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Green Synthesis of CaO Nanoparticles from Chicken Eggshell using Lemongrass Leaf Water Extract

Sintesis Hijau CaO dari Cangkang Telur Ayam Menggunakan Ekstrak Air Daun Sereh

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ABSTRACT

Sources of CaO in the synthesis of nanoparticles can be available from waste such as eggshell waste which has an average $CaCO_3$ content of 94%. This study aimed to synthesize nanoparticles with various compositions of CaO-0 and CaO-1 (50:50) derived from chicken eggshells, mediated by Cymbopogon citratus leaf extract, and to characterize the nanoparticles. Based on the results of phytochemical screening, *C. citratus* extract contained alkaloids, flavonoids, triterpenoids, and tannins. The results of characterization with UV-Vis spectrophotometer showed that CaO was formed at the highest wavelength with a composition of CaO-1 (50:50) with a value of 250-300 nm. Absorption band of FT-IR spectra at wave numbers 428 cm^{-1} and 711 cm^{-1} indicated Ca-O vibrational band and Ca-O bonds, respectively. The particle size of crystalline CaO-0 based on calculations using the Debye Scherrer formula ranged from 33.72 - 38.48 nm while the particle size of crystalline CaO-1 ranged from 28.93 - 70.75 nm with diposide and calcite phases based on XRD data. The surface morphology using SEM showed that CaO-0 was shaped like an irregular cube while CaO-1 forms irregular spherical lumps.

Keywords: CaO, green synthesis, *Cymbopogon citratus*, nanoparticles.

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1. INTRODUCTION

Alkaline earth metal oxide, calcium oxide (CaO), is an inorganic metal oxide that can be made in the form of nanoparticles. CaO for nanoparticle synthesis can be sourced from inexpensive and readily available waste, such as eggshell waste, fish scales, clam shell residues, and animal bones. Chicken eggshell is an abundant waste in Indonesia. In addition to coming from egg consumption waste by the community, eggshell waste is also produced by the chicken hatchery industry. The chemical composition of the egg shell is 94% $CaCO_3$, 1% $MgCO_3$, 1% $CaPO_4$, and the rest is organic material as much as 4% by weight of the egg shell (Stadelman, 2015). Calcium carbonate is the main composition in chicken eggshells which naturally forms calcite (Laca et al., 2017). Research conducted by Goli & Sahu (2018), reported that calcined eggshell powder contained 97.86 % CaO, 0.785 %

MgO, 0.387 % P_2O_5, and the rest was in the form of other metal oxides. CaO nanoparticles are interesting to research and develop because of their adsorption ability, catalytic activity, optical and electronic properties, antibacterial properties, and their ability as a drug delivery agent.

Nanoparticles can be synthesized by conventional chemical and physical methods. The disadvantage of the chemical method is that it requires a lot of reagents that are harmful and toxic to the environment, while the physical method requires a lot of energy and conditioning under controlled pressure (Kumar et al., 2019). Therefore, in recent years, plant-mediated nanoparticles synthesis has become increasingly important because it is simple and environmentally friendly. This kind of synthesis is known as green synthesis or biosynthesis (Marquis et al., 2016). Extracts from plants can act as both reducing and capping agents in the synthesis of nanoparticles. Iravani (2011) reported that this green synthesis process involves metal ions combining to form precursor building blocks which then self-assemble into nanoparticles. Based on this method, several metal nanoparticles such as Au, Ag, Pd, Pt, ZnO, and Fe have been successfully synthesized (Ain & Nor, 2013; Fatimah et al., 2020; Maringgal et al., 2020). Several studies believe that the presence of phytochemicals such as alkaloids, saponins, terpenoids, and tannins are involved in the synthesis of these nanoparticles (Poinern & Fawcett, 2019). CaO nanoparticles can be synthesized using plant extracts (Maringgal et al., 2020). The reducing properties of several different plant species play an important role in reducing Ca salts to CaO nanoparticles. Several biomolecules in plant extracts such as proteins/enzymes, vitamins, alcohol group compounds, polysaccharides, alkaloids, polyphenols, and other secondary metabolite compounds are involved in the bioreduction, formation and stabilization of metal nanoparticles.

CaO nanoparticles have been successfully synthesized using deionized water (DI water) solvent extract from Piper betel leaves, Trigona sp. honey, light green stems of *Cissus quadrangularis*, *Rhododendron arboretum* leaves, papaya leaves, green tea leaves, and *Ocimum sanctum* leaf extracts. (Anantharaman & George, 2016; Gurav et al., 2020; Maringgal et al., 2020; Marquis et al., 2016; Ramola & Joshi, 2019; Yoonus et al., 2020). One of the nanoparticles that can be synthesized through plant extracts is CaO nanoparticles. The plant extract that can be used is *Cymbopogon citratus* leaf extract. The *C. citratus* (lemongrass leaf) is also a plant that has the potential to be used as a mediation for the synthesis of nanoparticles because of its phytochemical compounds. The aqueous extract of *C. citratus* leaves, among others, contains flavonoid and phenolic compounds (Balakrishnan et al., 2014). The *C. citratus* is an Angiosperm plant that has been used to synthesize gold nanoparticles and act as a reducing agent (Smitha et al., 2020). The *C. citratus* as a reducing agent in the synthesis of CaO nanoparticles has never been reported. In this study, CaO nanoparticles were synthesized from chicken eggshells and mediated by *C. citratus* leaf extract.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this research are chicken eggshells from chicken hatchery in Caringin, West Java, *C. citratus* leaf (from Bogor, West Java), deionized water (Hanna), HCl (Merck), NaOH (Merck), $CaCl_2$ (Merck), universal pH indicator, and Whatmann 41 filter paper. The instruments used in this research are spectrophotometer UV-Vis (Bell Photomic UV-51), spectrometer Fourier Transform Infra-Red / FTIR (Shimadzu), X-Ray Diffraction / XRD (Shimadzu), Scanning Electron Microscope / SEM (Bruker).

2.2. Preparation of chicken eggshells into $CaCl_2$

Chicken eggshells are taken as waste from the chicken hatchery industry in the Caringin area, Bogor Regency, West Java. The eggshells were washed using warm distilled water until clean, then dried in an oven at 110 ºC for 3 hours. After drying, the eggshells were mashed into a powder measuring 100 mesh. Egg shell powder is converted into $CaCl_2$ by adding 12.5 grams of chicken egg shell powder to 250 mL of 1 M HCl and stirring using a magnetic stirrer until $CaCl_2$ is formed (Habte et al., 2019).

2.3. Preparation of CaO from chicken eggshell

$CaCl_2$ solution was added with 1 N NaOH drop by drop while stirring using a magnetic stirrer for 2 hours at a temperature of 60 ℃. The addition of NaOH was stopped until pH 12 and a precipitate formed in the solution. After 2 hours, the mixture was allowed to stand at room temperature for 3 - 5 days (cured) until the filtrate and precipitate separated. The precipitate was filtered using Whatmann No.41 filter paper and washed with deionized water. The precipitate obtained was then dried in an oven at 90 ℃ for 1 hour (Bano & Pillai, 2020). The weight of the precipitate which has stabilized is then calcined at 700 ℃ for 3 hours. CaO obtained was coded CaO-0. After calcination, the precipitate was cooled to room temperature and pulverized to become CaO powder. The CaO powder was then characterized for its structure and surface.

2.4. Preparation of C. citratus (Lemongrass) water extract

Fresh lemongrass leaves are washed with distilled water until clean and cut into small pieces. Lemongrass leaves are dried and crushed. The lemongrass leaf powder weighed as much as 50 grams and added with 100 mL of deionized water. This mixture was heated at 100 ℃ for 1 hour. After 1 hour, cooled down to room temperature. The mixture was then filtered using Whatmann filter paper. The filtrate obtained is lemongrass leaf extract which will be used in the synthesis of CaO nanoparticles. Lemongrass leaf extract was tested qualitatively for phytochemicals (phytochemical screening) according to the AOAC method (AOAC, 1990) include alkaloids, saponins, tannins, phenols, and flavonoids.

2.5. CaO synthesis using lemongrass leaf water extract

Lemongrass leaf extract and $CaCl_2$ with composition 1 = 50:50 v/v, the solution was stirred for 2 hours at 60 ℃. 20 mL of 1 N NaOH was added dropwise during stirring to pH 12 and a precipitate was formed. After 2 hours, the mixture was allowed to stand at room temperature for 3-5 days (cured) until the filtration and precipitate separated. The precipitate was filtered using Whatmann No.41 filter paper and washed with deionized water. The precipitate obtained was then dried in an oven at 90 ℃ for 1 hour (Bano & Pillai, 2020). The weight stabilized precipitate was then calcined at 700 ℃ for 3 hours. CaO obtained was coded CaO-1. After calcination, the precipitate was cooled to room temperature and pulverized to become CaO powder. The CaO powder was then characterized for its structure and surface.

2.6. Characterization of CaO nanoparticles

The *C. citratus* leaf extract, CaO-0 and CaO-1 solutions were characterized using a UV-Vis spectrophotometer at a wavelength of 200 - 600 nm to determine the maximum wavelength. CaO-0 and CaO-1 powders were characterized using spectrometer FT-IR with KBR pellet method at wave number 400 – 4000 cm^{-1}. CaO-0 and CaO-1 powders were characterized using XRD and SEM-EDX. Characterization of the structure, phase and determination of the crystal size of nanoparticles using XRD at an angle of 2θ 10 – 80° while the determination of the surface structure and particle size was carried out using SEM-EDX.

3. RESULTS AND DISCUSSION

3.1. C. citratus extracts phytochemical content

The *C. citratus* was extracted using DI water by a maceration method. Maceration method is an effective and simple method for the extraction of bioactive compounds in plant extracts (Ćujić et al., 2016). The *C. citratus* was extracted using deionized water (DI Water) by the maceration method. The results of phytochemical screening of *C. citratus* extract are shown in **Table 1**.

Table 1. Phytochemical screening test results

No.	Compound Group	Result
1	Alkaloid:	
	a. Dragendorf	-
	b. Mayer	-
	c. Wagner	+++
2	Flavonoid	+++
3	Saponin	+
4	Steroid/Triterpenoid	+++
5	Tanin	+++

Explanation:
(-) : Negative
(+) : Weak positive
(++) : Positive
(+++) : Strong positive

Based on the results of phytochemical screening, *C. citratus* extract contains alkaloids, flavonoids, triterpenoids, and tannins. This is in accordance with the research of Shah et al. which contains secondary metabolites in *C. citratus* in the form of triterpenoid, phenolic, and flavonoid compounds (Shah, et al., 2011). While Umar, et al. (2016) found alkaloid compounds, tannins, and alkaloids in the extract of *C. citratus*. These secondary metabolites have a role in the synthesis of CaO nanoparticles as reducing and capping agents (Kumar et al., 2019). The role of this capping agent is to control the growth, aggregation, and physical and chemical properties of nanoparticles (Sidhu et al., 2022). In addition, secondary metabolites in plant extracts also play a role in stabilization, shape and size of nanoparticles (Kumar et al., 2019).

3.2. CaO nanoparticles

CaO was synthesized from $CaCl_2$ solution derived from eggshell powder which was reacted with HCl according to the reaction:

$$CaCO_3\ (s) + 2HCl\ (aq) \rightarrow CaCl_2\ (aq) + H_2CO_3(aq)$$

The color of the eggshell powder obtained is light brown. Eggshell powder containing $CaCO_3$ can react with HCl to produce a $CaCl_2$ solution. In this reaction process H_2CO_3 is also formed which is characterized by CO_2 gas bubbles that appear during stirring. The $CaCl_2$ solution acts as a source of Ca which is used in the synthesis of CaO nanoparticles. Calcium oxide synthesized in this study included: CaO-0 from $CaCl_2$ solution by calcination method and CaO-1 synthesized from $CaCl_2$ solution by adding *Cymbopogon citratus* extract in a ratio of 50:50 CaO powder the resulting white.

3.3. UV-Vis spectra of CaO nanoparticles

The extracts of *C. citratus*, CaO-0 and CaO-1 were characterized using a UV-Visible spectrophotometer to determine the wavelength shift and the maximum wavelength. This confirms the formation of CaO nanoparticles. The UV-Vis spectra of CaO are shown in **Figure 1**. The synthesis was carried out by adding water extract of lemongrass leaves (CC extract) with $CaCl_2$ solution from eggshells. The formation of CaO particles is seen with the characteristics of the mixed color from green to white.

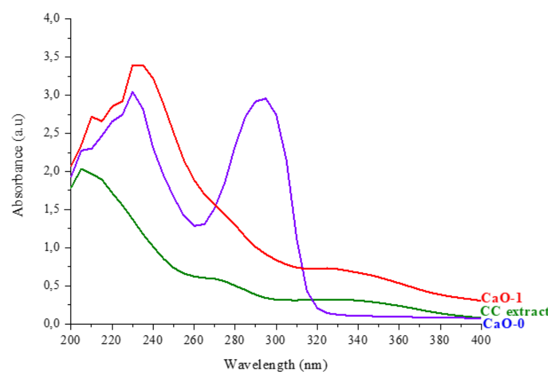

Figure 1. UV-Vis spectra of CaO nanoparticles.

The *C. citratus* extract has a maximum wavelength in the ultraviolet (UV) region. This maximum wavelength shifted when *C. citratus* extract was used to synthesize CaO nanoparticles. The more the amount of $CaCl_2$ solution used in the synthesis of CaO nanoparticles, the maximum wavelength shifts to the right and the absorbance is higher. The maximum wavelength with the highest absorbance is indicated by CaO-1. The process of biosynthesis of CaO particles using lemongrass leaf water extract is formed due to the reaction that occurs between polyphenol compounds and Ca^{2+}. This characterization is related to the analysis of surface plasmon resonance (SPR) that occurs in colloidal CaO particles. The maximum wavelength with the highest absorbance was shown by CaO-1 at 230 nm and 330 nm. Calcium oxide which was synthesized without using *C. citratus* extract (CaO-0) had two maximum wavelengths in the UV and visible regions, namely 230 nm and 295 nm. This is in accordance with previous research, the synthesis of CaO with broccoli extract which resulted in the absorption of UV-vis waves in the range of 297 nm (Osuntokun et al., 2018). From the shift in wavelength value, it can be concluded that the resulting CaO nanoparticles have been formed.

Infrared spectroscopy is a technique based on molecular vibrations or transitions in the vibrational and rotational levels of molecules. This spectroscopy provides information about the different functional groups that appear on the surface of the nanoparticles (Ramola & Joshi, 2019). Calcium oxide was analyzed using FT-IR to determine the functional groups and Ca-O bonds that occur. The FT-IR spectra of CaO-0 and CaO-1 are shown in **Figure 2.** The spectrum clearly shows the formation of CaO.

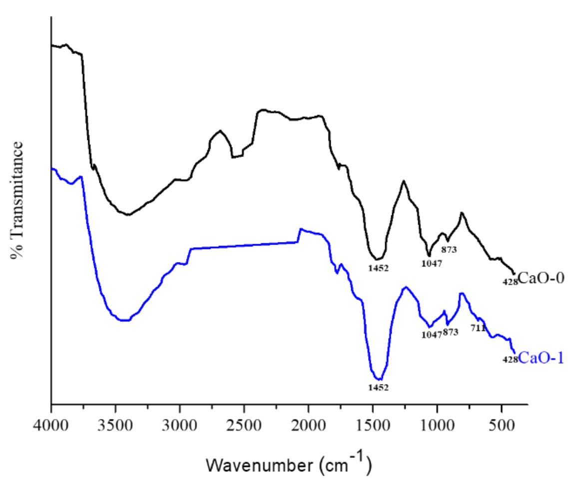

Figure 2. FT-IR spectra of CaO nanoparticles

Based on the resulting FT-IR spectra, a wide absorption band is formed at a wave number of 3400 cm^{-1}, indicating O-H bonds of water molecules on the CaO surface (Maringgal et al., 2020). The absorption bands that appear in the wave number region of 1452, 1047, and 873 cm-1 indicate the presence of C−O bonds which indicate that carbonation occurs in CaO nanoparticles (Habte et al., 2019). At a wave number of 428 cm-1, it shows the Ca−O vibrational band. In addition, the small absorption band that appears at the wavenumber 711 cm^{-1} also indicates the presence of Ca−O bonds that identify CaO (Bano & Pillai, 2020). The C=O bond is indicated by the absorption band at wave number 1795 cm^{-1}(Ramola & Joshi, 2019).

3.4. XRD diffractogram of CaO nanoparticles

XRD testing of the sample was carried out to identify the composition and phase of the sample. Structural characterization and identification of the CaO phase were carried out on CaO-0 and CaO-1. X-ray diffraction (XRD) is a non-destructive analysis which is very important for the characterization of materials. The XRD technique is based on X-ray diffraction on powders or crystalline materials and the resulting diffractogram represents the intensity at two theta (2θ) angle scattering. Structural characterization and identification of the CaO phase were carried out on CaO-0 and CaO-1. The XRD diffractogram is shown in **Figure 3**. The resulting diffractogram pattern still contains an amorphous phase, this indicates that the CaO obtained from the synthesis is not pure CaO. The main peak of CaO-0 appears at 2θ° = 18.0°; 29.4°; and 34.1° and another high-intensity peak appears at 2θ° = 31.8°; 39.4°; 43.1°; 47.1°; 50.8°; 54.3°; 62.8°; and 71.8°. The main peak of CaO-1 appears at 2θ° = 17.9°; 29.4°; 31.7°; and 34° and another high-intensity peak appears at 2θ° = 25.8°; 28.3°; 32.8°; 39.3°; 46.9°; 50.7°; 54.3°; 62.5°; and 71.4°. The XRD diffractograms of CaO-0 and CaO-1 exhibit similar patterns, however, slight differences in intensity are observed, and certain peaks appear in CaO-1 that are absent in CaO-0. The diffractogram peaks of $CaCO_3$, $Ca(OH)_2$ and CaO are seen in these two samples (Gurav et al., 2020; Nuryantini et al., 2019). This confirms that the resulting CaO is not pure CaO. The particle size of the calcium oxide nanoparticles was calculated using the Scherrer formula:

$$D = \frac{k\lambda}{\beta \cos\theta}$$

Where D is a measure of crystallinity (Å), which is an X-ray wavelength (Å), is a full width at half maximum / FWHM (rad), is a Bragg diffraction angle and k is a constant (0.94). The particle size of crystalline CaO-0 based on calculations using the Debye Scherrer formula ranged from 33.72 - 38.48 nm while the particle size of crystalline CaO-1 ranged from 28.93 - 70.75 nm.

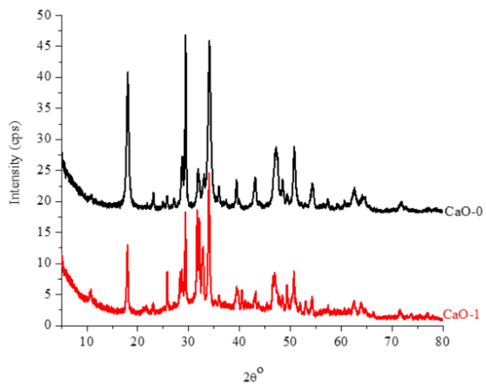

Figure 3. CaO nanoparticle diffractogram

The results of the characterization using XRD were analyzed using the *Match!* as shown in **Figure 4** and **Figure 5**. The figure shows the types of calcium oxide phases in CaO-0 identified, namely 48.7% calcite, 37.3% aragonite, and 14% calcium carbonate calcite. Identified calcite has a trigonal crystal system, aragonite has a triclinic crystal system, while calcium carbonate calcite has a rhombohedral crystal system.

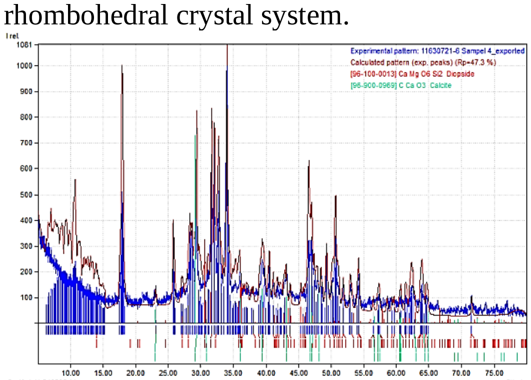

Figure 4. *Match!* result of CaO-0

Based on the results in **Figure 4** and **Figure 5**, the phases identified in the CaO-1 diffractogram are 56.8% diopside and 43.2% calcite. The crystal system in diopside is monoclinic while in calcite it is trigonal.

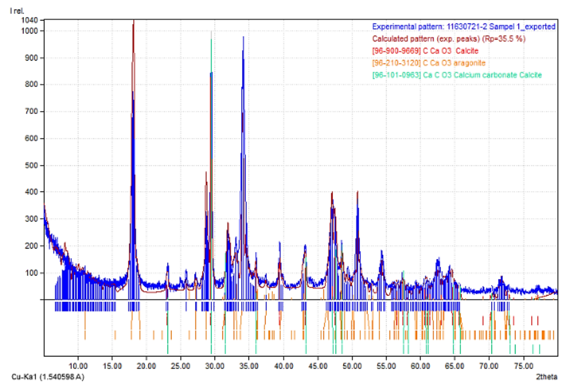

Figure 5. *Match!* result CaO-1

3.5. CaO surface morphology

The surface morphology of CaO-0 and CaO-1 were characterized using SEM-EDX. The resulting SEM micrograph is shown in **Figure 6**. Based on this figure, the shape of CaO-0 particles is more regular than CaO-1. The surface of CaO-0 appears as an irregular cube, while CaO-1 forms irregular spherical lumps, as observed in the SEM micrographs. This is consistent with the results of the XRD diffractogram which shows that CaO-0 has more ordered particles than CaO-1 and is more crystalline. Calcium oxide in CaO-1 obtained is not pure.

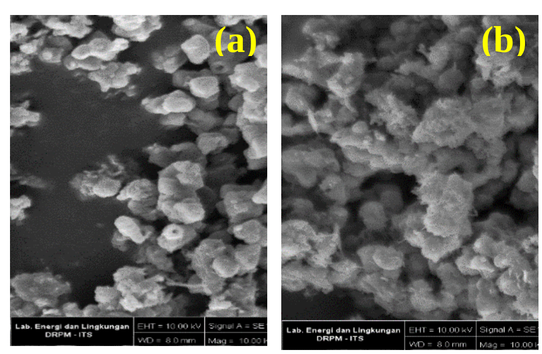

Figure 6. Micrograph SEM (a) CaO-0 and (b) CaO-1

Energy Dispersive X-Ray generally involves an X-ray spectrum for scanning the SEM region. The results obtained represent the elemental composition in CaO-0 and CaO-1 in the form of Ca and O (**Figure 7**). The percentage of Ca in CaO-0 is higher than in CaO-1, with values of 19.8% and 14.7%, respectively (**Table 2**). The lower Ca content in CaO-1 may be attributed to the interaction between calcium precursors and the bioactive compounds present in *C. citratus* extract. The presence of phytochemicals such as flavonoids, tannins, and triterpenoids could influence the reduction and stabilization of CaO nanoparticles, potentially leading to the formation of additional organic residues or secondary phases. Moreover, the XRD results indicate that CaO-1 contains a diposide phase, which suggests that the synthesis using *C. citratus* extract may have facilitated the incorporation of other elements, thereby reducing the overall Ca percentage compared to CaO-0.

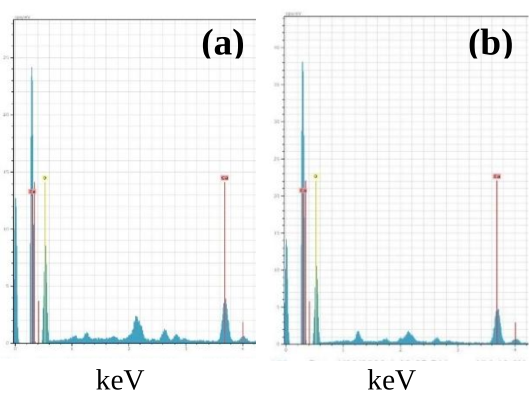

Figure 7. EDX spectra of (a) CaO-0 and (b) CaO-1

Table 2. Elemental composition of CaO

No.	Element	Composition (%)	
		CaO-0	CaO-1
1	Ca	19,8	14,7
2	O	80,2	85,3

4. CONCLUSIONS

The results showed that *C. citratus* can be used in the synthesis of CaO nanoparticles because it contains alkaloids, flavonoids, triterpenoids, and tannins. These compounds act as reducing and capping agents. Comparison of the composition of eggshell powder with the optimum *C. citratus* extract at 50:50 v/v. Characterization using a UV-Vis spectrophotometer showed that the maximum absorption of CaO nanoparticles occurred at wavelengths of 230 and 330 nm. The presence of *C. citratus* extract played a crucial role in shifting the absorption bands, indicating its involvement in nanoparticle stabilization and formation. Compared to CaO synthesized without the extract, the UV-Vis spectra of CaO-1 (50:50 composition) exhibited a more defined peak, suggesting enhanced nanoparticle dispersion and reduced aggregation. CaO-0 and CaO-1 have amorphous and crystalline structures containing a calcite phase with a crystal size of CaO-0 particles ranging from 33.72 - 38.48 nm while the crystal size of CaO-1 particles ranging from 28.93 - 70.75 nm. SEM-EDX results that the elemental composition on the surface of calcium oxide in the form of Ca and O is not homogeneous. CaO-0 is shaped like an irregular cube while CaO-1 forms rough spherical lumps.

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