Nanoparticle fabrication of calcium oxide (CaO) mediated by the extract of red dragon fruit peels (*Hylocereus Polyrhizus*) and its application as inorganic–anti-microorganism materials

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Abstract. The objective of this study was to biosynthesize calcium oxide (CaO) with the employment of the extract of red dragon fruit peels (*Hylocereus polyrhizus*) as the biologically reductive agent and to investigate the catalytic performance of the biosynthesized CaO on *Candida albicans* in aqueous conditions. In the initial process, the biosynthesis was carried out by conditioning the extract of dragon fruit peels reacting with CaCl2·2H2O as a precursor of calcium metal in the system. Continuing the characterization process of the biosynthesized results using Ultraviolet-visible spectroscopy (UV-Vis), it had maximum absorbance at 450 nm, which was indicated by the presence of Ca-O bond in the biosynthesized sample. Another characterization using Fourier-transform infrared spectroscopy (FTIR) showed the typical wave numbers that were observed at 505.35 and 540.07 cm⁻¹. This could be noticed on the IR absorption spectra of CaO crystals. The physical analysis using Scanning electron microscope (SEM) proved the morphology of the biosynthesized CaO which was in the rod shape (fiber), in which the Energy Dispersive X-Ray (EDX) confirmed the contents of the biosynthesized sample. The contents were calcium (29.06%) and oxygen (43.94%). Finally, X-ray diffraction (XRD) characterization showed that the average size of the biosynthesized CaO was 18.98 nm, which means that the produced materials can be classified into Nanoscale based on their sizes. The anti-microorganism activity of the biosynthesized CaO was observed using antifungal experiment against *Candida albicans* at various concentrations of 4500, 5900 and 6600 μg/mL under turbidimetry method. This indicated its inhibition percentage of 62.2%, 83.5% and 91.8%, respectively. This study revealed that the aqueous extract of the red dragon fruit peels (*H. Polyrhizus*) was successfully used as biological mediator on the CaO biosynthesis, and the biosynthesized CaO showed inorganic antifungal activity against C. Albicans.

Keywords: CaO nanoparticles, biosynthesis, dragon fruit peel, *Hylocereus polyrhizus*, *Candida Albicans*

1. Introduction

Term of nanomaterial refers to material sizes in the range of 1-100 nm. Nanomaterial been popularly used in many applications of life [1]. Recently, nanomaterials are interesting for many researchers due to their unique physical-chemistry characteristics for catalytic, optical, electronic, magnetic and antimicrobial materials [2]. Some preparation methods used on nanomaterial synthesis play important
role to ensure the chemical and physical properties (activity, size, shape) of the produced material which can have their characteristics as desired product. Generally, there are three (3) common methods used on nanoparticle synthesis: chemical, physical and biological methods. As it has been explained by many researchers, there are some weak points by employing chemical and physical methods on nanomaterial synthesis, such as high energy consumption, and producing some chemical wastes at their final step, so that both of these conventional preparation methods are considered to be inefficient from environmental and economic aspects [3, 4]. Considering the environmental and economic aspects as mentioned above, recently, biosynthesis method becomes an alternative method used for nanomaterial synthesis. In practical application, some biomaterial plants have been popularly used as mediator or reductive agent and they have been developed continuously by many researchers. This is recognized widely as green nanotechnology [3, 5].

Calcium oxide (CaO) is the most important materials which has been popularly used in many fields, such as catalyst, cosmetic and ceramic. It is also applied as inorganic antimicrobial material for controlling microorganisms. Based on its chemical properties, CaO exist in the alkaline earth group on periodic table [6, 7]. On the other hand, concerning the rich biodiversity of tropical plants found in Indonesia, the uses of tropical biomass or their extracts for CaO biosynthesis will be a good scientific challenge due to more efficient and eco-friendly method for CaO biosynthesis. It has been well known that metabolite compounds found in plant materials (biomasess or their extracted compounds) can act as biological reductor on metal synthesis [3, 4], where flavonoid compounds had been noticed as one of the most useful groups of secondary metabolite in plant tissues applied as reductive agent for metal ions. Most flavonoid compounds naturally contained natural pigment with various colours, such as red, pink and yellow depending on the kind of the plants [8].

Red dragon fruit (H. Polyrhizus) is a tropical plants found widely in Indonesia. It well known for its contents which are rich in flavonoid and phenolic compounds [8]. This fruit is usually consumed directly or processed into fruit juice, in which its peels are usually thrown away as by-product (organic wastes). Due to the fact that there is a high flavonoid and phenolic compounds found in red dragon peels (H. Polyrhizus), and there are still lack of scientific reports on applying the extract of red dragon fruit peels (H. Polyrhizus) as biologically reductive agent on CaO biosynthesis, we propose a new innovative work for CaO synthesis with the employment of secondary metabolite compounds extracted from H. Polyrhizus peels and its study regarding anti-microorganism activity on Candida albicans.

2. Experimental Procedure

2.1. Preparing H. Polyrhizus Extract
In this experiment, the secondary metabolite compounds were extracted from H. Polyrhizus peels in aqoues solvent using maceration method. Phytochemical identification of the secondary metabolite compounds was conducted based on scientific procedure [9]. Then, the extracted solution was used as biological agent to mediate CaO biosynthesis.

2.2. Biosynthesis of CaO Nanoparticles
CaO nanoparticles were prepared by adding calcium metal precursor (CaCl$_2$·2H$_2$O) 0.2 M to aqueous extract of H. Polyrhizus (1:1 of volume ratio). In this process, NH$_4$OH as pH controller was also used, with the reaction time of 24 hours in room temperature. This was followed by centrifuge process at 10,000 rpm for 15 minutes. After that, its precipitation and supernatants which had been separated completely were calcined at 700°C for 3 hours. The produced material (powder) will be characterized using XRD, SEM, FTIR, and its application for the study of its anti-microorganism activity on C. Albicans used turbimetry method referring to the previous scientific experiment [5, 10].
3. Results and Discussion

3.1. Phytochemistry Screening on H. Polyrhizus Peel Extract
To prevent water vaporization since it used solvent and to allow optimal amount of extracted compound, the maceration process was run at 50°C for 30 minutes. As it is shown table 1, the extract was changed from hyaline to yellow colour and formed yellow precipitate on phytochemistry screening process. It indicated some phenolic group (such as flavonoid) which were contained in the aqueous extract of H. Polyrhizus peel biomass.

| No | Phytochemical groups | observation | Physical observation |
|----|----------------------|-------------|----------------------|
| 1  | Phenolic             | +           | Yellow               |
| 2  | Flavonoids           | +           | Yellow precipitate   |

The phytochemical results of this experiment are in line with the previous research, in which the contents of the main compound in H. Polyrhizus peel extracts are phenolic and flavonoids. Based on the phytochemical test as its mentioned above, it was concluded that the extracted compound containing phenolic and flavonoids groups existing some of hydroxyl group (OH-) on their compound structures.

3.2. Biosynthesis of CaO Nanoparticles

![Figure 1](image_url)

Figure 1. (a). UV-Vis Spectra, (b) FTIR Spectra of stable Complex between Ca ions and Secondary Metabolite Compound Extracted from H. Polyrhizus Peels.

Based on the experimental observation, the colour of aqueous extract of H. polyhirzus changed from red to brown after CaCl2·2H2O was introduced in the solution system. It could be assumed that the Ca ions which existing in the solution system interacted with OH sides on secondary compounds and formed a stable complex of Ca ions-the hydroxyl in the system was indicated by brown colour. As it can be seen on Fig. 1, the UV-Vis spectrophotometer was used for monitoring whether these calcium ions were reacted with hydroxyl (OH) functional groups of the secondary compound which was extracted from H. polyhirzus extract. Fig. 1 showed the optimal absorbance of the UV-vis on the prepared sample was detected at 450 nm, where this result are in line with previous research reported [5], explaining that the specific UV-vis of Ca-OH complex on the secondary metabolite compound was clearly detected the specific absorption of UV-vis in the wavelength range of 400-450 nm. Based on these UV Vis spectra, it could be concluded that the Ca ions reacted with the hydroxyl site forming Ca-OH complex in this experiment. In order to support that Ca ions reacted with the OH functional group of the secondary compound existing in the system, the FTIR analysis was also applied in this experiment. The FTIR
analysis was measured in the range of 500-4000 cm\(^{-1}\), where it indicated vibration peak at 505.35 cm\(^{-1}\), 557 cm\(^{-1}\) and 711.73 cm\(^{-1}\). This could be predicted come from specific vibration of Ca-O due to the formation of Ca ion chemical bonding on the CO functional group as it was hypothesized previously [11].

Another physical analysis using scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) was carried out in order to obtain information about the surface morphology, size, porosity and shape of the synthesized CaO. The results of SEM image is shown in Fig. 2(a).

![SEM image](image1)

**Figure 2.** (a) SEM image, and (b) EDX’s diffractogram of the biosynthesized CaO.

As can be seen on SEM image analysis and EDX diffractogram above, it could be understood that these particles shape of the biosynthesized CaO which were resulted on this experiment were in fibre shape, these particles sizes were distributed around 50-121 nm, with average of 81.45 nm, so that it could be classified into nanoparticles material (<100 nm). Based EDX diffractogram as shown in Fig. 2(b) above, it could be noted that the synthesized CaO material mostly consist of 29.06% (w/w) of Calcium (Ca) and 43.94% (w/w) of oxygen (O), and 27.01% (w/w) of other elements consisting of 21.33% (C), 0.29% (Mg), 0.48% (Al), 3.24% (Cl), 0.12% (K) and 1.55% (Zr). This could be assumed that the elements naturally come from *H. Polyrhizus* peel biomass, as it is reported by Norashikin and Muhammad Azlan [12], who explained that *H. Polyrhizus* peel naturally containing 24 minerals, in which calcium and magnesium are two highest components found in the peel biomass.

The XRD pattern as shown in Fig. 3 completely supported about our conclusion that CaO produced successfully in this experimental work, which appearing in the specific pattern of 2\(\theta\) at 18.0166\(^{\circ}\), 34.1182\(^{\circ}\) and 50.8479\(^{\circ}\) reflected on the specific XRD pattern of CaO crystal. Based on the three highest intensities, it had been detected at 2\(\theta\) of 34.1182\(^{\circ}\), 18.0166\(^{\circ}\) and 50.8479\(^{\circ}\). The particles sizes were calculated using the Debye-Scherer equation, in which these particle size were 17.57; 18.01 and 21.34 nm respectively, with the average of their particle size was 18.98 nm.
3.3. Study of Synthesized CaO Anti-Organism Activity on C. Albicans

Anti-microorganism activity of the biosynthesized CaO on *C. Albicans* was performed using turbidimetry technique. Turbidimetry is known as a method to measure the inhibition anti-microorganism by evaluating turbidity of solution based on their UV-Vis absorption at the wavelength of 600 nm. In this experiment, the anti-microorganism activity of the biosynthesized CaO on *Candida albicans* was designed at various concentration of 4500, 5900 and 6600 μg/m. This showed the inhibition percentage of 62.2%, 83.5% and 91.8%, respectively. Referring to the scientific report by Becker et al. [13], alkaline earth metal oxides (such as CaO) has been a large surface area and important materials to produces Reactive Oxygen Species (ROS) such as O$_2^-$, and 'OH, where those ROS components could be active to inhibit *C. Albicans*.

4. Conclusion

The aqueous extract of *H. Polyrhizus* peel biomass has been successfully used as biological mediator to biosynthesize CaO nanoparticle. It has fibre shape, with the average size was 18.98 nm. The biosynthesize CaO nanoparticle shown their anti-microorganism activities on *C. Albicans*.

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