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Preparation of Celery Leaf Extract, Phytochemical Screening, and Nanoparticle Formulation in Antiaging Cream Development

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A B S T R A C T

This study examines the preparation of bioactive extracts from celery leaves (*Apium graveolens* L.) and their transformation into nanoparticulate forms for potential application in antiaging formulations. The celery leaves were extracted using the maceration method with 96% ethanol. The phytochemicals of celery extract were examined using a unique reagent, which shows the celery extract contains flavonoid, alkaloid, and triterpenoid. These compounds are usually used as active agents in antiaging creams. We employed the ultrasonication probe-homogenizer to produce nanosized particles of celery extracts. The particle size analyzer data showed that the particle size significantly reduced from 6,757.2 nm (bulk) into nanoparticle when probe homogenization was employed, with averages size 583.8 nm (30 minutes homogenization), 219.9 nm (60 minutes homogenization), 161.7 nm (90 minutes homogenization). These values indicate that within time, the bulk active compound in celery could be used to formulate an antiaging cream for the next application.

Contribution to Sustainable Development Goals (SDGs):
SDG 3: Good Health and Well-being
SDG 9: Industry, Innovation, and Infrastructure
SDG 12: Responsible Consumption and Production

1. INTRODUCTION

1.1. Research Background

Premature aging is an unavoidable physiological process. It is generally caused by free radicals from exposure to air pollution, UV radiation, and various other factors. Preventing premature aging can be achieved through multiple measures, including anti-aging cosmetic creams[1]. Although numerous anti-aging creams are available on the market, concerns regarding the safety of their active compounds persist. Consequently, research into natural active compounds derived from plants continues to evolve. Celery (*Apium graveolens* L.) contains flavonoids such as luteolin and apigenin, which exhibit antioxidant activity capable of inhibiting the aging process in the skin[2]. However, the potential of these compounds is limited due to the low solubility of plant-derived active compounds, making them less effective for direct use as antiaging agents in cosmetic formulations. By employing nanotechnology, these active compounds can be engineered into nanoscale particles, offering improved bioavailability compared to their bulk counterparts [3].

Although natural active compounds such as flavonoids and polyphenols exhibit high biological activity, they face challenges due to their purifying complexity, such as utilizing toxic solvents. The application of nanotechnology in cosmetics represents a groundbreaking advancement in the industry. One of the primary advantages of nanotechnology in cosmetic products is the enhanced stability and durability of the formulations, achieved through the homogeneous distribution of nanosized active ingredients compared to their bulkier counterparts, which are less soluble in formulations[4]. Nanotechnology approaches from biomass such as nanocellulose from pineapple leaf waste [5] and hemp [6], nano-starch from yam bean [7] and sago [8], and nanocatechin from gambir [9] have been reported in our previous work. This can be a reference for the development of new products by utilizing nanotechnology from celery waste biomass to produce nanosized extract which can be used for the manufacture of herbal cosmetics with nanosized active ingredients that have high antiaging activity, this is because nano-sized active ingredients have higher antioxidant activity, the distribution in the cream is more homogeneous, and are stable at higher temperatures compared to their bulky size, so that to obtain the same antioxidant activity only requires less nano active ingredients compared to their bulky ingredients.

Another benefit of nanosized active compounds is their more efficient skin penetration due to their extremely small size (100–1000 nm), allowing the active ingredients to reach the targeted skin layers for optimal effectiveness [10]. To date, numerous antiaging cream formulations have been studied and developed. However, many papers reported that pure molecules as active compounds were used as active agents in antiaging-formulation. This method is likely ineffective due to toxic solvent utilization and the complicated procedure. Nanotechnology is a new pathway to enhance the benefits of active compounds in celery extract. Here, we proposed the new method for producing nanosized active compounds from celery in a simple way and also relatively safe for cosmetic application in compounds derived from celery extract.

1.2. Literature Review

Premature skin aging is typically caused by free radicals originating from air pollution, photo-oxidation of the skin by ultraviolet (UV) rays, and unhealthy lifestyle choices. In addition to affecting physical appearance, premature aging is a concern because it may indicate the onset of various diseases, such as skin cancer. Preventive aging is often achieved with antiaging creams in cosmetic formulations. Currently, the active compounds found in commercially available antiaging creams are primarily derived from chemical synthesis or isolation, which comes with drawbacks such as high production costs, rendering these products unaffordable for many. Furthermore, the production processes often involve solvents that can harm the skin. Consequently, herbal cosmetics are popular due to their relatively low cost, safety, and high skin tolerability [11].

Various plants containing antioxidant compounds, such as extracts from mangosteen peel [12], citrus[13], and other plant sources, have been widely developed. However, these plants are seasonal and not always available throughout the year. On the other hand, Celery (*Apium graveolens* L.) is a horticultural vegetable that is abundantly grown, readily available year-round, and widely cultivated by farmers. In everyday practice, celery is commonly used as a vegetable, seasoning for soups, or ingredient in other dishes. However, the older leaves of celery are often discarded as waste. These older leaves contain higher antioxidants, including saponins, flavonoids (apigenin and luteolin), and polyphenols. Utilizing celery leaf waste could provide a valuable source of active compounds to produce antiaging agents in cosmetics. Flavonoids in celery, particularly luteolin and apigenin, are known to exhibit high antioxidant activity, making celery a promising candidate for antiaging cream formulation [14].

The hydroxyl groups in apigenin and luteolin serve as scavengers of free radicals, which are the primary contributors to premature aging through oxidative reactions. Free radicals are atoms or molecules with unpaired electrons, making them highly reactive and capable of damaging other molecules, including those in living cells, causing degenerative diseases such as cell damage. Compared to synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate [15], which are known for their high toxicity, flavonoids are relatively safer alternatives.

1.3. Research Objective

This study aims to produce nanosized active compounds (100–1000 nm) from ethanolic extract derived from celery leaf waste. Phytochemical screening was performed on the extract using specific reagents. These extracts were converted into nanosized particles once confirmed to contain active compounds such as flavonoids.

2. MATERIALS AND METHODS

2.1. Extraction of Celery

Celery leaves were air-dried for two days, indicated by the wilting of the leaves. Subsequently, they were oven-dried at 50 °C for 6 hours until the leaves became brittle. The dried leaves were then ground to a uniform particle size of 60 mesh to facilitate extraction. A total of 500 g of powdered celery leaves was placed in a container, followed by adding 1 L of 96% ethanol as a solvent. The mixture was left to macerate for 72 hours, stirring every 24 hours to ensure optimal extraction. The mixture was then filtered using Whatman No. 41 filter paper, and the solvent was evaporated using a rotary vacuum evaporator until a concentrated celery leaf ethanol extract was obtained. The evaporation was conducted at 35 °C under a pressure of 100 mbar.

2.2. Phytochemical Screening

The following tests were conducted to identify the bioactive compounds in the celery leaves [16]:

2.2.1. Flavonoid Test

One gram of celery leaf powder was added to 100 mL of hot water, boiled, and filtered. The filtrate was mixed with 0.05 g of magnesium powder and 1 mL of concentrated HCl. A positive result was indicated by a color change to red, yellow, or orange, confirming the presence of flavonoids.

2.2.2. Alkaloid Test

One gram of celery leaves was mixed with 1 mL of 2N HCl and 9 mL of distilled water, heated, cooled, and filtered. The filtrate was divided into three test tubes, each treated with Mayer's, Buchardat's, and Dragendorff's reagents. A positive result was indicated by forming a precipitate or turbidity in at least two of the three tests, confirming the presence of alkaloids.

2.2.3. Steroid Test

One gram of celery leaves was placed in a test tube and mixed with 2 mL of acetic anhydride, followed by a drop of concentrated

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sulfuric acid. A color change (e.g., green) indicated the presence of steroids.

2.2.4. *Triterpenoid Test*

One gram of celery leaves was mixed with acetic anhydride and concentrated sulfuric acid. A color change indicated the presence of triterpenoids.

2.2.5. *Tannin Test*

One gram of celery leaves was placed in a test tube, added to 10 mL of hot water, and boiled. The filtrate was treated with 2–4 drops of $FeCl_3$. The change of color to bluish-green or blackish-green indicated a positive result for tannins.

2.2.6. *Nanoparticle production from celery extract*

The crude extract was transferred to a 100 mL beaker, and the nano-active ingredients were prepared using the ultrasonic probe homogenizer method, following the procedure outlined by Ref. [9]. The celery extract was dissolved in 500 mL of solvent and subjected to size reduction using an Ultrasonic Probe Homogenizer with time variations of 30 minutes, 60 minutes, and 90 minutes. The particle size were examined using particle size analyzer Horiba SZ100Z2.

3. RESULT AND DISCUSSION

3.1. *Extraction of celery extract*

The extraction of bioactive compounds from celery leaves (*Apium graveolens* L.) was employed to isolate these active compounds using ethanol as a solvent. The mature celery leaves, often considered waste, were sorted, cleaned, and dried. This initial treatment was critical to remove impurities, including dirt and pesticide residues, ensuring a pure extract.

Once the leaves were dried for two days in the open air, in an oven at 50 ^{o}C for six hours, they ground into a fine powder. The particle size is filtered through a 60-mesh sieve; this small size of starting material allows for increased surface area, which is essential for effective extraction. The ethanol 96% can solubilize a wide array of bioactive constituents while maintaining stability. The extraction process involved macerating the leaf powder in ethanol for three days at room temperature, allowing for optimal leaching of phytochemicals [15]. This prolonged contact ensured that the active compounds, particularly flavonoids such as luteolin and apigenin, were efficiently dissolved into the solvent without degradation from heat. Upon completion of the extraction, the ethanol was evaporated to yield a concentrated extract ready for subsequent analysis.

3.2. *Phytochemical screening*

The phytochemical screening confirmed the presence of these active compounds, which are the desired antiaging properties of the cream. The positive results regarding flavonoid content underline the potential of celery leaf extract as a natural antioxidant resource. The phytochemical analysis of celery leaves (*Apium graveolens* L.) includes alkaloid, phenolic, flavonoid, steroid and triterpenoid as general screening for secondary metabolite presence in celery leaves extract. The alkaloid test demonstrated the presence of alkaloids in celery leaves, indicated by the formation of an orange precipitate when tested with Dragendorff's reagent.

In contrast, tests for phenolic compounds and tannins yielded negative results, as indicated by the lack of a brownish-green or blue-black color upon adding $FeCl_3$. This absence suggests that celery leaves may not be rich in phenolic compounds. The flavonoid test showed that celery contains high flavonoids, as the orange color showed, and flavonoid compounds like luteolin and apigenin, known for their strong antioxidant effects, may be present. This suggests that they could serve as valuable active ingredients in antiaging formulations.

Additionally, the steroid test revealed that celery leaves do not contain steroids; conversely, triterpenoids were found in celery leaves; the phytochemical screening is shown in Figure 1.

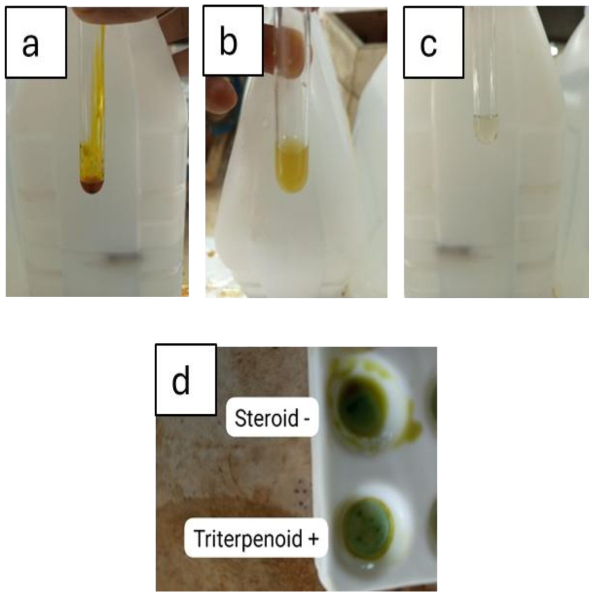

Figure 1. Phytochemical screening of celery leaves, a. alkaloid test, b. phenolic test, c. Flavonoid test, and d. steroid and triterpenoid test

This active content is essential for the formulation of antiaging cream. Even if it's not purified, this extract can be used by employing nanotechnology to increase the surface contact and enhance the dispersion of active compounds in the cream.

Table 1. Summary of phytochemical contents in celery leaves extract.

No	Secondary metabolites	contained
1	Alkaloid	+++
2	Phenolic	-
3	Flavonoid	++
4	Steroid	-
5	Triterpenoid	+

3.3. *Nanoparticle Fabrication of Celery extract*

The method of nanoparticle production using an ultrasonic probe homogenizer follows a top-down approach by breaking bulk particles into nanosized particles, with desired particle sizes within the range of 100-1000 nm. The average particle size of the crude celery extract was found to be significantly large, approximately 6,757.2 nm, reflecting a broad particle size distribution that included sizes like 3,205 nm, 3,621.48 nm, 4091,63 nm, and 9,615 nm, as shown in Figure 2.

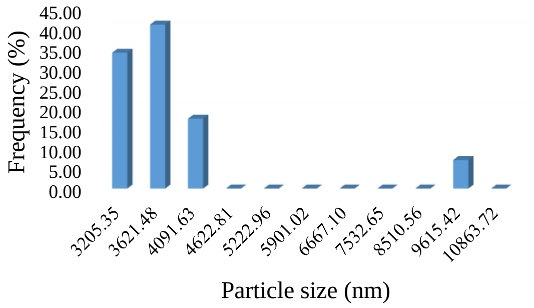

Figure 2. Particle size of celery raw extract

This method involves the mechanical breakdown of larger aggregates through high-acoustic waves, reducing the particle size and improving the homogen distribution of nanoparticles. The homogenization process for 30, 60, and 90 minutes, as shown in figures below (Figure 3, Figure 4, and Figure 5).

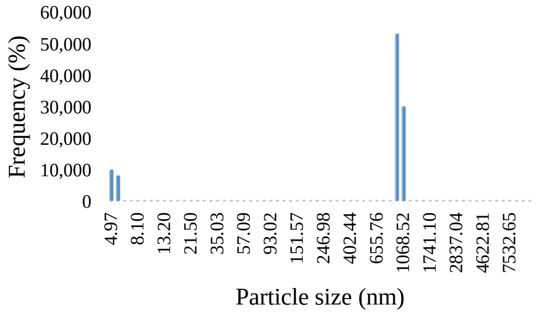

Figure 3. Particle size of celery extract with homogenization for 30 minutes

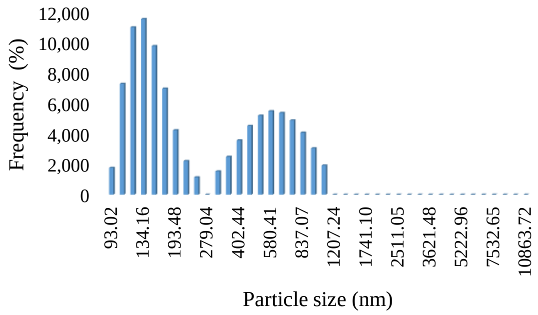

Figure 4. Particle size of celery extract with homogenization for 60 minutes

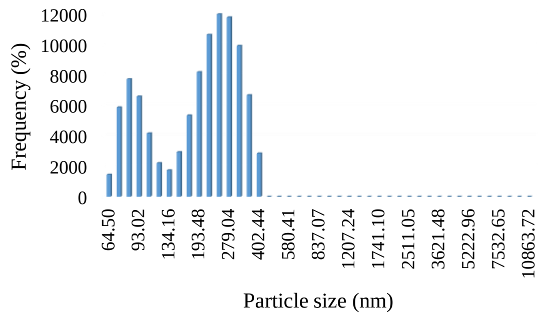

Figure 5. Particle size of celery extract with homogenization for 90 minutes

The probe homogenizer effectively creates a more uniform bioactive compound dispersion in the nano-scale range. Such a reduction in particle size enhances the bioavailability and bioactivity of the active ingredients, allowing for improved permeation and interaction with biological tissues. The particle size of celery extract was significantly reduced from 6,757.2 nm (bulk) into nanoparticle when probe homogenization was employed, with average size of 583.8 nm (30 minutes homogenization), 219.9 nm (60 minutes homogenization), 161.7 nm (90 minutes homogenization). These values showed that probe homogenization reduced particle size within the sonication time.

Furthermore, nanoparticles can lead to more homogeneous spreading of the active ingredients across the skin surface, which is essential for the effectiveness of antiaging creams. By employing probe homogenization, this research successfully transformed the bioactive compounds from celery leaves from bulk size into nanoparticle size, which will potentially be used in antiaging cream applications. This methodological innovation paves the way for future research and development of nanotechnology in cosmetic formulations, optimizing the delivery and efficacy of natural bioactive compounds [15].

4. CONCLUSION

Nanoparticles can lead to a more homogeneous spreading of the active ingredients across the skin surface, which is essential for the effectiveness of antiaging creams. This research successfully transformed the bioactive compounds from celery leaves into nanosized particles by employing an ultrasonication probe homogenizer. This methodological innovation paves the way for future research and development of nanotechnology in cosmetic formulations, optimizing the delivery and efficacy of natural bioactive compounds.

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