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Green Synthesis of Calcium Oxide Nanoparticles and Their Catalytic and Antibacterial Activity

Enas Almadani[a]*, Fatimah Omar[b], Amyra Mohammed[a]

[a]Chemical Department, Faculty of Science, Omar Al-Mukhtar University, Al Bayda, Libya.
[b]Chemical Department, Faculty of Science, University of , Derna, Libya

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ABSTRACT

Metal oxide nanoparticles have been widely used to catalyse various types of organic reactions. The most widely used metal oxide as a heterogeneous catalyst is calcium oxide, due to its various properties, such as high activity, low cost, and environmental friendliness. This study aims to utilise the biosynthesised material (CaO) as a catalyst for the esterification of acetic acid and to study its antibacterial activity. Calcium oxide was prepared from waste chicken bones by calcining them at 800 °C for 7 h. The sample was then characterised by FTIR, N_2 sorption, TEM, SEM, EDX, and XRD. The catalytic activity was tested for the esterification of acetic acid with ethanol. The antibacterial effect was tested against two types of Gram-positive bacteria. The esterification reaction was carried out at different temperatures (40, 60, and 80 °C) and reaction times (60, 120, and 180 min). The prepared catalyst was able to achieve an acid conversion of up to 70%. The reusability test was conducted under the same reaction conditions; the catalyst was reused and achieved an acid conversion of about 69%, which was very close to the original CaO. This indicates that the catalyst did not lose its catalytic activity during the reaction. The antibacterial activity of CaO against Staphylococcus aureus and Streptococcus pyogenes was evaluated by exposure to CaO at concentrations ranging from 25 to 100 mg/mL. The antimicrobial efficacy of CaO was concentration-dependent; as NP concentration increased, the inhibition zone increased.

التخليق الأخضر لجسيمات أكسيد الكالسيوم النانوية ونشاطها الحفزي والمضاد للبكتيريا

إيناس المدني[a]*، فاطمة عمر[b] واميرة محمد[a]

[a]قسم الكيمياء، كلية العلوم، جامعة عمر المختار، البيضاء، ليبيا.
[b]قسم الكيمياء، كلية العلوم، جامعة درنة، درنة، ليبيا.

الكلمات المفتاحية:
اكسيد الكالسيوم.
عظام الدجاج.
محفزات غير متجانسة.
تفاعل الاسترة.
النشاط المضاد للبكتيريا.

الملخص

استُخدمت جسيمات أكاسيد المعادن النانوية على نطاق واسع لتحفيز أنواع مختلفة من التفاعلات العضوية، وأكثر أكاسيد المعادن استخدامًا كمحفزات غير متجانسة هو أكسيد الكالسيوم؛ نظرًا لخصائصه المتنوعة مثل النشاط العالي والتكلفة المنخفضة بالإضافة إلى كونه صديقًا للبيئة. تهدف هذه الدراسة إلى الاستفادة من المادة المتاحة (CaO) من المواد المصنعة حيويًا كمحفز لأسترة حمض الخليك ودراسة نشاطها المضاد للبكتيريا. تم تحضير أكسيد الكالسيوم من نفايات عظام الدجاج عن طريق تحميص عظام الدجاج عند درجة حرارة 800 درجة مئوية لمدة 7 ساعات. ثم تم دراسة خواص العينة باستخدام FTIR و N2-sorption و TEM و SEM و EDX و XRD. تم اختبار النشاط التحفيزي لتفاعل أسترة حمض الخليك مع الإيثانول. تم اختبار التأثير المضاد للبكتيريا لنوعين من البكتيريا موجبة الجرام. تم إجراء تفاعل الأسترة عند درجات حرارة تفاعل مختلفة (60, 40 و80 درجة مئوية) وفي أوقات تفاعل مختلفة (60 , 120 و180 دقيقة). كانت المحفزات المحضرة قادرة على إعطاء تحويل حمضي يصل إلى 70٪. تم إجراء اختبار قابلية إعادة الاستخدام في نفس ظروف التفاعل، وتم إعادة استخدام المحفز وكان قادرًا على إعطاء تحويل حمضي حوالي 69٪، وهو ما كان قريبًا كثيرًا من CaO الأصلي،

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*Corresponding author.
E-mail addresses: enas.almadani@omu.edu.ly, (F. Omar) fatimah.alqadeeri@uod.edu.ly, (A.Mohammed) amyraalhassan@gmail.com

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وهذا يشير إلى أن المحفز لم يفقد نشاطه التحفيزي أثناء التفاعل. النشاط المضاد للبكتيريا لـ CaO ضد المكورات العنقودية والمكورات العقدية، من خلال التعرض لـ CaO بتركيزات تتراوح (25 , 50 و75 و100 مجم / مل). كانت الفعالية المضادة للميكروبات لـ CaO تعتمد على التركيز، مع زيادة تركيزات NPs، تزداد منطقة التثبيط.

1. Introduction

Calcium oxide can be obtained by thermal decomposition of materials such as limestone or seashells that contain calcium carbonate ($CaCO_3$; mineral calcite) in a lime kiln, using a process known as calcination. This process starts with thermal decomposition at high temperatures, while keeping the temperature below the melting point of the material. Calcium carbonate is calcined at temperatures ranging between 1070 °C and 1270 °C. The products formed from the reaction are burnt lime and carbon dioxide. The carbon dioxide formed is immediately removed so that the reaction proceeds until completion of the process in accordance with Le Chatelier's principle.

Calcium oxide is an important inorganic compound extensively used for a wide range of applications. It can be used as a catalyst for many types of reactions, a toxic waste treatment agent, and an additive in the refractory and paint industries [1,2]. Calcium is essential for living organisms, as it is a component of bones, shells, and teeth. The most common calcium compound is calcium carbonate, which is used as a source of calcium oxide for glaze production.

Calcium oxide can be obtained from waste-derived materials and utilised as an economical and environmentally friendly catalyst in transesterification for biodiesel production [3]. Another bio-resource of CaO is the thermal decomposition of calcium-rich materials from chickens (*Gallus gallus domesticus*) and goats (*Capra hircus aegagrus*) [4]. In addition, calcium carbonate in waste chicken bone can be converted to calcium oxide and used in biodiesel production via transesterification of waste cooking oil (WCO) with methanol [5,6]. Calcium oxide has also been obtained from waste fish bones [7,8]. Other natural sources, such as crab shells and eggshells, have been characterised and evaluated in the transesterification of waste cooking oil [9]. CaO obtained from chicken, duck, and quail eggshell wastes has been applied as a raw material for the preparation of heterogeneous catalysts [10,11].

Bones are one of the most important bio-resources, consisting of cells, fillers, and fibres. These contain proteins, mineral salts, calcium phosphate, calcium carbonate, magnesium phosphate, calcium fluoride, water, and red and yellow marrow. Removal of these organic materials by heat does not significantly alter the bone structure but reduces the weight due to loss of water content [12]. Chicken bone has been used as a source of calcium oxide through chemical and thermal treatment and then modified using $FeCl_3$ solution. This catalyst showed a significant effect on biodiesel production (22%) [5].

Calcium oxides have been widely used to catalyse the esterification and transesterification reactions of vegetable oils with alcohols. Transesterification of soybean oil with methanol has been carried out using CaO obtained from different sources [13]. Calcium oxide has also been used to catalyse the transesterification of fresh and waste sunflower oil with methanol [14–16]. In other applications, CaO from eggshells was impregnated with $ZnCl_2$ (CaO/ZnO) and used as a catalyst for biodiesel production via esterification of waste cooking oil with methanol [13,17].

The increasing prevalence of microbial diseases and multidrug-resistant bacteria has driven the development of new antibacterial agents. One promising approach is the use of metal oxide nanoparticles, which exhibit strong antibacterial properties [18,19].

The aim of this work is to develop a catalyst that is inexpensive, non-polluting, and readily available for use in esterification reactions under optimum conditions, and to evaluate its antibacterial activity against Gram-positive bacteria, namely *Staphylococcus aureus* and *Streptococcus pyogenes*.

2. Experimental

2.1 Calcium Oxide Preparation

Catalyst is prepared by collecting the waste chicken bone, then washing and drying it in a normal oven. Chicken bones are ground until they become a powder (Grinding). The powder was calcinated in a burning oven to dry for 7 hours at 800°C. Then the prepared samples were characterized by FTIR, UV-spectroscopy, N_2-sorption, TEM, SEM, EDX and XRD.

2.2 Catalytic Activity of the Catalyst

The catalytic activity of the unmodified CaO and modified samples was studied by the esterification reactions. The reactions were conducted in a stirred batch reflux system according to the method by [20,21], where a two-neck round-bottom flask was equipped with a water-cooler condenser, a thermometer and a magnetic stirrer. The reaction system consisted of acetic acid and ethanol, which were mixed and heated up until they reached the reaction temperature. After that, 0.2 g of catalyst (modified CaO or unmodified CaO) was added to the reaction flask, and the reaction was conducted for 3 hours. The percentage of acetic acid conversion to ester was determined by titration with standardized 1.0 M NaOH. The samples were withdrawn from the reaction flask every subsequent hour for 3 hours. The conversion of the Acetic acid was calculated using the following equation (1) [21].

Percentage of conversion (%) = $X\ acid = ((ai-at))/(ai) \times 100$

where (ai) is the initial acidity of the reaction mixture at t0 and (at) is the acidity at (t) time

2.3 Antibacterial Activity of CaO NPs

The prepared CaO was tested against pathogenic bacteria; Gram-positive bacteria: *Staphylococcus aureus* and *Streptococcus pyogenes*, were obtained from Al-Bayda hospital. Pathogenic bacteria were cultured on sterilized Mueller-Hinton agar plates. A pure culture was prepared and incubated at 37 °C for 24 hours to obtain uniform bacterial growth. The agar well diffusion method was used to determine the antibacterial activity. Nutrient agar plates were swabbed with sterile cotton swabs, and wells were made in each of these plates using a sterile cork borer. Approximately 100µl of CaO was added to the wells using a sterile syringe. The antibacterial activity of CaO was evaluated at different concentrations (25, 50, 75, and 100 mg/ml); the procedure was repeated for all tested bacterial strains [22].

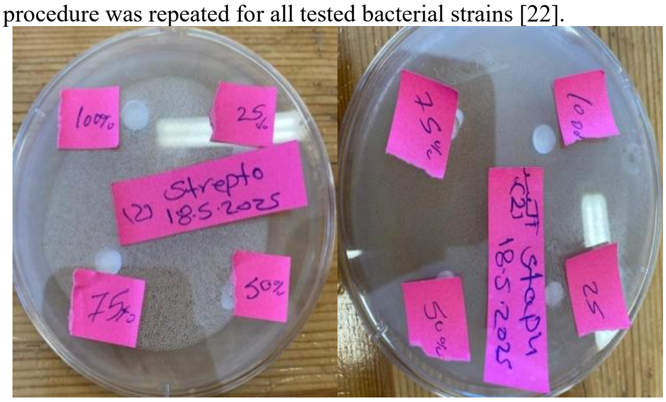


Fig.1: Bacteria with CaO NPs

3. Results and Discussion

3.1 FT-IR Spectroscopy

The FTIR spectra of CaO from 4000 to 500 cm^{-1} are shown in Figure 2. The Figure showed peaks at 3496 and 3570 cm^{-1}; these peaks correspond to the OH functional group, which indicates the presence of physisorbed and coordinated water in CaO. This is due to the hygroscopic property of CaO, and also, calcium oxide can easily absorb water vapor from the air [8]. The Figure also showed no sharp absorption in the range from 700-900 cm^{-1}, which indicated that the calcium carbonate, which is the major component in the chicken bones, is already converted to calcium oxide [11]. Peak at 1415 cm^{-1} corresponding to O-C-O attached to the calcium oxide surface [4, 23]. Other peaks at 470, 876 cm^{-1} are attributed to the bond of Ca-O [7, 24].

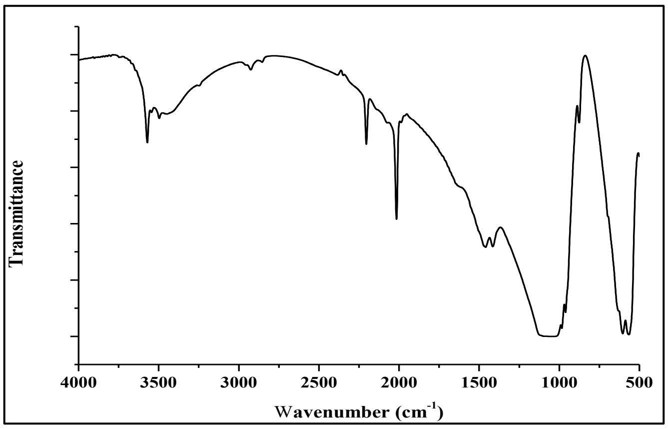


Fig. 2: FTIR spectra of unmodified and modified CaO

3.2 Scanning Electron Microscopy (SEM)

The surface morphology of unmodified and modified CaO samples was identified using SEM at a magnification of 1.50 k as shown in Figure 3. The SEM image showed that the calcium carbonate from chicken's bones forms calcium oxide at calcification temperature, and this was consistent with previous work [4]. The SEM images of CaO from chicken's bones calcined at 800 °C, showed that the surface of CaO has irregular particles (a non-uniform size of particles), which contain small flaky particles and large particles, indicating the formation of CaO [5, 25]. The small particles observed were due to the large amount of CO_2 gas produced during the calcination process (the decomposition of $CaCO_3$ to CaO and CO_2), which was also observed by Mmusi et al. 2021 [25].

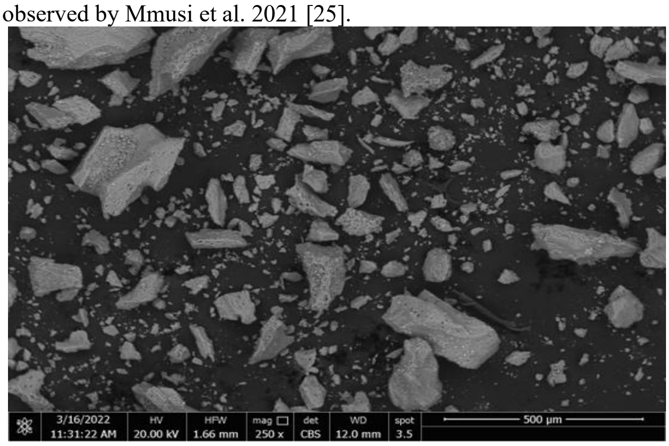


Fig. 3: SEM images of CaO with magnification of 1.50 K

3.3. Energy Dispersive X-ray Spectroscopy (EDX)

The energy dispersive X-ray spectroscopy EDX can identify the elements in the sample, as shown in Table 1. The percentage atomic compositions in all samples showed the existence of nine elements, i.e., carbon (C), oxygen (O), sodium (Na), magnesium (Mg), aluminium (Al), silicon (Si), potassium (K) and calcium (Ca). These elements represented the components of chicken bone waste; the results indicated the presence of CaO in the chicken's bones and also indicated that CaO was formed in high concentration [25].

Table 1: Elemental compositions (%) from EDX analysis of CaO

Elemental Compositions (%)	CaO
Ca	28.96
O	47.53
C	16.44
Na	1.18
Al	0.67
K	0.57
Mg	0.78
P	12.64

3.4. Textural Properties

3.4.1 Nitrogen Sorption Isotherms

The textural characterisations of the CaO sample, which consist of the surface area, pore volume and pore size distribution, were determined using BET analysis. Figure 4 shows the nitrogen sorption isotherm of the CaO. It was clear that the isotherm of CaO belonged to Type IV according to the BET classification. Type IV isotherm can be associated with the mesopore type [11]. Type IV isotherm is typical for mesoporous adsorbents. At low pressures, first, an adsorbate monolayer is formed on the pore surface, which is followed by the multilayer formation at higher pressures. The samples had a closure point at P/Po (~ 0.4). This meant that the complete monolayer formation took place slowly, and there was also an effective contribution of micropores to the adsorption on the samples. Consequently, these features of the sorption isotherms reflected the mesoporous character of the sample. However, the N_2 adsorption capacity, the volume of adsorption (cc/g) in CaO was ≈ 60 cc/g.

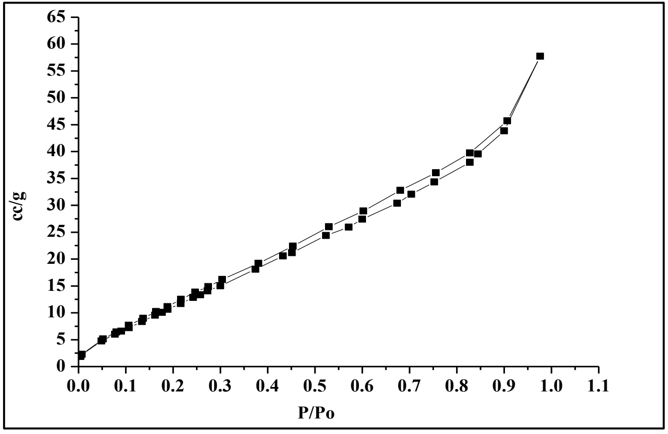


Fig. 4: Nitrogen sorption isotherm of CaO

3.4.2 Surface Area Measurement

The textural data of the samples, including the BET surface area, pore volume and pore radius obtained from the conventional analysis of nitrogen isotherms, are shown in Table 2. To calculate the BET surface area of the sample by plotting X P/Po against Y 1 / [W ((Po/P) -1)] (the BET-plots). From Table 2, it can be seen that the CaO had the highest BET surface area (48.3318 m^2/g), which almost agreed with previous studies. Zarubica et al. (2015) [26] and Widiarti et al. (2017) [8] suggested that the higher calcination temperature from 800 °C to 1000 °C will increase the crystal size, where the higher calcination temperature will break the crystal structure of calcium oxide.

Table 2: Textural properties of unmodified CaO and modified samples

Sample	Surface area (m^2/g)	Pore volume (cc/g)	Pore radius (nm)
CaO	48.3318	0.0895	28.214

3.4.3.Pore Size Distribution

Pore size distribution (PSD) was calculated by the BJH method developed by the Barret, Joyner and Halenda model. Pore size distribution curves from the adsorption isotherm of the CaO sample are illustrated in Figure 5. As discussed previously, the isotherm of the samples belonged to the mesoporous type, with pore sizes between 2-50 nm. According to IUPAC, the definition of these pores is related to the mesopores range, the material micropore (<2 nm), mesoporous (2-50 nm), and macropore (> 50 nm) [11].

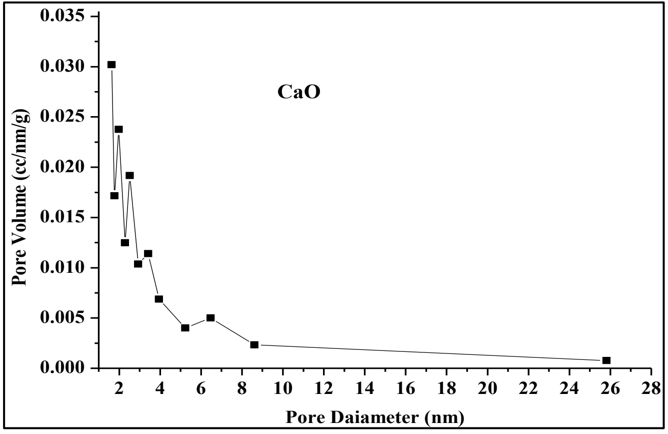


Fig. 5: Pore size distribution of the CaO sample

3.5. Transmission Electron Microscopy (TEM)

The TEM image of CaO shows agglomerated particles [27] with a size range from 46.7 nm to 66.7 nm, which confirms the nanoscale nature of the sample [28]. The crystallite size obtained from XRD was 5.94 nm, which is significantly smaller than particle size observed in TEM.

This difference is due to XRD measure the crystallite size which represent the size of a single crystalline domain, whereas TEM measures the overall particle size. The large size observed in TEM attributed to the aggregation of several crystallites forming large particles (agglomerates).

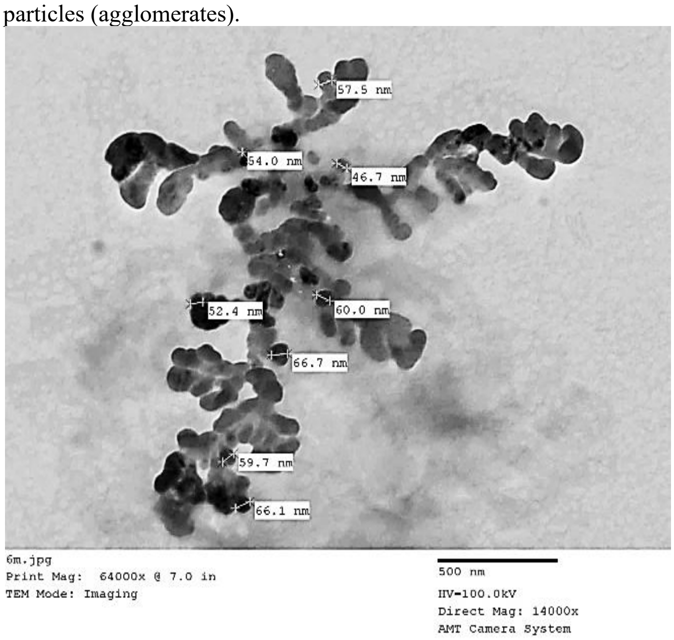


Fig. 6: TEM image of the CaO sample

3.6. X-ray diffraction (XRD)

The XRD pattern, as shown in Figure 7, the Figure showed that the most intense diffraction peaks were observed at 2θ = 32.222°,35.377°°, 46.635 ° which, corresponding to the barvais lattice plane (111), (200), (220) respectively. These were very close to the results obtained by [29,30]. Using Scherrer equation to calculate the crystallite size ($D = K^* \lambda / \beta \cos \theta$) Eddy et al., 2024 [29], where D is the average crystal size (nm), k is Scherrer's constant (0.98), λ Cu Kα radiation (1.54060Å), β the width of all diffraction peaks (FWHM) and θ the Bragg angle which corresponding to the maximum peak intense using this equation it was found that the average crystal size of CaO NPs was 5.94 nm.

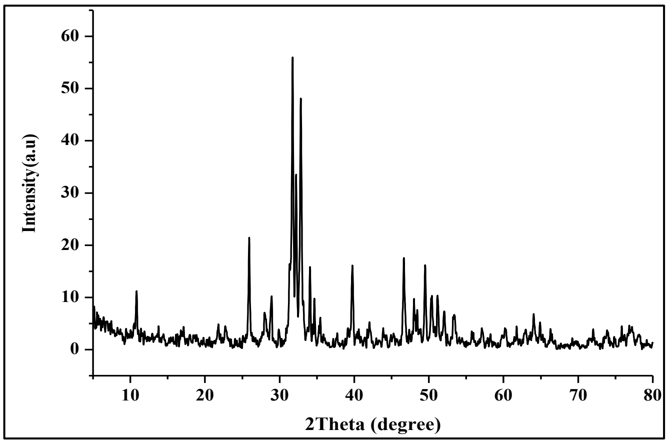


Fig. 7: XRD of CaO sample

3.7. The Catalytic Activity

The test of catalyst activity was done using the conventional method of varying one parameter at-a-time approach.

a) Effects of Catalysts

The reactions were studied in the absence of a catalyst and using CaO while fixing the other conditions. The esterification of acetic acid with ethanol using catalysts at 80 °C is shown in Figure 8. It can be observed that the reaction occurred in the absence of a catalyst, and CaO gave 43.6 and 71.42% acid conversion, respectively. This explained that the esterification reaction can be done in the absence of a catalyst, but gives a small acid conversion value [30], while the use of CaO gives about 72% acid conversions. This may be attributed to the properties of CaO as discussed previously, as the CaO showed a high surface area, which suggests that the CaO can be a potential catalyst for industrial applications.

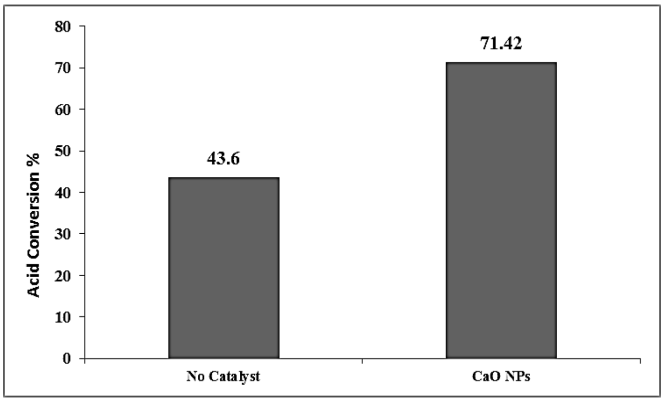


Fig. 8: Effect of catalyst on esterification of acetic acid with ethanol at 80 °C after 3 hours

Reusability Test

The reusability test was conducted using the same reaction conditions as mentioned above. After the reaction was done, the catalyst was separated from the reaction products; the catalyst was then washed with distilled water. The catalyst was dried in an oven at 150 °C and further used in the next esterification reaction. The reused catalyst gave acid conversion about 69.52 % as shown in Figure 9, which was much closed to the original CaO NPs, and this indicates that the catalyst did not lose its catalytic activity during the reaction.

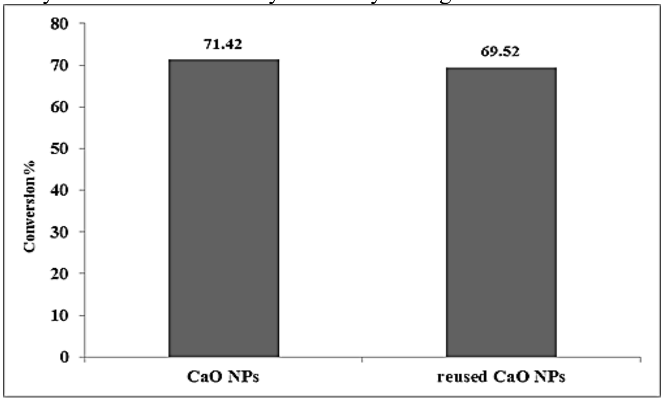


Fig. 9: The esterification of acetic acid with ethanol at 80 °C for 3 hours using the original of CaO and reusing catalyst.

b) Effect of Temperature

The temperature of the reaction had a strong effect on the esterification reaction. In this study, the esterification reactions were carried out at different reaction temperatures while keeping the other parameters constant. Figure 10 illustrates the influences of temperature on the esterification of acetic acid with ethanol at three different reaction temperatures: 40, 60 and 80 °C. From the Figure, it can be seen that the increase in the reaction temperature had a positive effect on the percentage of conversion of acetic acid. The percentage increased with increasing the reaction temperature from 40 °C (68.80) to 80 °C (71.42%). It can be seen; the optimum reaction temperature was 80 °C for the esterification reaction of acetic acid with ethanol. This might be due to the alcohol used, as the boiling point of ethanol is 78.37 °C and the reaction temperature was achieved under reflux to avoid the vapour loss during the reaction [31, 32].

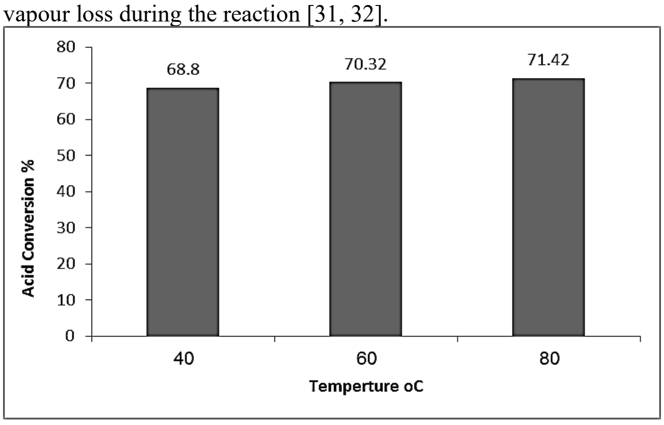
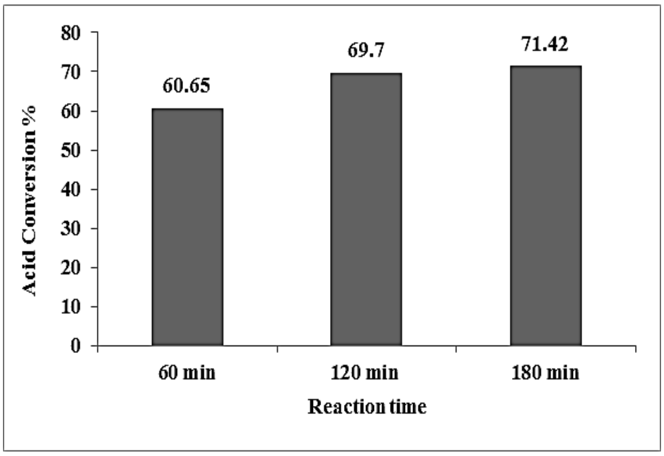


Fig. 10: Effect of temperature on esterification of acetic acid with ethanol. Reaction condition:/CaO, reaction time: 3h.

c) Effect of Reaction Time

Esterification reactions were carried out at different reaction times while keeping the other parameters constant. Figure 11 showed the reaction time profile for the esterification reaction of acetic acid with ethanol catalyzed by CaO NPs at 80 ^{o}C. The percentage value of acid conversion fluctuates from time to time, as the nature of the esterification reaction is a reversible reaction [20]. The conversion of acetic acid increased with the increase in reaction time in the esterification with ethanol. From the first 1 hour of the reaction time, the conversion percentage with ethanol was 70 % after that, it remained almost constant until 3 hours, with the maximum value at 71.42 %. The esterification reaction was expected to have a gradual increase in acid conversion with the increase in the reaction time [33]. As the esterification reaction is reversible, the increase in the reaction time might have cause the shifted the reaction to shift backwards to the (ethanol and acetic acid), as reported by [32] Syazwani et al., 2019]. Thus, the three hours were chosen as the optimum reaction time.



1. **Fig.11:** Effects of reaction time on esterification of acetic acid with ethanol and ethanol. Reaction condition: Catalyst; CaO NPs, Reaction temperature 80 ^{o}C.

3.9. The Antibacterial Activity

The results of the antimicrobial activity by diffusion against the tested microorganisms in Figures 12 and 13 showed that the CaO NPs extract from chicken bones had the highest inhibition zone in Staphylococcus aureus bacteria (10mm) at 100% concentration, and the lowest inhibition zone was (4mm) % at 25%. In addition to Streptococcus pyogenes, the highest inhibition zone was achieved (8mm) at a concentration of 100%, while the lowest inhibition zone was obtained (2mm) at 25%. In this study, the antimicrobial efficacy of CaO NPs was concentration-dependent. If the concentrations of NPs increase, the inhibitory zone will be more effective. The results reveal that the diameter of the inhibitory zone increases with increasing nanoparticle concentration for the tested strains and increasing CaO concentration results in the decrease in the microbial cell growth, the bacteria have different cell wall compositions and structures, the Gram-positive bacterium with a thick peptidoglycan layer and a thick peptidoglycan layer [34].

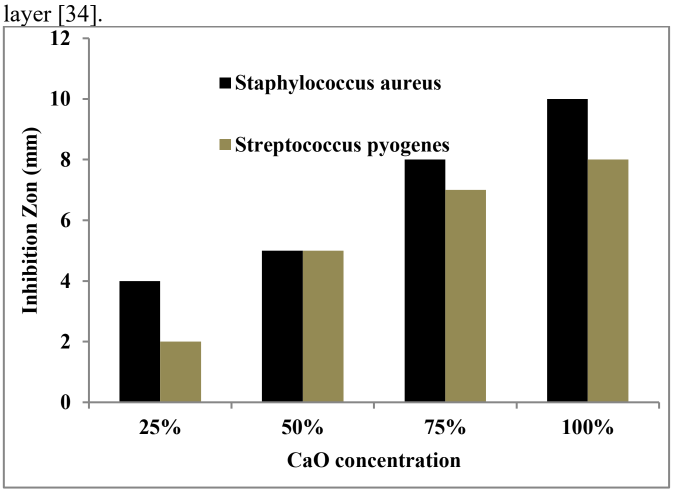


Fig.12: Antibacterial Inhibition Zone

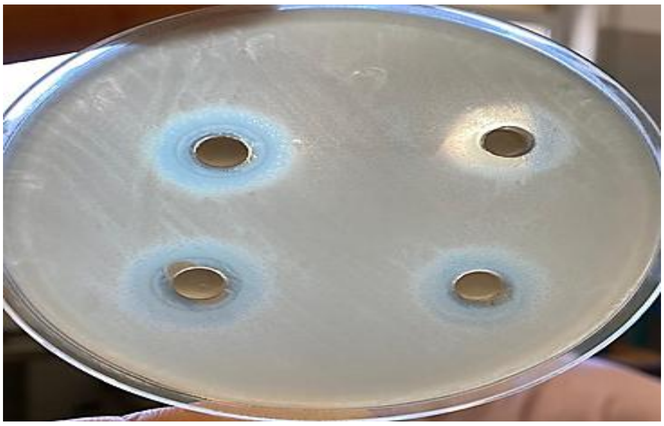


Fig.13 a: Effects of CaO on *Staphylococcus aureus*

Fig.13 b: Effects of CaO on *Streptococcus pyogenes*

4. Conclusions

Calcium oxide (CaO NPs) was successfully synthesised from biological resources (waste chicken bones). The catalyst showed outstanding results in esterification reactions and was able to achieve high acid conversions of up to 71%. The use of green sources such as waste chicken bones may provide an alternative solution to problems associated with multidrug-resistant bacteria and may also contribute to preventing the growth of microorganisms and food spoilage.

It can be concluded that calcium oxide proved to be an inexpensive and efficient heterogeneous catalyst, with the potential to replace the use of potentially hazardous homogeneous catalysts. This is in addition to the advantages of CaO, such as low cost and environmental friendliness, as well as its use as a catalyst in many organic syntheses.

5. Acknowledgement

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