Stretching (Hydroxyl Group) in the higher wavenumber region corresponds to O-H stretching vibrations. Hydroxyl groups are commonly found in alcohols, phenols, and water. In your sample, this signal could suggest the presence of surface-adsorbed water molecules or hydroxyl groups on the calcium oxide (CaO) nanoparticles. This is frequent because CaO is highly hygroscopic, which means it easily absorbs moisture from the atmosphere. 1409.46 cm⁻¹: C-O Stretching or Symmetric O-C-O Stretching This peak could correspond to the asymmetric or symmetric stretching of the O-C-O bond in carbonate ions (CO₃²⁻). This might indicate the presence of calcium carbonate (CaCO₃), a by-product of CaO's reaction with CO₂ from the air, forming a surface layer of CaCO₃.It could also be linked to bending vibrations from C-H groups in organic compounds, particularly if there are remaining organic components from the Ormocarpum cochinchinense plant extract utilized in nanoparticle formulation. This peak is typically associated with the stretching vibrations of the C-O bond1074.10 cm⁻¹: C-O Stretching (Alcohols, Ethers, or Esters), often found in alcohols, ethers, and esters. In the context of green synthesis, it could arise from plant-based organic molecules present in the Ormocarpum cochinchinense extract. The Ormocarpum cochinchinense extract may act as a stabilizer or capping agent for the CaO nanoparticles, preventing aggregation, and this peak could be associated with those organic stabilizers. This peak is characteristic of C-H out-of-plane bending 4. 868.21 cm⁻¹vibrations, often observed in aromatic compounds. The presence of aromatic rings suggests the plant extract contains aromatic hydrocarbons, which could play a role in the reduction and stabilization of the nanoparticles during green synthesis.

559.09 cm⁻¹ and 541.72 cm⁻¹ These low-frequency peaks are typical of metal-oxygen (M-O) stretching vibrations, and in this case, they are most likely due to Ca-O bonds. These bands confirm the presence of calcium oxide (CaO) nanoparticles in the sample, and match to the expected metal-oxygen bond vibrational modes in the calcium oxide crystal lattice.

## Nanoparticle Stabilization

The existence of peaks associated with organic functional groups (such as O-H and C-O) may indicate that the Ormocarpum cochinchinense extract not only functions as a reducing agent during nanoparticle formation, but also stabilizes the CaO nanoparticles. Plant extracts frequently contain polyphenols, terpenoids, and flavonoids that can attach to nanoparticle surfaces, reducing agglomeration. The lack of prominent peaks in the 2800-3000 cm⁻¹ area suggests minimal aliphatic C-H stretching (common in simple hydrocarbons). This could imply that the organic compounds in the plant extract are more likely involved in stabilizing the nanoparticle surface rather than staying free and unbound chemicals. The large signal at 3640.52 cm⁻¹ shows the presence of moisture or hydroxyl groups, possibly from the environment or plant extract. Because of the material's high affinity for moisture, many CaO nanoparticle samples exhibit some degree of water adsorption. The FTIR spectra confirms the creation of calcium oxide nanoparticles, indicating Ca-O bonds at low wavenumbers (559 cm⁻¹ and 541 cm⁻¹). The peaks at 3640.52 cm⁻¹ and 1409.46 cm⁻¹ indicate interaction with moisture or carbonate production, potentially due to CaO's high reactivity with ambient CO₂. Peaks at 1074 cm⁻¹ and 868 cm⁻¹ suggest the presence of organic chemicals, possibly from the Ormocarpum cochinchinense extract, that help stabilize the nanoparticle surface. This analysis lends support to the concept that a green synthesis technique using Ormocarpum cochinchinense extract was successful in producing CaO nanoparticles, with organic compounds from the plant perhaps acting as capping and stabilizing agents.

# CONCLUSION

The study shows that Ormocarpum cochinchinense CaO NPs had substantial antioxidant and anti-inflammatory properties across a variety of assays. The results were consistently equivalent to those of conventional references, demonstrating CaO NPs' potential as a natural therapeutic agent. Given their similar activity levels, these nanoparticles may provide a potential alternative to traditional antioxidants and anti-inflammatory drugs in medicinal applications.

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