ECO-FRIENDLY SYNTHESIS OF CERIUM OXIDE NANOPARTICLES FOR UV PROTECTION

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Abstract

Long exposure of UV radiation increases risk of skin diseases such as skin cancer. Cerium oxide nanoparticles have great potential to be made into sunscreen for Ultraviolet radiation (UV) protection purposes. However, the conventional method of synthesizing cerium oxide nanoparticles uses calcination. Although it is effective, it is extremely energy-intensive and economically unfriendly. In the present study, cerium oxide was synthesized using leaf extracts of *Cordyline fruticosa* and peel extracts of dragon fruit. The cerium oxide nanoparticles synthesized were evaluated in terms of their in vitro SPF and antioxidative properties. Results showed that cerium oxide nanoparticles synthesized are spherical in shape and have sizes less than 5 nm. Cerium oxide nanoparticles synthesized using *Cordyline fruticosa* leaf extracts has higher in vitro SPF and greater antioxidative properties than that synthesized using dragon fruit peel extracts. Sunblock containing cerium oxide nanoparticles synthesized using *Cordyline fruticosa* leaf extracts have UV absorption property comparable to that of commercial sunblock and has great potential to be a safe and effective alternative to commercial sunscreen containing harmful chemicals like oxybenzone.

1. Introduction

Sun radiation constantly impacts the earth with approximately 50% visible light, 40% infrared radiation (IR) and 10% ultraviolet radiation (UV). Ultraviolet rays can be divided into UVA rays (320 to 400nm), UVB rays (290 to 320 nm), and UVC (100 to 290nm). Fortunately, the most damaging UV radiation, UVC rays, do not reach the Earth's surface, as it is completely absorbed by ozone (Khazaeli & Mehrabani, 2008).

The destructive influence of UVA and UVB rays occur on the biochemical, the cellular and on the functional level of the human skin. UVA rays contribute to skin cancer via indirect DNA damage, resulting in single-strand breaks in DNA. UVB rays, on the other hand, not only causes sunburn but also some forms of skin cancer. Both UVA and UVB also destroy vitamin A in skin, which may cause further damage (Fonseca & Rafaela, 2013).

In order to alleviate the harmful effects of UV radiation, sunscreens have been developed. Sunscreens typically contain inorganic agents such as titanium dioxide or zinc oxide and organic agents such as oxybenzone (Fonseca & Rafaela, 2013). However, the high refractive indices of titanium dioxide and zinc oxide can make the skin look unnaturally white when incorporated into the products. In addition, their high photocatalytic activity facilitates the generation of reactive oxygen species, which can oxidize and degrade other ingredients in the formulation, raising safety concerns. Oxybenzone, on the other hand, causes allergic skin reactions, may disrupt hormones and is associated with endometriosis in women and alters sperm production in animals (Kunisue et al., 2012).

Cerium is a lanthanide series rare earth element, and is the most abundant of these elements, present at about 66 parts per million in the earth's crust. Cerium can exist in oxide form and can cycle between cerium(III) and cerium(IV) oxidation states. Both oxides of cerium strongly absorb UV light (Hu et al., 2006). In addition, as cerium oxide nanoparticles has good transparency in the visible range, has no known toxicity and appears natural on the skin (Herrling et al., 2013), it is a promising material for use as a UV filter in sunscreen cosmetic products.

2. Objectives and hypotheses

The objectives of this study are to

- synthesize cerium oxide nanoparticles using an ecofriendly method where extracts of *Cordyline fruticosa* leaves and dragon fruit peel were used as capping agents in the synthesis.
- evaluate the in vitro SPF and antioxidative properties of cerium oxide nanoparticles synthesized.
- develop a sunblock containing cerium oxide nanoparticles as the UV absorbing material.

Hypotheses:

- Cerium oxide nanoparticles can be synthesized using extracts of *Cordyline fruticosa* leaves and dragon fruit peel.
- Cerium oxide nanoparticles synthesized using *Cordyline fruticosa leaf extract* possess greater UV absorption and antioxidative properties than that synthesized using dragon fruit peel extracts.
- Sunblock containing cerium oxide nanoparticles has UV absorption property comparable to that of commercial sunblock.

3. Materials and Methods

3.1 Materials

Sodium hydroxide was purchased from GCE Chemicals. Cerium nitrate, ABTS and Folin-Ciocalteau agents were purchased from Sigma Aldrich. Leaves of *Cordyline fruticosa* were obtained from school while dragon fruit peels were obtained from local fruit stalls.

3.2 Extraction of extract from leaves/fruit peel

Dragon fruit peel and leaves of *Cordyline fruticosa* were chosen as both contain anthocyanin, a reducing agent and antioxidant which could potentially act as capping and stabilizing agents during the synthesis of cerium oxide nanoparticles. *Cordyline fruticosa* is a plant with purple leaves which is commonly found in Singapore (figure 1).

Figure 1. *Cordyline fruticosa* plant

A sample of 10 g of finely cut dried leaves/peel was added to 100 ml of deionised water and boiled for 15 min. The mixture was filtered, centrifuged and stored in refrigerator for further usage.

3.3 Synthesis of cerium oxide nanoparticles

1 ml of cerium (III) nitrate (10 mM), 1 ml of aqueous leaf/peel extract and 7 ml deionised water were first mixed at room temperature. 1 ml of sodium hydroxide (30 mM) was then added drop-wise to the mixture. The suspension was stirred for 48 h, when it became clear. A control with cerium(III) nitrate and sodium hydroxide but without plant extract was included for comparison.

The mixtures were finally sonicated for 10 min to obtain well-dispersed colloidal cerium oxide nanoparticles.

Cerium oxide nanoparticles were characterized using UV-VIS spectrophotometry, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM).

3.4 UV absorption properties of cerium oxide nanoparticle in terms of in vitro SPF

The colloidal solution of cerium oxide nanoparticles prepared by each extract was used to obtain a UV-Absorption spectrum by scanning in the range of 290-320 nm at 5 nm intervals. Readings were taken in triplicates for each point. Calculation of SPF was performed using the Mansur equation (Mansur et al., 1986):

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where EE(λ) is the erythemal effect spectrum, I(λ) is the solar intensity spectrum, Abs (λ) is the absorbance of cerium oxide nanoparticles; CF is the correction factor (=10). The values of EExI are constant, as shown in table 1.

Table 1. Normalized product function used in the calculation of in vitro SPF (Mansur et al., 1986)

Wavelength (nm)	EExI (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
315	0.0839
320	0.0180

3.5 Antioxidative properties of cerium oxide nanoparticles (ABTS assay)

Free radical scavenging activity of colloidal cerium oxide nanoparticles was determined by ABTS radical cation decolorization assay **(**Rajurkar & Hande, 2011**).** ABTS·+ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use.

2.5ml of ABTS Solution was transferred into a cuvette using a micropipette. This was followed by the addition of 0.5ml of cerium oxide nanoparticles. Thereafter, the cuvette was capped and mixed. The reaction was allowed to occur for 25 minutes, after which the absorbance was measured at 743 nm. Control was prepared by adding 2.5 ml of ABTS solution to 0.5 ml of deionized water. Absorbance of control is the initial absorbance.

The radical scavenging activity of cerium oxide nanoparticles is expressed in terms of the percentage inhibition of ABTS radical. Percentage of inhibition is calculated using the formula:

$$\text{Percentage inhibition} = \frac{A_{initial} - A_{final}}{A_{initial}} \text{ x } 100\%$$

The higher the percentage of inhibition, the greater the scavenging activity of cerium oxide nanoparticles.

3.6 Total polyphenol content of plant extracts

The total phenolic content of the peels was determined using the Folin-Ciocalteau assay with gallic acid as the standard. An aliquot (1 ml) of peel or leaf extract was added to a 25 volumetric flask containing 9 ml of deionised water. A reagent blank using deionised water was also prepared. One milliliter of the Folin-Ciocalteau's phenolic reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% (w/v) sodium carbonate solution was added to the mixture. The solution was diluted to 25 ml with deionised water and mixed. After incubation for 90 min at room temperature, the absorbance against the prepared reagent blank was determined at 750 nm with a UV-VIS Spectrophotometer. Calibration curve was prepared using gallic acid of concentrations 20, 40, 60, 80 and 100 mg/l. The total phenolic contents of the extracts were expressed as milligrams of gallic acid equivalents (GAE) per litre of extract (mg GAE/l). All samples were analysed in triplicates.

3.7 Preparation and evaluation of sunblock containing cerium oxide nanoparticles

The ingredients required to make the sunblock can be divided into:

- Oily phase consisting of 3 g of beeswax, 5 g of olive oil, 15 g of mineral oil and 12 g emulsifying wax
- Aqueous phase consisting of 35 g of deionised water and 10g of glycerine

Ingredients from each phase was mixed and heated to 80°C, until a homogeneous mixture was obtained. Then the 2 phases were mixed together to obtain a homogeneous cream. Finally, cerium oxide nanoparticles of varying concentrations (5%-10% w/w) were added and the mixture mixed to give the final product.

UV absorption property of sunblock was tested using a UV-VIS spectrophotometer. A fixed mass (0.4 g) of sunblock was spread onto one side of a cuvette which was then inserted into the spectrophotometer. The absorbance was measured at 300, 320, 340 and 360 nm. Results were compared with control (sunblock without any cerium oxide nanoparticles) and commercial sunblock.

4. Result and Discussions

4.1 Characterisation of cerium oxide nanoparticles

4.1.1 UV VIS spectrum

The UV VIS spectrum of cerium oxide nanoparticles synthesized using leaf extract of *Cordyline fruticosa* is shown in figure 2, where an intense surface plasmon resonance (SPR) absorption peak at 280 nm corresponding to cerium (III) oxide was observed. The result is similar to that reported by Dutta et al., (2016). In contrast, UV VIS spectrum of cerium oxide nanoparticles synthesized using dragon fruit peel extract does not show evident peak around 280 nm.

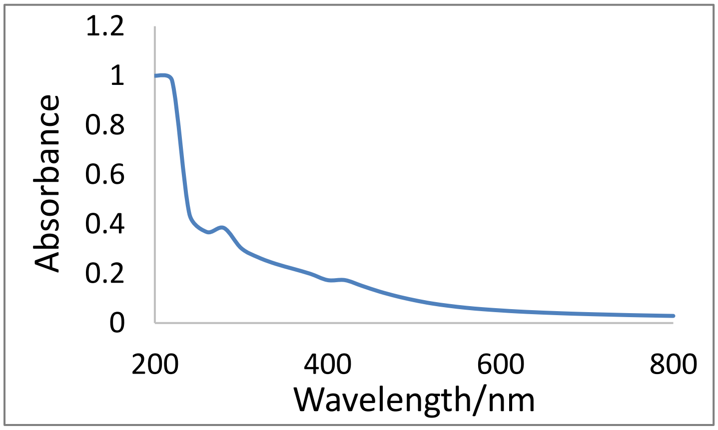
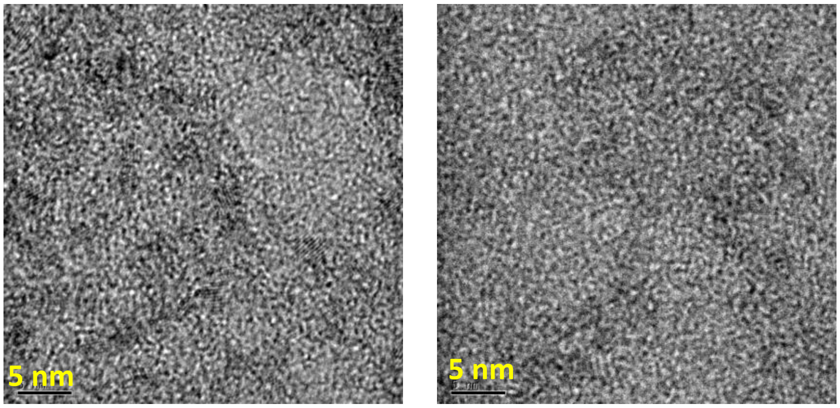


Figure 2: UV VIS spectrum of cerium oxide nanoparticles synthesized using leave extract of *Cordyline fruticosa*

4.1.2 SEM images of cerium oxide nanoparticles



(a) (b)

Figure 3: TEM image of cerium oxide nanoparticles synthesized using (a) *Cordyline fruticosa* leaf extract, (b) dragon fruit peel extract

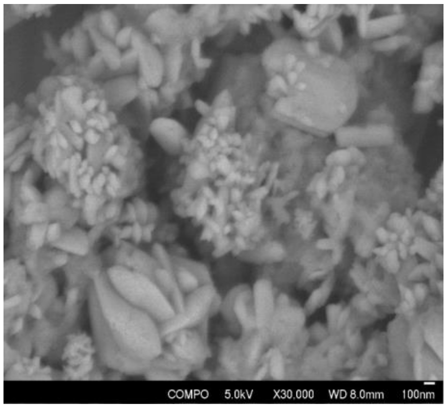


Figure 4: SEM image of cerium oxide nanoparticles synthesized without plant extracts

Cerium oxide nanoparticles synthesized using *Cordyline fruticosa* leaf and dragon fruit peel extracts were spherical and less than 5 nm (figure 3). The result was similar to cerium oxide nanoparticles synthesized using *Aloe ve*ra extract where the nanoparticles range from 2-3 nm with spherical shapes (Dutta et al., 2016).

In contrast, cerium oxide nanoparticles synthesized without the plant extracts produced particles with a much larger size of 2-3 µm (figure 4). This is due to the biomolecules present in the plant extracts which act as capping agents for the cerium oxide nanoparticles (figure 5), stabilizing them and preventing them from aggregating (Dutta et al., 2016), hence resulting in particles with much smaller sizes.

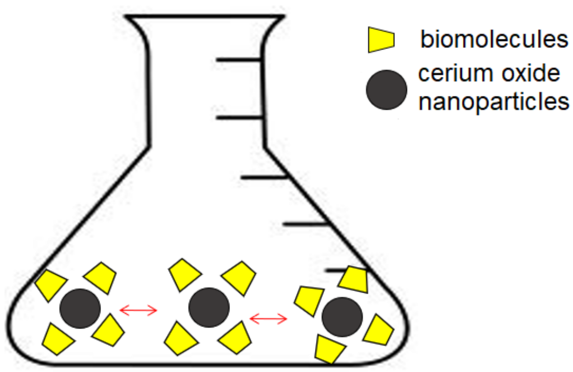


Figure 5: Biomolecules in plant extracts acting as capping and stabilizing agents for cerium oxide nanoparticles

4.2 UV Absorption property of cerium oxide nanoparticles

Evaluation of the efficiency of a sunscreen by means of SPF has been assessed for a long time through in-vivo test which is performed on human volunteers (Ebrahimzadeh et al., 2014). Although useful and precise, this type of determination is time-consuming, complex and expensive (Dutra et al., 2004). The in vitro SPF which is determined using UV spectrophotometry is useful for screening test during the product development, as a supplement of the in vivo SPF (Korac and Khambholija, 2011).

As shown in figure 6, cerium oxide nanoparticles synthesised using leaf extracts of *Cordyline fruticosa* and dragon fruit peel extract have higher in vitro SPF than their corresponding extracts. Cerium oxide synthesized using leaf extracts of *Cordyline fruticosa* has the highest in vitro SPF while the cerium oxide nanoparticles synthesized without plant extract has the lowest in vitro SPF. Due to the small particle size, the cerium oxide nanoparticles synthesized using leaf extract are very effective in UV absorption owing to high surface area for interaction with light. The absorption edge of cerium oxide nanoparticle lies between 350-370 nm, providing good transparency in the visible range and good absorption in the UV-B and UV-C range (Gopinathan et al., 2015).

In contrast, cerium oxide synthesized without plant extract has very low in vitro SPF. Without the presence of biomolecules from plant extract to act as stabilizing agent, cerium oxide nanoparticles are likely to aggregate, resulting in larger particle size, as supported by SEM analysis (figure 4). Hence, they are not as effective in the absorption of UV.

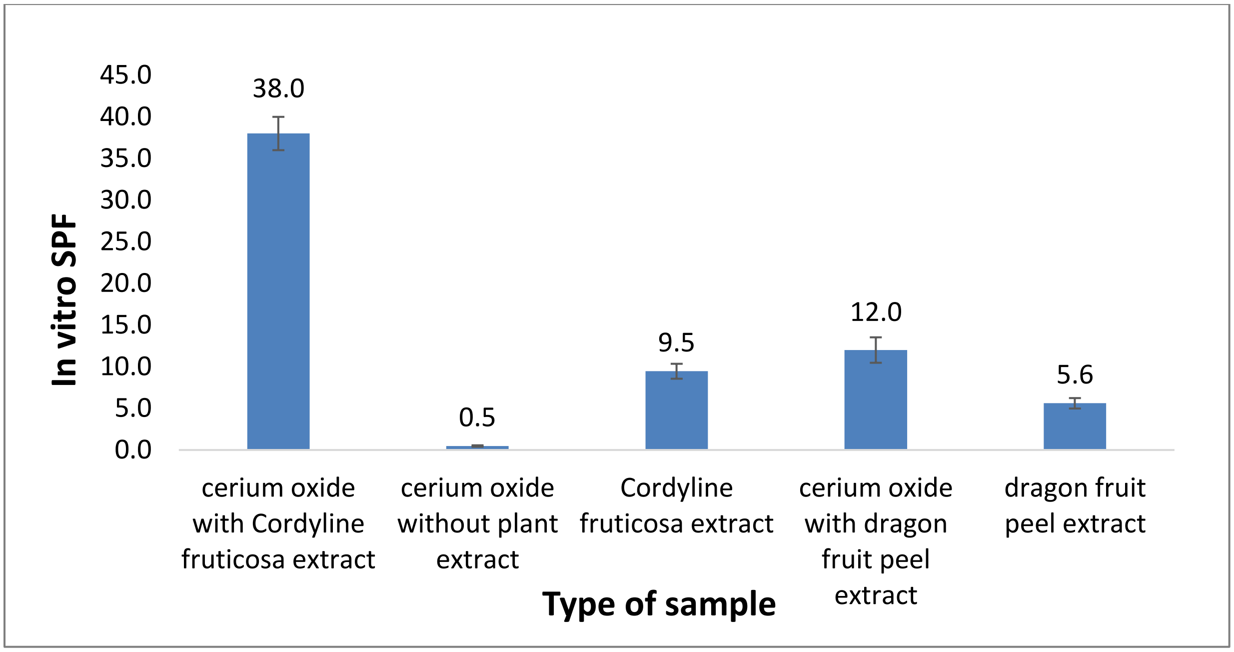


Figure 6: In vitro SPF of cerium oxide nanoparticles synthesized with and without plant extracts

4.3 Antioxidative properties of cerium oxide nanoparticles

UV rays have the capacity to generate reactive chemical species, including free radicals which could lead to photoaging and cancer. Besides being able to absorb UV, it would be an added advantage if cerium oxide nanoparticles are able to scavenge free radicals.

Figure 7 compares the antioxidative properties of cerium oxide nanoparticles synthesized using different extracts.

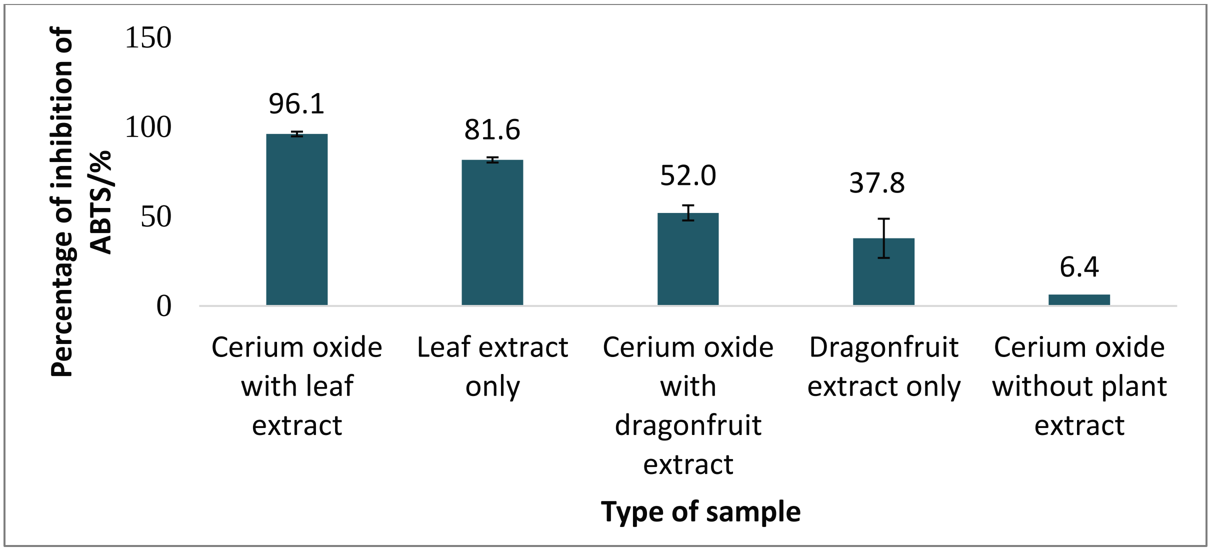


Figure 7: Antioxidative properties of cerium oxide nanoparticles synthesized with and without plant extracts

Figure 7 shows that the cerium oxide nanoparticles synthesized using leaf extract of *Cordyline fruticosa* scavenged ABTS by about 96% and this is 15 times greater than that of cerium oxide nanoparticles without plant extract. The Ce^{3+} from the cerium oxide nanoparticles are able to scavenge the ABTS radicals, and during the reaction gets oxidized to Ce^{4+} (Dutta et al., 2016). The leaf extract of *Cordyline fruticosa* and peel extract of dragon fruit peel also possess good antioxidant property, due to the polyphenols such as anthocyanin which are present in the leaf and peel extracts (Figure 8). The total polyphenol content of *Cordyline fruticosa* leaf extract is about 7 times higher than that in dragon fruit peel extract, explaining why the radical scavenging ability of cerium oxide nanoparticles synthesized by *Cordyline fruticosa* leaf extract is much higher than that synthesized by dragon fruit peel extract.

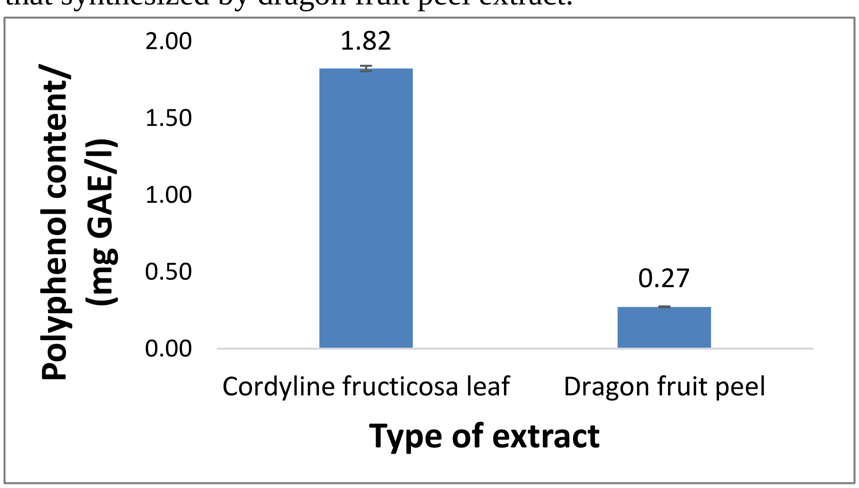


Figure 8: Total polyphenol contents of *Cordyline fructicosa* leaf and dragon fruit peel extracts.
GAE: Gallic acid equivalents

4.4 UV Absorption property of sunblock containing cerium oxide nanoparticles

Sunblock containing cerium oxide nanoparticles synthesized using *Cordyline fruticosa* leaf extracts is comparable to commercial sunblock in absorbing UV (figure 9), suggesting that it has great potential to be further developed into a commercial product. Optimum concentration of cerium oxide nanoparticles to be incorporated is 10%.

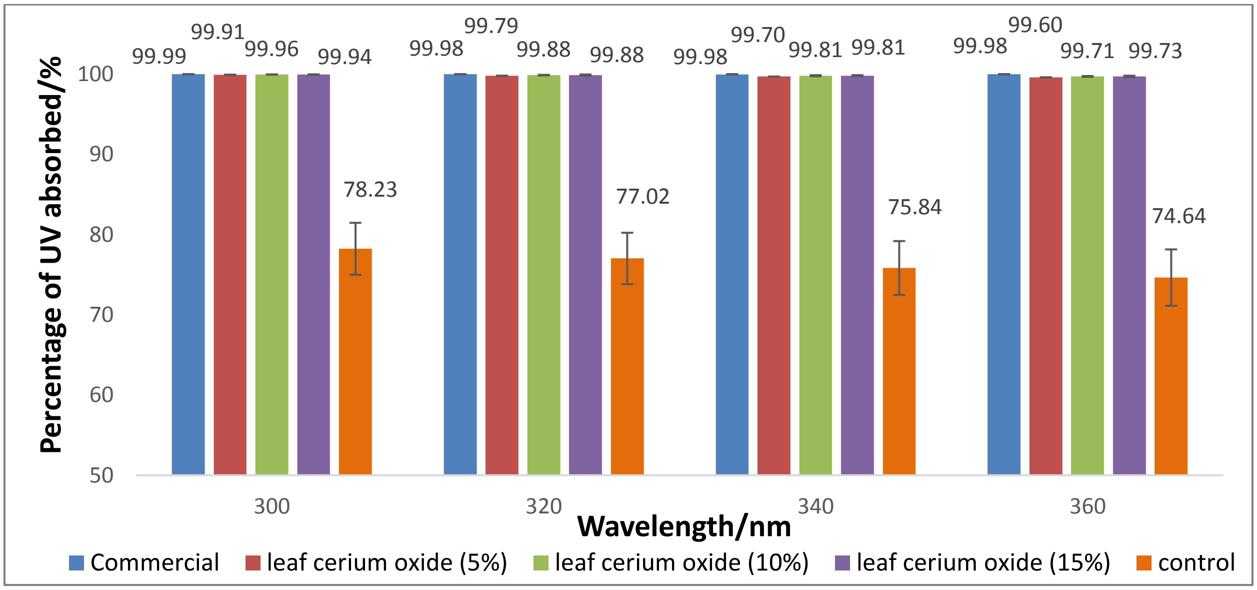


Figure 9: UV absorption property of sunblock containing cerium oxide nanoparticles

5. Conclusion

Cerium oxide nanoparticles have been successfully synthesized using *Cordyline fruticosa* leaf and dragon fruit peel extracts via a simple and eco-friendly method. Cerium oxide nanoparticles synthesized using *Cordyline fruticosa* leaf extracts have greater in vitro SPF and antioxidative properties as compared to those synthesized using dragon fruit peel extracts. Its polyphenol content is also 7 times that of dragon peel extract. Incorporating the cerium oxide nanoparticles synthesized using *Cordyline fruticosa* leaf extracts into sunblock enhances its UV absorption property which was comparable to that of commercial sunblock. The optimal concentration of cerium oxide nanoparticles was determined to be 10%.

6. Future Work

In the future, the sunblock containing cerium oxide nanoparticles can be tested on artificial skin such as the episkin. The stability of cerium oxide nanoparticles can also be evaluated to determine the practicality of using it in sunblock.

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