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An Insight into the Calcium Oxide Nanoparticle Fabrication through Physio-Chemical and Greener Mode

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Abstract: Nanotechnology has dominated in all the sectors in the recent few years. The nanoparticles are the core of the nanotechnology and applied in medicine, agriculture, remediation of environment and other fields. Nanoparticles are usually one nanometre to 100 nanometres range and owns different morphologies. The nanoparticles contain newer and enhanced biological and physio-chemical properties that are absent in the bulkier parts of the same matter. Fabrication of nanoparticles is executed by opting physio-chemical to biological protocols. The physical as well as the chemical protocols are unfit for harnessing nanoparticles as they dictate various flaws such as requirement of greater pressure, temperature, working equipment and noxious solvents which pose environmental harm when discharged improperly. In the present review we have described the possible methodologies of creating calcium oxide nanoparticles (CaO NPs). The greener, plant-based harnessing of CaO NPs is safer, cost-effective and feasible. The secondary metabolites of the phyto-extracts along with other natural chemicals functions as bio-reducer, stabilizer and capping factor in the phyto-wrapped designing of CaO NPs. In addition to physio-chemical protocols, the designing of CaO NPs using the extract of various sections of different medicinally important plants, along with its characterization and the bioactivities displayed by them was also discussed. Moreover, the phyto-created CaO NPs were non-toxic and biologically compatible and exploited in the drug targeting and anti-proliferative therapies.

Keywords: Nanotechnology, Nanoparticles, Physio-chemical, CaO NPs, Biocompatible

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Introduction

The advancement in the sector of nanotechnology has entirely revolutionized the scientific and other sectors. This field brings the manufacturing of nano-dimension particles that generally ranges from 1 nm to 100 nm through modifying the bulkier material at their molecular phases. The nanoparticles inherit enriched and distinctive traits that are not identified in their bulkier parts

(Alharbi and Alsubhi, 2022). The nanoparticles have magnetism, chemical reactiveness, optical, physical durability and electrical conductivity. These properties are as a consequence of their nano-dimension, definite quantum confinement and huge ratio of surface to volume (Adebayo-Tayo *et al.*, 2019).All the above-mentioned nature contributes to the unique biological as well as physio-chemical properties to the nanoparticles. The nanoparticles appear in various morphologies such as tubular, flat, core-shell, triangular, spiral, conical cylindrical and spheroidal (Castañeda-Aude *et al.*, 2023;Khan *et al.*, 2022). Owned to the dimensionality the nanoparticles are named as 0-D (zero-dimensional), 1-D (one-dimensional), 2-D (two-dimensional) and 3-D (three-dimensional). Generally, the nanoparticle types are organic, carbon-based, inorganic and hybrid nanoparticles (Harish *et al.*, 2022). Mostly nanoparticles are applied in the food sectors, cultivation, bio-remediations, cosmetics, textile and biomedicines (drug targeting, biosensors and bio-labelling) (Keskin *et al.*, 2023).

Mainly there are two approaches of harnessing nanoparticles which include bottom-up (biological and chemical protocols) and top-down (physical protocols) approaches. In the bottom-up engineering methodology the molecules or the atoms are gathered to create nanoparticles whereas in the top-down approaches the bulkier matter is crushed into tinier size crafting nanoparticles (Karunakaran *et al.*, 2023). The physical as well as chemical protocols employed to harness the nanoparticles needs huge energy, pressure, temperature, complexed equipment and harmful reducing chemicals which dumps noxious side-products into the eco-system (Ying *et al.*, 2022). The biogenic designing of nanoparticles has become famous as it creates lower environmental toxicity, sustainable and environmental-friendly thereby becoming an alternative to physio-chemical methodologies (Aarthye and Sureshkumar, 2021). In the greener harnessing methodology of nanoparticles usually microbes (fungi, algae, yeast and bacteria), enzymes and extracts of plants are applied. The plant extract applied synthesis of nanoparticles is beneficial in comparison to microbial synthesis which necessitate the involvement of culture maintenance and biohazard problem (Liaqat *et al.*, 2022; Noah and Ndangili, 2022).

The extract of herbs/medicinal plants synthesis of various forms of metallic as well as metal oxide forms of nanoparticles has been used in various studies that include platinum, silver, zirconium, gold, nickel, copper oxide, iron oxide and cobalt oxide (Bordiwala, 2023). Different organs of plants like leaves, stem-bark, seeds, root, flower, peel and fruits are utilized for designing metallic oxide nanoparticles (Shafey, 2020). Flavonoids, polyphenols, terpenoids, alkaloids, carbohydrates and proteins functions as bio-reduction converting the metallic salts into their corresponding metallic oxide nanoparticles forms. Moreover, the phytochemicals of plant stabilize and caps the metallic oxide nanoparticles in an efficacious way (Makarov *et al.*, 2014; Aigbe and Osibote, 2024). The shape, dimension, geometrical features and stableness of the nanoparticles hugely impacted by the pH, incubation duration, temperature of the reaction, concentration of phytogenic extract and the precursor metallic salt (Siddiqi and Husen, 2016; Singh *et al.*, 2019). The phyto-based procured metallic nanoparticles are apt for functioning in the biomedical field owned to non-toxicity (Osman *et al.*, 2024). The fabrication of nanoparticles occurs in three distinctive phases namely, activation-phase where metal ions are reduced and nucleated. This is followed by growth phase where nanoparticles are stabilized and finally attains a defined shaped in the termination phase (Jayachandran *et al.*, 2021). The phyto-extract applied harnessed diverse nanoparticles of metal oxide has presented antiviral, antifungal, antibacterial, anti-radical, anti-proliferative and anti-inflammatory performances (Naikoo *et al.*, 2021; Singh *et al.*, 2023).

Calcium oxide (CaO) in contrast to other metallic oxide are cheaper and corrosion free and functions as catalyst and applied as additives in the factories, pollution emission controls and gas desulfurization process. It is also used in the synthesis of calcium carbonate (Atchudan *et al.*, 2022). Calcium oxide nanoparticles (CaO NPs) are compatible to the living system and displays microbial activity (Silva *et al.*, 2020).They are also used in the delivery of the drugs (chemotherapeutic medicines) to the destinated location in the tissues, diagnosis of cancerous cells, PDT (photodynamic therapy) and PTT (photothermal therapies), bio-labelling owned to their non-hazardous nature (Kumari *et al.*, 2023).

Physiochemical methods of harnessing CaO NPs

Adopting thermal decomposition methodology, fabrication of CaO NPs was done. In this protocol mingling of 1 M and 2 M of sodium hydroxide solution with calcium hydroxide was done. To this solution added drop-wise calcium chloride (0.5 M) with strong and continuous stirring at 80 ℃ in the presence of argon gas over the surface. A whitish precipitate appeared for the calcium hydroxide which was collected carefully and washed 5 times in the distilled water and then finally dried in the desiccator (Mirghiasi *et al.*, 2014).

Tang *et al.* (2008) also followed thermal decomposition protocol and fabricated CaO NPs. In his methodology, calcium nitrate hexahydrate (11.81 g), sodium hydroxide (12.5 ml) and ethylene glycol (25 ml) were mingled together in a vigorous manner and stirred for 10 min to procure nanoparticles of uniform dimension and morphology. A gel like consistent solution obtained was retained under static condition for 5 h and washed applying distilled water and dried thoroughly in a vacuum drier. The fabricated nanoparticles had aggregation and owned 14 nm as mean size with high crystalline nature (Tang *et al.*, 2008).

Using micro-wave irradiation protocol CaO NPs were harnessed. For this, exactly sodium hydroxide (0.7 M) and calcium nitrate hexahydrate (0.5 M) were individually mingled in the 50 ml of de-ionized water and stirred continuously for 10 min at ambient temperature. A whitish gel formed was irradiated through micro-wave energy in a kitchen micro-wave oven at 2.45 GHz frequency under ambient condition for 10 min. The entire content was cooled to attain the room temperature and the precipitate was washed in ethanol and dried at 80 ℃. The obtained CaO NPs had cubic morphology and crystalline nature (Roy and Bhattacharya, 2011).

Sol-gel protocol was followed for the fabrication of CaO NPs. Hen's egg shell powdered form was used as a source of calcium carbonate which was mingled with HCl (hydrochloric acid) to extract the calcium carbonate. The procured suspension was filtered and the precipitate of calcium carbonate was washed multiple times in a deionized water to eradicate the impurities and traces of acid. Then dried in the oven at 150 ℃ to achieve a constant weight and calcined for 900 ℃ for 3 h. At end, the calcined product was made into fine powder and sieved (100 μm) mesh used. The nanoparticles of calcium oxide were characterized (Muleta *et al.*, 2024).

Applying the solution combustion protocol, designing of CaO NPs was done. For harnessing CaO NPs, exactly 6 g of urea and 14.16 g of calcium nitrate hydroxide were completely diluted in the distilled water (100 ml) and the mixture was calcined in the muffle furnace operated at 600 ℃. The obtained CaO NPs were crystalline featured and had cubic crystal shape (Madhusudhana *et al.*, 2012). Ulakpa *et al.* (2024) synthesized CaO NPs using hydrothermal methodology. The preparation of hugely active calcium oxide catalyst was done through various steps applying the shell of snail. The shell was washed, dried, crushed, calcined at 900 ℃ for 3 h. The harnessed CaO were refluxed in distilled water at 80 ℃. The content was stirred in the magnetic stirrer and retained for settlement for 122 h and the whitish coloured product developed was collected through filtration and dried at 120 ℃ and the produced content was calcined for 2 h at 700 ℃. The synthesized CaO NPs displayed irregular morphology through SEM and the agglomeration was captured clearly in the TEM images.

Moreover, the grain size of the CaO NPs was 50 nm (Chukwuemeke Ulakpa *et al.*, 2024).

Green synthesis of CaO NPs and its bioactivities

For preparing the CaO NPs the leaf aqueous extract of *Moringa oleifera* was boiled to 60 ℃ on a hot magnetic stirrer plate and then added calcium nitrate (2 g) to the boiling solution. To retain a basic pH, addition of 2M sodium hydroxide (few drops) was done and boiled. The thick yellowish paste developed was collected and heated to 400 ℃ in a muffle furnace for 3 h. Post heating, a whitish coloured powder of CaO NPs was formed. The XRD spectral outcome dictated the cubic structural feature of the fabricated CaO NPs. SEM showed the created CaO NPs were agglomerated owning spheroidal morphology with 30 nm to 40 nm average dimension. Moreover, the fabricated CaO NPs were bactericidal towards *Staphylococcus aureus* and *Escherichia coli* (Jadhav *et al.*, 2022).

Mazher *et al.* (2023) tailored CaO NPs applying the fruit extract of *Citrullus colicynthis* and found that the procured CaO NPs owned crystalline nature and the TEM photographs authenticated the spheroidal morphology owing 35.93 nm as mean size. The FTIR displayed the bioactive ingredients of plants extract and its involvement in the phyto-engineering of CaO NPs. In the bioactivities studies it was identified that the CaO NPs and its amalgamated form with the fruit extract displayed profound antibacterial traits towards the *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Microccus luteus*. Additionally, the CaO NPs displayed antioxidant, anti-hemolytic and anti-proliferative properties (Mazher *et al.*, 2023).

Ramli *et al.* (2019) applied aqueous extract of *Hylocereus polyrhizus* (Red dragon) fruit peel and procured CaO NPs. For preparing the CaO NPs, the fruit extract of *Hylocereus polyrhizus* was blended with equal proportion of calcium chloride. Then ammonium hydroxide was poured into the reacting solution to regulate the pH and retained for reaction for 24 h at ambient environment. After centrifugation (10,000 rpm) of the reacted solution for 15 min, the collected precipitate was calcined in the muffle furnace (700 ℃). The UV spectral emerged from 400 nm to 450 nm with intensified peak at 450 nm. The SEM presented fibrous shaped CaO NPs with 81.45 nm as mean size. EDX spectra confirmed calcium (29.06%) and oxygen (43.94%) along with carbon (21.33%), magnesium (0.29%), potassium (0.12%) and chloride (3.24%). Moreover, the harnessed CaO NPs were fungicidal towards *Candida albicans* (Ramli *et al.*, 2019).

Adnan and Mohammed (2023), blended juice of lemon (60 ml) with calcium nitrate solution (20 ml) through drop-by-drop addition and continuously stirrer on a hot-plate magnetic stirrer operated at 80 ℃ for 2 h. A brownish coloured solution formed justified the creation of CaO NPs. Post cooling, centrifugation of the developed solution was done for 15 min at 5,000 rpm. The collected precipitate was washed, calcined and characterized. The TEM micrographic images proved the nano-rod shape of the created CaO NPs. The dimensional range of CaO NPs was 30-60 nm identified via SEM. The FTIR spectral peak at 674.16 cm^{-1} notified the calcium-oxygen vibrational stretching. The fabricated calcium oxide nano-rods were cytotoxic towards the A549 cell line (lung cancer cell line) after 72 h of incubation. Through agar-well diffusion protocol it was confirmed that the designed nano-rod of calcium oxide were able to lyse the two bacterial strains namely, *Streptococcus mutans* and *Escherichia coli* with 21 nm as well as 22 nm, respectively as inhibitory zones at 100 µg/ml. Furthermore, the anti-radical activity of calcium oxide nano-rod was 52.5% at 104.7 µg/ml. All the bioactivities investigated had dose-related performances (Adnan and Mohammed, 2023).

Aarthe and Rashika (2022) in a greener form created CaO NPs using *Cymbopogon citratus* leaf water extract (30 ml) and calcium chloride (3 g as precursor metal salt). Moreover, sodium hydroxide was also added in the reacting content to retain the pH of the solution. The precipitate of calcium oxide nanoparticles procured after the whole reaction was subjected to physio-chemical to microscopic characterization. In the XRD spectra it was proven the crystalline feature of the designed CaO NPs. The spheroidal and agglomerated form of CaO NPs was disclosed by SEM. Moreover, the harnessed CaO NPs were able to inhibit the development of *Klebsiella pneumonia*, *Enterococcus* spp., *Escherichia coli* and *Staphylococcus aureus* when screened at 50 µl as well as 100 µl doses with maximum zone of inhibition at 100 µl (Aarthe and Rashika, 2022).

Synthesis of CaO NPs in a phyto-engineering form was executed by mingling the leaf aqueous solution of *Crescetia cujete* with equal quantity of calcium chloride solution (1 mM). The solution was retained for reaction without any hindrance for 24 h at ambient environment. The pellet was obtained through centrifugation for 15 min at 5,000 rpm and dried in hot-air oven for 30 min at 60 °C. The procured CaO NPs displayed UV-vis spectral peak at 287 nm with a stability of -23 mV identified through zeta potential. Through SEM, it was divulged that the harnessed CaO NPs owned spherical shape with 62 nm as mean diameter. Zebrafish cytotoxicity assay (*in vivo* assay) proved that the applied doses of CaO NPs provided dose-dependency type of toxicity towards the zebrafishes. This bio-study revealed the biological compatibleness of the harnessed CaO NPs (Eram *et al.*, 2021).

Gandhi *et al.* (2021) created CaO NPs using the water leaf extract of *Ocimum tenuiflorum* (5 ml) and calcium chloride (0.1 M, 95 ml) and the greyish to brownish colour signified the creation of CaO NPs and in the UV spectral the peak developed at 332 nm. The SEM pictures dictated the irregularity in the shape of the harnessed CaO NPs with agglomeration features and the EDX confirmed 14.88% of calcium and 60.50% of oxygen in the k-shell, whereas other elemental ingredients (sodium and chloride) are also appeared in the EDX signalling. The fabricated CaO NPs exposed antibacterial activity towards four pathogenic bacteria namely *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis* (Gandhi *et al.*, 2021).

Anantharaman and George (2016) adopted phyto-engineering methodology and synthesized CaO NPs using the water leaf extract of Papaya and green tea. Calcium nitrate solution (10 ml) was mingled continuously with the leaf water extracts (10 ml from each plant). Sodium hydroxide few drops was also added to the mixture and the whitish precipitate of calcium hydroxide procured was repeatedly rinsed using distilled water to wipe out the impure contents. Finally, calcination of the precipitate at 500 °C offered CaO NPs. The procured CaO NPs had 3.44 eV band-gap. The XRD conveyed the cubic-phase of the synthesized CaO NPs with grain sizes of 24 nm for green tea mediated designed CaO NPs and 23 nm for papaya extract applied design CaO NPs. Both the extract derived CaO NPs were efficacious against the functioning of pathogenic bacterial strains and also displayed photocatalytic performance causing degradation of CR (congo red) dye (Anantharaman and George, 2016).

In another study utilizing the leaf aqueous extract of papaya (30 ml), calcium chloride (30 ml) and sodium hydroxide (15 ml added drop-wise) to make the pH 10 was added together followed by stirring constantly at 37 °C for 30 min and the acquired precipitate was harvested through 10 min centrifugation process after discarding the supernatant solution. Washing of precipitated pellet was done using distilled water to wash out the organic impure contents and then dried for 6 h at 100 °C in a hot-air oven. After calcination in the muffle furnace at 400 °C, the CaO NPs obtained was ground to a fine powdery material in the mortar and pestle. The XRD diffraction peak displayed crystalline characteristic CaO NPs with no extra peaks justifying the pureness of the fabricated CaO NPs with a grain size of 16.2 nm. Moreover, the EDX presented 58.86% of calcium and 41.14% of oxygen in the CaO NPs. The CaO NPs had efficacious antibacterial trait against the two infective bacteria *(Dhivya et al.*, 2020).

Osuntokun *et al.* (2018) created CaO NPs using the broccoli water extract. Different volumes (10 ml, 20 ml and 30 ml) of broccoli water extract as a bio-reducer was amalgamated with precursor salt (50 ml, 0.1 M calcium chloride) and constantly stirred in a magnetic stirrer for approximately 40 min followed by sodium hydroxide (2 M) drop-wise addition to balance the pH as 12. Until the formation of whitish precipitate (calcium hydroxide) happened stirring was done for 2 h. The precipitate was separated from the solution mixture via centrifugation for 15 min at 4,000 rpm and the pellet was rinsed four times in distilled water for removing the contaminants. Hot-air oven drying of the pellet at 90 °C for 8 h created CaO NPs which on calcination (650 °C) yielded CaO NPs in a pure and powdery form. The highly crystalline and spheroidal morphology CaO NPs displayed efficacious degradation of BG (bromocrescol green) (Osuntokun *et al.*, 2018).

Harnessing of CaO NPs was executed by adopting continuously mixing the aqueous extract of *Rhododendron arboretum* leaf (50 ml) and calcium nitrate (50 ml) along with drop-by-drop addition of solution of sodium hydroxide (0.1 N) in the reaction mixture. The FTIR spectral report conveyed the peaks and bands of the functional groups of the leaf extract that served as potent bio-reducer, stabilizer and capping factor in the phyto-adopted harnessing of CaO NPs whereas the XRD diffraction peaks evinced the crystalline nature of the CaO NPs with cubic-phase. The synthesized CaO NPs owned needle-like morphology which was captured in the SEM photographs and the EDX study gave 32.16% of calcium, 61.28% of oxygen and 6.56% of carbon. Furthermore, the CaO NPs were bactericidal towards *Proteus vulgaris*, *Escherichia coli* and *Streptococcus mutans* (Ramola and Joshi, 2019).

Gurav *et al.* (2020) engineered CaO NPs using the aqueous extract of Tulsi (*Ocimum sanctum*) leaves, calcined shell of egg (45 ml) solution and concentrated hydrochloric acid (10 ml). The entire content was heated for half an hour. After cooling the solution was centrifuged and the pellet procured was washed in double distilled water and calcined. The XRD diffraction spectra presented peaks apt for the CaO NPs. The size of the CaO NPs ranged from 35 to 70 nm. FTIR conveyed the existence of hydroxyl group as well as CaO NPs creation owned to the O-H group and Cao_O group vibrational stretching. Bacterial strains (gram-positive and gram-negative) were susceptible to the CaO NPs treatment (Gurav *et al.*, 2020).

By amalgamating the leaf aqueous extract of *Piper betel* (120 ml) and calcium nitrate aqueous solution (50 ml, 0.1 M) designing of CaO NPs was done. In the diffraction spectra of XRD, the peaks were narrower and highly intensified dictating the enhanced crystallinity of CaO NPs. The grain size of the fabricated crystalline CaO NPs was 24.9 nm calculated through the Debye-Scherrer's formula. The SEM and TEM showed the spheroidal shape of the designed CaO NPs with dimension ranging from 24 to 30 nm. A sharp peak formed at 875 cm^{-1} proved the formation of CaO NPs in the FTIR spectrum and the UV-visible spectrum developed at 262 nm (band-gap 1.95 eV). The synthesized CaO NPs were able to kill the growth of *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Staphylococcus aureus* through the creation of reactive oxygen species (superoxide anion) which caused the disruption of cellular wall of bacteria. The bactericidal performance was concentration-based. Furthermore, through MTT assay it was identified that the CaO NPs were toxic towards the survival of A-549 cells when screened from 1 μg/ml to 100 μg/ml in a dose-related form with an IC_{50} value of 92.08 μg/ml (Yoonus *et al.*, 2020).

Phyto-designing of CaO NPs in a budget-friendly route was performed by mingling the fruit extract of *Ficus carica* (50 ml) and calcium nitrate solution (50 ml) at a pH of 5.7. Stirring of the solution was done for 2 h at 60 °C until the dark-brownish coloured formed. The whole reacted mixture was subjected to centrifugation at 10,000 rpm for 15 min and then the pellet developed was placed in muffle furnace (500 °C) for calcination. At 360 nm a intensive peak was formed for CaO

NPs in the UV-vis spectral study. The bio-reducers phytometabolites functional group involvement was authenticated by FTIR. In the EDX spectra, 31.40% of calcium and 58.68% of oxygen was confirmed. From SEM microscopic pictures, the CaO NPs were found to be irregularly shaped with 84.78 nm dimension along with agglomeration. The synthesized CaO NPs were able to inhibit the creation of biofilm by the *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Khan *et al.*, 2023).

Pasupathy and Rajamanickam (2019) in a phyto-mode engineered CaO NPs using the leaf aqueous extract of four different plants namely, *Acalypha indica*, *Cisscus quadrangularis*, *Phyllanthis niruri* and *Solanum nigrum*. Here, the precursor of calcium was procured from the egg shell of chicken. Through agar-well diffusion methodology, it was cleared that the CaO NPs were efficiently able to inhibit the bacteria growth by forming the inhibitory zones against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. The bactericidal role was dose-based. As the concentration of CaO NPs wrapped with the phyto-extract raised from 25 µg/ml to 100 µg/ml, the bactericidal performance increased (Pasupathy and Rajamanickam, 2019).

Ijaz *et al.* (2017) harnessed CaO NPs in a greener form by mingling the water leaf extract of *Mentha pipertia* and calcium chloride. In the SEM images, the CaO NPs appeared to be disc shaped with agglomeration and EDX conveyed the intensified peaks for both oxygen and calcium. The antibacterial performance of the procured CaO NPs was checked applying the MIC (minimum inhibitory concentration) and agar-well diffusion technique. From the antibacterial assays it was disclosed that the CaO NPs were efficacious in killing *Escherichia coli* (Ijaz *et al.*, 2017). Devanesan *et al.* (2024) designed CaO NPs utilizing the seed aqueous extract of *Eleusine coracana* and 1 mM of calcium chloride solution. The change in the solution colour from brownish to off-whitish or pale yellow conveyed the creation of CaO NPs. FTIR spectral outcome showed the participation of polyphenolics, proteins and carbohydrates as bio-reducer in the fabrication of CaO NPs. The micrographic images of CaO NPs taken through SEM showed agglomeration of CaO NPs. The formed CaO NPs were bactericidal towards the four bacterial strains. Moreover, the CaO NPs wrapped with the seed extract demonstrated anti-inflammatory traits when assessed through protein denaturation inhibiton assay and nitric oxide inhibition assay (Devanesan *et al.*, 2024).

Using the bulb extract of *Tulbaghia violacea* seed (20 ml) and calcium chloride (50 ml, 0.1 M) synthesis of CaO NPs was executed in an eco-benefited mode. In the reaction between the bulb extract and the precursor salt, sodium hydroxide was added to adjust the pH to neutral via drop-by-drop addition. For two hours continuously stirring of the solution was done and the white-milk solution formed was centrifuged to pertain the pellet. Washing of pellet was done in distilled water and then ethanol and calcined for 2 h at 650 °C. In the TEM, the spheroidal and agglomerated nature of the synthesized CaO NPs was captured. In the EDX spectra two strong peaks developed for calcium (21.92%) and oxygen (58.41%). The fabricated CaO NPs presented cytotoxicity towards the tested HEK-293 as well as HeLa cell line in a dose-based form. Additionally, antibacterial activity was also identified (Mbenga *et al.*, 2023).

By amalgamating the aqueous extract of *Mentha arvensis* leave (10 ml) and calcium nitrate (10 ml) fabrication of CaO NPs was achieved. A prominent peak at 265 nm developed for the CaO NPs in the UV-vis spectrophotometric study. The synthesized CaO NPs were irregularly shaped, highly porous and agglomerated when visualized through SEM images. The anti-inflammatory activity of the phyto-harnessed CaO NPs was assessed through two different assays namely, nitric oxide scavenging assay and protein denaturation inhibition assay. The CaO NPs dictated concentration proportional anti-inflammatory performance with maximum anti-inflammatory activity at 250 µg/ml. Moreover, the CaO NPs were able to impact the survival of the breast cancer cells (MDA-MB-231 cells) in a dose-based mode. Also, the CaO NPs were profoundly able to degrade methyl orange (dye) at 125 µg/ml (Shanmuganathan *et al.*, 2023).

Calcium nitrate solution (80 ml) was blended with the extract of *Linum usitatissimum* seed (20 ml) which was added drop-b-drop to the precursor salt solution and heated for 12 h at 60 °C. The lemon yellowish coloured gel consistency calcium hydroxide obtained was dried for 4 h at 120 °C and calcined for 2 h at three varying temperatures. The whitish coloured calcium oxide nanoparticles were obtained and characterized. The crystalline and spheroidal morphology CaO NPs with average dimension of 59 nm were cytotoxic towards the CT-26 cell line in a dose-based manner with highest cancerous cell killing at 1000 ppm, at which only 55% of the CT-26 cells were alive (Tabrizi Hafez Moghaddas *et al.*, 2024).

Conclusion

In this review we have presented the various physio-chemical methodologies for designing CaO NPs. As the physio-chemical methods are unsafe, costlier and needs special requirements they are replaced by the biological methods. Various herbs or medicinal plant's extract was applied for designing the required CaO NPs in its purest form and the confirmation of the synthesis was done by executing characterization studies. Along with the characterization reports, the bioactivities of CaO NPs was also discussed.

Ethical Statement

This is a review article hence no Ethical Approval needed.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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