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Original Research Article

Combination of Candlenut (*Aleurites mollucana* (L.) Wild) Oil and Celery (*Apium graveolens*) Extract Stimulates Hair Growth: Formulation and Activity Test of Spray Preparation

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ABSTRACT

Hair serves both aesthetic and protective purposes. Alopecia (excessive hair loss) is frequently addressed with synthetic products that may cause adverse effects. Celery extract and candlenut oil are natural alternatives that can provide safer hair growth stimulation. This study aimed to assess the efficacy and safety of aerosol formulations of celery extract and candlenut oil. Aerosols of celery extract and candlenut oil were formulated at concentrations of 0.1% (F1), 0.5% (F2), and 1% (F3). The total flavonoid and phenolic contents of celery extract were evaluated colorimetrically. Fatty acid composition of candlenut oil was identified by gas chromatography-mass spectrometry (GC-MS). Acute dermal irritation test was performed by assessing erythema and edema on rabbits' skin following spray application. The hair growth stimulating activity was assessed *in vivo* using rabbits. GC-MS analysis of candlenut oil revealed the main fatty acids as oleic acid (20.81%), linoleic acid (20.80%), and palmitic acid (20.79%), while celery extract contained flavonoids (50.41 ± 2.01 mg QE/g) and phenolic compounds (20.00 ± 0.01 mg GAE/g). Skin irritation test showed no erythema or edema on application of the aerosols, with a primary irritation index of 0.0 ± 0.0. The treatment groups exhibited a significant increase in hair growth compared to the control ($p < 0.05$), with F3 exhibiting the highest average hair growth rate of 0.64 mm/day. The results indicate that the combination of celery extract and candlenut oil can effectively stimulates hair growth without causing skin irritation, thereby substantiating its potential as a safe, natural alternative for alopecia treatment.

Keywords: *Aleurites moluccana* (L.) Wild, *Apium graveolens*, Hair growth, Natural products, Spray activity.

Introduction

The hair is a critical component of the aesthetic aspect of the skin, and acts as a natural protector of the cranium, particularly from external factors like air pollution and ultraviolet radiation. The quality of life and self-confidence of an individual can be considerably impacted by hair loss or alopecia, which is a prevalent condition.[1,2] Causes of hair loss are typically classified into two primary categories: internal and external factors. Exposure to environmental pollution, ultraviolet radiation, excessive hair care, and the use of inappropriate hair care products are all external factors. Meanwhile, internal factors encompass hormonal changes, nutritional deficiencies, genetic predisposition, and autoimmune disorders.[3,4] In recent decades, the development of cosmetic preparations to address hair loss has generally relied on synthetic constituents, including minoxidil. Although minoxidil has been demonstrated to be effective in promoting hair growth, it is also known to cause a variety of adverse effects, including dandruff, dermatitis, local irritation, and erythema.[5–7] Therefore, the current research trends are centered on the utilization of natural ingredients that are believed to be safer and have minimal adverse effects compared to synthetic products.

One of the innovations that can be developed is a spray formulation that is effective in stimulating hair growth and preventing baldness, and is derived from natural constituents.[8–10] The objective of this investigation is to develop and assess the potential of an aerosol formulation consisting of celery extract (*Apium graveolens*) and candlenut (*Aleurites moluccana*) oil as a hair growth stimulant.

Various bioactive compounds, including apigenin, apiin, mannitol, vitamin B8 (inositol), asparagine, glutamine, choline, linamarin, potassium, and sodium, are known to be present in celery. These compounds have the potential to stimulate hair follicles and exhibit antifungal activity.[11–13] On the other hand, candlenut is rich in active compounds, including saponins, flavonoids, tannins, oleic acid, amino acids, and phenolics, which are responsible for the nourishment, and darkening of the hair.[14–17] This study is the first to incorporate these two natural ingredients into a singular hair care spray formula. The potential synergy between two herbal ingredients that have not been previously examined in the context of stimulating hair growth and prevention of alopecia is the novelty of this research.

In order to evaluate the formula's capacity to promote hair growth, this research employed an experimental approach that includes the extraction of natural ingredients, the formulation of a spray preparation that is a combination of celery and candlenut extracts, and efficacy testing on experimental animals. This method is unique in the sense that it enables researchers to assess the biological activity as a potential hair growth stimulant, and the physicochemical properties of the formulation.[18–20] This method is anticipated to yield a natural

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preparation that is both safe, effective, and has the potential to be developed as a non-synthetic alternative therapy for hair loss.

Materials and Methods

Chemicals and Equipment

The chemicals/reagents used in this assay include; Gallic acid (Sigma-Aldrich, USA; ≥99%), Folin-Ciocalteu reagent (Sigma-Aldrich, USA), sodium carbonate (Merck KGaA, Germany; ≥99.8%), toluene (Merck KGaA, Germany; ≥99%), methanol p.a (Merck KGaA, Germany; ≥99.8%), n-hexane (Merck KGaA, Germany; ≥95%), hydrochloric acid (Merck KGaA, Germany; ≥37%). All reagents and chemicals used were of analytical grade. The equipment used include; analytical balance (Ohaus Pioneer PX124, Ohaus Corporation, USA); drying exhauster and incubator (Memmert, Germany); hot press (MTI Corporation, USA); oven (Universal Oven UN55, Memmert, Germany); Brookfield viscometer (Model DV1, AMETEK Brookfield, USA); borosilicate glassware (Iwaki Pyrex®, Iwaki Glass Co., Japan); water bath (Maspion S-301, PT Maspion, Indonesia); magnetic stirrer (SP1422020-33Q, Thermo Scientific, USA); benchtop pH meter (Ohaus ST3100-F, Ohaus Corporation, USA); stainless steel maceration vessel (locally manufactured, Indonesia); gas chromatography-mass spectrometer (GC-MS; QP-2010 Plus, Shimadzu Corporation, Japan); UV-Visible spectrophotometer (UV-1280, Shimadzu Corporation, Japan); hematology analyzer (Sysmex KX-21®, Sysmex Corporation, Japan); ELISA reader (ELx800 Epoch™, BioTek Instruments, USA); refrigerated centrifuge (Rotina 200R, Hettich Zentrifugen, Germany); non-refrigerated centrifuge (SelectSpin™ 21, Bioproducts Laboratory, UK); and additional centrifuge (Sorvall™ ST 16, Thermo Fisher Scientific, USA).

Plant collection and identification

The leaves and stems of celery (*Apium graveolens*) which were three months old were collected from Ngadioro Hamlet, Berjo Village, Ngargoyoso District, Karanganyar Regency, Central Java, Indonesia (GPS coordinates: 7.6503° S, 111.1334° E). Candlenut (*Aleurites moluccana*) seeds were collected from Gondosuli Village, Tawangmangu District, Karanganyar Regency, Central Java, Indonesia (GPS coordinates: 7.6580° S, 111.1252° E). The plant materials were identified at Setia Budi University Laboratory, Surakarta, Indonesia, and voucher numbers 101/DET/UPT-LAB/13.03.2024 and 118/DET/UPT-LAB/19.05.24 were assigned for celery and candlenut, respectively.

Extraction of plant materials

Powdered celery leaves (100 g) were extracted by maceration in 96% ethanol (1 L) at room temperature (25-30ºC) for 5 days with constant stirring at least once daily. The extract was filtered by vacuum filtration. The filtrate was then exhaustively evaporated in a drying chamber for 24 hours to produce a thick extract.

Candlenut seeds were sorted, cleaned with water and then dried in a 50ºC oven for 5 days. The dried seeds were chopped into smaller pieces. Thereafter, the chopped candlenut seeds were placed into a hot press machine to obtain candlenut oil. The oil obtained was store in the refrigerator until ready for use.

Determination of total flavonoid content

Total flavonoid content was determined according to the method described by Ismiyati *et al*. (2025).[21] To a 1 mL standard solution of quercetin with concentrations ranging from 10 to 50 ppm was added 0.1 mL of 10% $AlCl_3$ and 0.1 mL of 1 M potassium acetate, to the reaction mixture was added 2.8 mL of 96% ethanol to make a final volume of 4 mL. The final mixture was incubated at room temperature (25°C) for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm using a UV-Vis spectrophotometer. Celery leaf extract (1 mL of 50 mg/mL) was subjected to the same procedure described above for quercetin. The determination was done in triplicate. The total phenolic content was quantified in milligrams of quercetin equivalent per gram of sample (mg GAE/g) from the equation of the quercetin calibration curve.

Determination of total phenolic content

Total phenolic content of celery extract was assessed using the Folin-Ciocalteu colorimetric method as described by Syafitri (2024).[22] Standard gallic acid solution (0.5 mL) with concentrations ranging from 10 to 50 ppm was combined with Folin-Ciocalteu reagent (2.5 mL) and incubated at room temperature for 5 minutes, followed by the addition of 7% Na_2CO_3 solution (2 mL). the resulting mixture was incubated at 40°C for 30 minutes. The absorbance of the reaction mixture was measured at 745 nm using a UV-Vis spectrophotometer. A calibration curve of absorbance versus concentration was prepared. The celery leaf extract (0.5 mL of 50 mg/mL) was subjected to the same procedure described above for gallic acid. The determination was done in triplicate. The total phenolic content was quantified in milligrams of gallic acid equivalent per gram of sample (mg GAE/g) from the equation of the gallic acid calibration curve.

GC-MS analysis of candlenut oil

Candlenut oil (0.15 mL) was mixed with toluene, methanol, and concentrated HCl (8%), followed by incubation at 40°C for 60 minutes. Subsequently, the mixture was extracted with n-hexane. The examination of volatile constituents was performed using gas chromatography - mass spectrometry (GC-MS), equipped with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm), using helium (≥99.99%) as the carrier gas at a steady flow rate of 1 mL/min. the injection volume was 1 µL and the injector temperature was 250°C. The oven temperature was programmed at 80ºC for 1 minutes, then 10ºC/min to 300ºC for 8 minutes. The MS transfer line was maintained at 280ºC. The ionization mode used was electron ionization at 70eV and ion source temperature of 230ºC. Compound identification was assessed using Total Ion Count (TIC) at start mass of 40 amu and end mass of 500 amu. The total analysis duration was 37 minutes. The Spectra of the separated compounds were compared with the database of the NIST Reference Spectra Library.

Formulation of celery and candlenut extract spray

Each ingredient in the formulation has a specified maximum usage limit as follows: glycerin (2–10%), propylene glycol (5–20%), Tween 80 (1–5%), propyl paraben (maximum 0.4%), methyl paraben (maximum 0.14% when used alone; maximum 0.8% when used in combination), and distilled water (70–90%) as required. The spray composition comprising celery leaf extract and candlenut oil is detailed in **Error! Reference source not found.**. The physical assessment of the formulation encompasses organoleptic evaluation (appearance, colour, odour), homogeneity (uniformity of the mixture), pH (calibrated with pH 4.01 and 7.01 buffer solutions), specific gravity, spreadability (under a 5 cm load), adhesion (measured after 10 seconds), viscosity (utilizing a Brookfield DV1 viscometer, spindle 64, AMETEK Brookfield, USA), drying time, and the pattern and quality of the spray (at a distance of 2 cm) to evaluate the overall efficacy of the spray product.

Animals

Forty (40) healthy male New Zealand rabbits (*Oryctolagus cuniculus*) aged 8 – 12 weeks, each weighing between 2.5 – 3.0 kg were obtained from a local breeder located in Dusun II, Kagokan, Gatak District, Sukoharjo Regency, Central Java, Indonesia. The rabbits were housed individually in a cage measuring 70 × 50 × 50 cm, maintained under standard laboratory conditions. The rabbits were acclimatized to the laboratory condition for two weeks. The animals were fed with commercial pellets (Munafeed®), and had access to drinking water twice daily.

Ethical approval

The study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Muhammadiyah University of Surakarta (KEPK FK UMS) with approval reference number 5243/A.1/KEPK-FKUMS/VII/2024. All the test procedures were executed in compliance with the Guide for the Care and Use of Laboratory Animals,[23] and the Indonesian National Agency of Drug and Food Control Regulation Number 10 of 2022 regarding Guidelines for Nonclinical *In Vivo* Toxicity Testing.[24]

Table 1: Spray formula comprising celery extract and candlenut oil

Ingredient	Amount added				Function
	Condition	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)	
Celery Extract (g)	Non-toxic	0.1	0.5	1	Active Ingredients
Candlenut Extract (g)	Non-toxic	0.1	0.5	1	Active Ingredients
Glycerin (mL)	2-10	10	10	10	Humectant
Propylene glycol (mL)	5-20	15	15	15	Solubilizer
Tween 80 (mL)	1-5	3	3	3	Surfactant
Propylparaben (g)	< 0.4	0.1	0.1	0.1	Preservative
Methylparaben (g)	< 0.8	0.2	0.2	0.2	Preservative
Distilled water (mL)	70-90	ad 100	ad 100	ad 100	Solvent

Acute dermal irritation test in rabbits

Fifteen (15) male rabbits were randomly assigned into five groups of three animals per group (n = 3), and treated as follows:

Group C (Control): No treatment was applied to this group, which served as a baseline.
Group C^- (Negative Control): Received a spray base formulation that did not contain celery leaf extract or candlenut oil.
Group F1 (Formula 1): Received a spray formulation containing 0.1% celery leaf extract and 0.1% candlenut oil.
Group F2 (Formula 2): Received a spray formulation containing 0.5% celery leaf extract and 0.5% candlenut oil.
Group F3 (Formula 3): Received a spray formulation containing 1% celery leaf extract and 1% candlenut oil.

The treatment was applied as a patch on an area of rabbit skin measuring approximately 4 cm^2.[25] For formulations suspected to be irritative, an initial test was conducted on one rabbit with 10 spray applications, followed by a 4-hour observation period. If no severe irritation signs were observed, the testing was continued on two additional rabbits. Meanwhile, for the formulation estimated to be safe, a single spray was administered to three rabbits, and the skin response such as erythema and edema was observed at 0, 24, 48, and 72 hours. Observation continued until day 14 to evaluate the possibility of irritation reversibility.[24] Skin reactions were evaluated and classified using the irritation scoring system listed in Table 2, and categorized based on the level of irritation response as shown in Table 3.[26]

Table 2: Classification of irritation response in rabbits

Average Score	Response category
0.0 – 0.4	Very mild irritation (negligible)
0.5 – 1.9	Mild irritation (slight)
2.0 – 4.9	Moderate irritation
5.0 – 8.0	Severe irritation

Table 3: Skin irritation scoring system

Formation of Erythema	Score
No erythema	0
Erythema is very small (almost indistinguishable)	1
Erythema is clearly visible	2
Moderate to severe erythema	3
Severe erythema (flesh and blood) until the formation of scars which inhibits the assessment of erythema	4
Formation of edema	**Score**
No edema	0
Edema is very small (almost indistinguishable)	1
Small edema (clearly visible area boundaries)	2
Edema of medium level (increases in area by about 1 mm)	3
Severe edema (area increases by more than 1 mm and widens beyond the area treated by the test preparation	4

Calculation of primary irritation index

The severity of the lesion in each test animal was determined by calculating the irritation scores for erythema and udema after 24, 48, and 72 hours from the time the patch was removed. These scores were used to determine the results of the acute dermal irritation test. The primary irritant index, also referred to as the irritation score, is the aggregate of all observations made during the test. This primary irritation index was calculated using the following formula (equation 1).[24]

$$\text{Primary irritation index} = \frac{A - B}{C} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where;

A = Sum of erythema and edema scores of all sample observation points at 24, 48, and 72 hours.

B = Sum of erythema and edema scores of all control observation points at 24, 48, and 72 hours.

C = Total number of animals observed.

Hair growth stimulation activity test in rabbits

Twenty-five (25) male New Zealand rabbits were randomly assigned into five groups of 5 rabbits per group (n = 5). The rabbits were treated as follows:

Group C (Control): Rabbits received no treatment and served as the untreated baseline group.

Group C⁻ (Negative Control): Rabbits were treated with a spray formulation base devoid of both celery leaf extract and candlenut oil.

Group F1 (Formula 1): Rabbits received a spray formulation containing 0.1% celery leaf extract and 0.1% candlenut oil.

Group F2 (Formula 2): Rabbits received a spray formulation containing 0.5% celery leaf extract and 0.5% candlenut oil.

Group F3 (Formula 3): Rabbits received a spray formulation containing 1% celery leaf extract and 1% candlenut oil.

To commence hair growth assessment, a 4 cm × 4 cm area on the dorsal region of each rabbit was shaved using an electric clipper. The shaved area was further divided into five equal sections, allowing for localized application of each treatment. Treatments were applied twice daily for 18 days. Prior to each application, the skin surface was cleaned using alcohol-soaked cotton swabs to ensure optimal absorption.[27]

Hair growth was evaluated by monitoring the length of hair every three days, specifically on days 3, 6, 9, 12, 15, and 18 post-treatments. On each observation day, 20 hairs were randomly selected from each treatment area, and the 10 longest hairs were measured using a micrometer to assess the mean hair length.[28] The distribution of treatment areas on the rabbits' backs is illustrated in Figure 1.

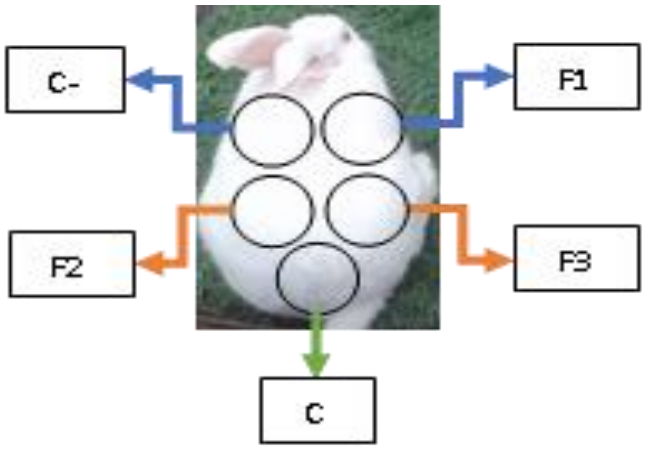

Figure 1: Distribution of treatment areas on rabbit's back

Statistical analysis

Data were presented as mean ± standard deviation (SD) of triplicate determination. Data were analyzed using the Shapiro-Wilk normality test, and Levene's homogeneity test. significant differences between means were determined by One-Way analysis of variance (ANOVA) test, followed by a Tukey HSD post hoc test. $P < 0.05$ was regarded as significant. The analysis was conducted using IBM SPSS Statistics version 29.0 (IBM Corp., USA; release 2022).

Results and Discussion

Celery and candlenut oil extraction yield

The extraction of celery leaf powder resulted in 18 g extract corresponding to a percentage yield of 12.33%, surpassing the minimum standard efficient extraction yield of 10%.[29]The water loss on drying was 7.79%, which is below the maximum limit of 10%, suggesting that the secondary metabolite content is not harmed by the drying process. On the other hand, the extraction of candlenut seeds resulted in the production of 40 g of pure oil corresponding to a percentage yield of 26.70%, suggesting a high level of efficiency in the extraction of vegetable oil. This efficiency is a critical initial stage, as the success of the topical formulation is contingent upon the quality of the extracted raw materials. The stability of flavonoid, phenolic, and essential fatty acids, which have hair growth stimulatory activity, will be maintained through optimal extraction.

Total flavonoid content

The objective of this study was to identify the potential bioactive compounds that could contribute to the pharmacological activity of the plants, including their function in stimulating hair growth, by determining the total flavonoid content in celery leaf extract. Flavonoids are phenolic compounds that are recognized for their potent antioxidant and anti-inflammatory properties. They have the ability to inhibit the activity of the enzyme 5α-reductase, which in turn suppresses the production of the hormone dihydrotestosterone (DHT), which is highly associated with hair loss.[30,31,32]

In this study, the total flavonoid content was determined using the UV-Vis spectrophotometric method with aluminium chloride reagent and quercetin as the reference standard. The standard curve of quercetin demonstrated a linear relationship between concentration and absorbance, with absorbance values increasing from 0.116 at 2 ppm to 0.177 at 10 ppm (Table 4, Figure 2).

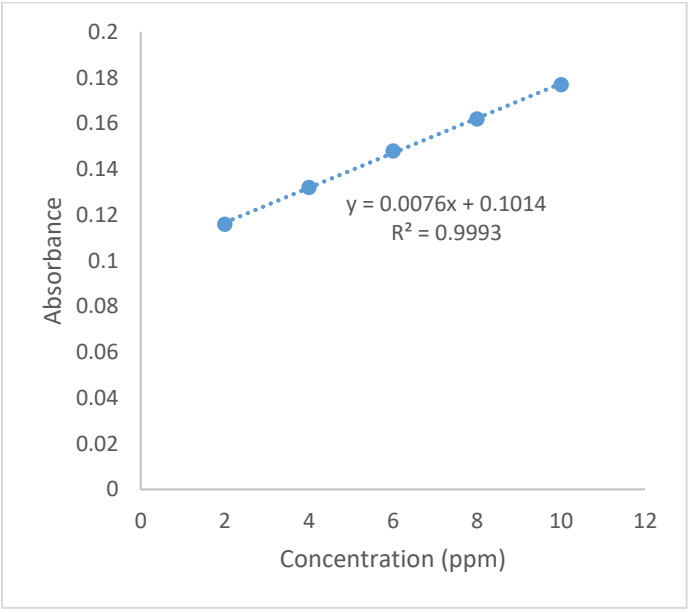

Figure 2: Quercetin calibration curve for estimation of total flavonoid content

The celery leaf extract exhibited absorbance values of 1.105, 1.136, and 1.153 in three replicates. These values were subsequently converted into total flavonoid content of 48.23 mg QE/g, 50.79 mg QE/g, and 52.20 mg QE/g, respectively. The average total flavonoid content of 50.41 ± 2.01 mg QE/g (Table 4) was classified as high when compared to extracts of other plants in comparable literature.[5]
The high flavonoid content indicates that celery leaves have tremendous potential as an active ingredient in natural-based hair care products. The anagen phase, which is essential for hair regeneration, is extended by the flavonoids in celery extract, particularly apigenin and luteolin, which are known to enhance the proliferation of dermal papilla cells. Consequently, the efficacy of celery extract in hair growth spray formulations is substantiated by its high flavonoid content.

Total phenolic content
The Folin-Ciocalteu method was employed to assess the total phenolic content of celery leaf extract, with gallic acid serving as the reference standard. As illustrated in Table 5, the absorbance values of the standard gallic acid solution at concentrations ranging from 10 to 50 ppm exhibited a linear correlation between concentration and absorbance. The absorbance increased from 0.324 at 10 ppm to 0.553 at 50 ppm, suggesting that the calibration curve is valid and suitable for the determination of total phenolics. The absorbance values of 10 mg/mL celery leaf extract were 0.336, 0.341, and 0.360, with an average absorbance of 0.346 (Table 5, Figure 3).

The extract's total phenolic content was 20.00 ± 0.01 mg GAE/g (milligrams gallic acid equivalent per gram of extract) as determined by calculations from the equation of the standard curve

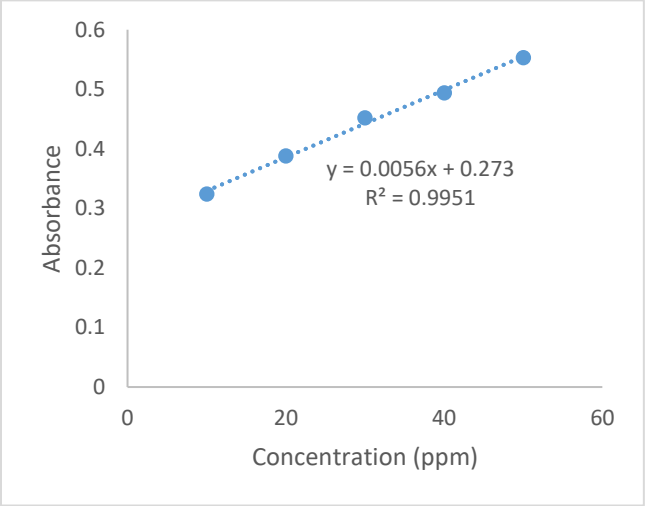

Figure 3: Gallic acid calibration curve for estimation of total phenolic content

Table 4: Absorbance readings and total flavonoid content of celery leaf extract

Standard			Sample		
Concentration (ppm)	Absorbance (A)	Replicates	Absorbance (A)	Total Flavonoid Content (mg QE/g)	Average Total Flavonoid Content (mg QE/g)
2	0.116	Replicate 1	1.105	48.23	
4	0.132	Replicate 2	1.136	50.79	50.41 ± 2.01
6	0.148	Replicate 3	1.153	52.20	
8	0.162				
10	0.177				

Table 5: Absorbance readings and total phenolic content of celery leaf extract

Standard			Sample		
Concentration (ppm)	Absorbance (A)	Replicates	Absorbance (A)	Total Phenolic Content (mg GAE/g)	Average Total Phenolic Content (mg GAE/g)
10	0.324	Replicate 1	0.336	11.25	
20	0.388	Replicate 2	0.341	12.14	12.98 ± 2.26
30	0.452	Replicate 3	0.360	15.54	
40	0.494				
50	0.553				

The substantial antioxidant potential of celery leaf extract is suggested by its high phenolic content. Phenolic compounds are recognized for their ability to extend the anagen phase (the hair growth phase), inhibit the enzyme 5-α-reductase, which is involved in the formation of DHT (dihydrotestosterone), and stimulate growth factors such as IGF-1 and VEGF, by counteracting free radicals that induce oxidative stress.

Fatty acid content of candlenut oil
The successful separation of fatty acid components was demonstrated in the gas chromatographic-mass spectrometric (GC-MS) analysis of candlenut oil. The resulting chromatogram (Figure 4) identified three major fatty acids, including oleic acid, linoleic acid, and palmitic acid which were distinguished by their relatively high peak areas of 20.815, 20.805, and 20.790, respectively (Table 6).
The GC-MS analysis results indicated that oleic acid (C18:1) is the primary monounsaturated fatty acid, possessing anti-inflammatory properties and contributing to the maintenance of scalp health and moisture. Meanwhile, linoleic acid (C18:2), a polyunsaturated fatty acid, plays a vital role in enhancing the penetration of active substances into the epidermis and strengthening hair follicles. Palmitic acid (C16:0), despite being classified as a saturated fatty acid, also functions as an emollient that preserves the epidermis barrier's integrity.

Table 6: Fatty acids identified in candlenut oil by GC-MS analysis

Peak No.	Retention Time (min)	Relative Peak Area	Resolution	Compound Name
1	1.675	20.665	1.580	Palmitic acid
2	1.685	20.675	1.665	Linoleic acid
3	1.815	20.785	1.785	Oleic acid

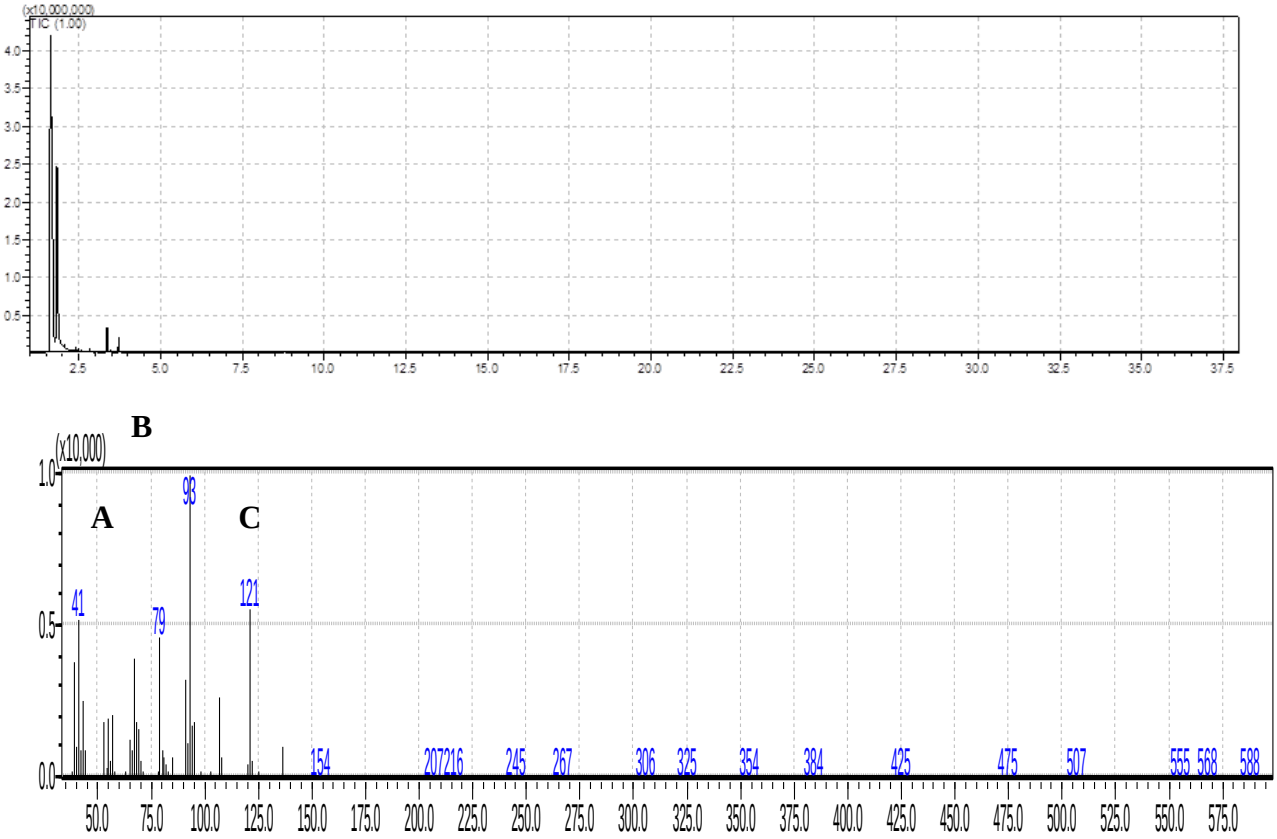

Figure 4: GC Chromatogram and MS spectrum of fatty acid content in candlenut oil
A: Oleic acid peak area; **B:** Linoleic acid peak area; **C:** Palmitic acid peak area

These three fatty acids play a key role in the effectiveness of candlenut oil as a lipid-based solvent, supporting not only the development of topical formulations, but also promoting biological activities such as hair growth, and epidermal regeneration.[33,34] Figure 4 presents the chromatogram displaying three prominent peaks corresponding to the identified fatty acid components. The chromatogram, along with the associated mass spectra, confirms the presence of three fatty acids such as oleic acid, linoleic acid, and palmitic acid as the dominant compounds separated through GC-MS.

Organoleptic and physical properties of celery extract and candlenut oil spray

The assessment of physical characteristics indicates that all formulations exhibit satisfactory stability and physical performance. The organoleptic properties remained consistent throughout storage, and no phase separation was observed, suggesting that the formulations are homogeneous and stable. The average pH value of 5.79 is within the normal range of 4.5–6.5, which is acceptable for human use.[35,36]

The spray preparations from the three formulas (F1, F2, and F3) exhibited similar organoleptic characteristics, including a clear yellowish-green colour, a distinctive herbal aroma, a bitter taste, and a texture that ranges from liquid to semi-solid (Figure 5, Table 7).

Formula 1 Formula 2 Formula 3

Figure 5: Formulation product of celery extract and candlenut oil

Table 7: Organoleptic properties of spray formulation of celery extract and candlenut oil

Formula	Organoleptic properties				
	State	Colour	Smell	Taste	Texture
F1	Liquid	Yellowish green and clear	Distinctive odour of the extract	Bitter	Liquid
F2	Liquid	Yellowish green and clear	Distinctive odour of the extract	Bitter	Thick
F3	Liquid	Yellowish green and clear	Distinctive odour of the extract	Bitter	Thick

The pH of all three formulations were 5.64 (F1), 5.82 (F2), and 5.91 (F3), which are all within the safe range for the epidermis and are visually homogeneous. The weight uniformity test indicates excellent consistency, as evidenced by a low CV% value (<2%). The formula's adhesion remains relatively stable at approximately 13 seconds, while its spreadability increases with concentration, from 6.40 cm (F1) to 7.60 cm (F3). Formula F1 was the most viscous with a viscosity of 774 cP, while formula F3 was the least viscous with a viscosity of 521 cP. The discharge pattern exhibited an even distribution with F3 showing the highest spray pattern area. The drying time was relatively uniform (91–94 seconds) (Table 8). These findings suggest that all formulations possess favourable physical attributes and are compatible with topical application.

Table 8: Physical properties of celery extract and candlenut oil spray formulation

Parameter	Value		
	F1	F2	F3
Homogeneity	Even	Even	Even
pH	5.64 ± 0.13	5.82 ± 0.13	5.91 ± 0.13
Weight uniformity (CV%)	1.56 ± 0.15	1.75 ± 0.15	1.87 ± 0.15
Spreadability (cm)	6.40 ± 0.61	7.20 ± 0.61	7.60 ± 0.61
Adhesion (seconds)	13.15 ± 0.33	13.20 ± 0.33	13.75 ± 0.33
Viscosity (cP)	774.00 ± 128.05	682 ± 128.05	521.00 ± 128.05
Drying time (seconds)	91.00 ± 1.53	93 ± 1.53	94.00 ± 1.53
Spray pattern	0.07 ± 0.09	0.0832 ± 0.09	0.09 ± 0.09

Values are mean ± standard deviation (SD), (n = 3).

Acute dermal irritation in rabbits

The objective of the skin irritation test was to assess the topical safety of an aerosol formulation that contains celery leaf extract and candlenut oil. All three formulas (F1, F2, and F3) exhibited modest erythema reactions at the 3rd minute and the first hour, with scores of 1.0 ± 0.5 (F1) and 1.0 ± 0.0 (F2 and F3), according to observations up to 72 hours (Table 9, Figure 6). Conversely, the control groups (C and C−) did not exhibit any reaction (0.0 ± 0.0). The erythema was absent by the fourth hour (0.0 ± 0.0 for all formulations) and did not reappear until the 72nd hour. The edema score remained at 0.0 ± 0.0 for the duration of the observation period.

Mild erythema with a score of 0.2 ± 0.5 was observed in all three formulations at the 24th, 48th, and 72nd hour. This erythema is assumed to be the result of mechanical irritation from shaving, rather than the active components of the formula. This was corroborated by a primary irritation index (PII) value of 0.0 ± 0.0, which suggests that the formulation does not induce primary irritation (Table 9).

Despite the presence of methyl paraben and propyl paraben in the formula, which have been reported to cause mild irritation, the concentrations used are still within safe limits and do not induce significant reactions.[35] Consequently, the spray formula, which is composed of celery extract and candlenut oil, has been certified as dermatologically safe and is appropriate for use as a topical hair care preparation derived from natural ingredients.

Hair growth stimulation activity

The hair growth stimulation activity test was conducted for 18 days on rabbits, with hair length measurements taken every 3 days. The results showed that formulas F1, F2, and F3 significantly increased hair growth compared to the control groups (C and C−) (Figure 7 and Table 10)

Table 9: Irritation effect of celery extract and candlenut oil spray formulation

Time	Irritation Effects	Test Group (Score)				
		C	C-	F1	F2	F3
3rd minute	Erythema	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.5	1.0 ± 0.0	1.0 ± 0.0
	edema	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1st hour	Erythema	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.5	1.0 ± 0.0	1.0 ± 0.0
	edema	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4th hour	Erythema	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	edema	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
24th hour	Erythema	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.5	0.2 ± 0.5	0.2 ± 0.5
	edema	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
48th hour	Erythema	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.5	0.2 ± 0.5	0.2 ± 0.5
	edema	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
72nd hour	Erythema	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.5	0.2 ± 0.5	0.2 ± 0.5
	edema	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Primary Irritation Index				0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Values are mean ± standard deviation (SD), (n = 15). Information: Erythema = Redness of the skin; Edema = Swelling of the skin

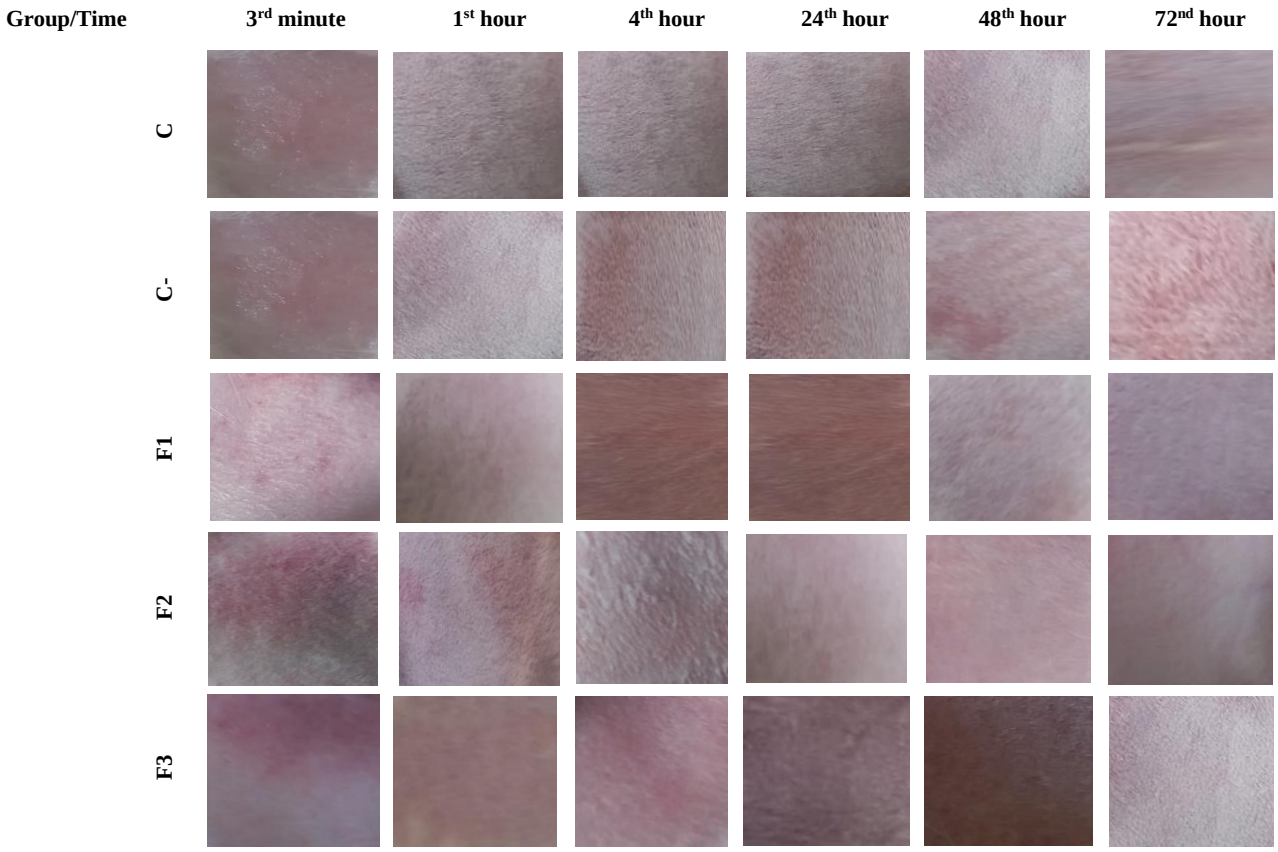

Figure 6: Photographic images of rabbits' skin allowing clear visual assessment of erythema and edema responses over a 72-hour period. Photos were captured with a standard smartphone camera with 2x magnification.

On the third day, formulas F1, F2, and F3 each showed hair lengths of 2.58 mm, 2.63 mm, and 2.72 mm, respectively, while the C− group only showed 0.57 mm and C showed 0.37 mm. This trend continued to increase until day 18, where F1 reached a length of 11.36 mm, F2 11.41 mm, and F3 the highest at 11.47 mm. On the other hand, C− only reached 2.46 mm and C 0.96 mm. The average daily growth rate in F1 and F2 was 0.63 mm/day, and F3 was 0.64 mm/day, much higher compared to C− (0.14 mm/day) and C (0.05 mm/day) (Table 9). Compared to the literature, celery leaf extract alone is reported to produce hair growth rate of about 0.25 mm/day,[5] and candlenut oil 0.32 mm/day.[36] For comparison, 2% minoxidil, which is the standard medical therapy for alopecia has been shown to exhibit hair growth rate of 0.65 mm/day.[37]

Table 10: Hair growth stimulation activity of celery extract and candlenut oil spray formulation

Treatment	Average daily hair growth (mm)						Hair growth rate
	3rd day	6th day	9th day	12th day	15th day	18th day	(mm/day)
C	0.37	0.52	0.78	0.85	0.92	0.96	0.05
C-	0.57	0.92	1.28	1.85	2.12	2.46	0.14
F1	2.58	4.45	5.68	7.67	9.80	11.36	0.63
F2	2.63	4.49	5.72	7.75	9.87	11.41	0.63
F3	2.72	4.58	5.78	7.86	9.96	11.47	0.64

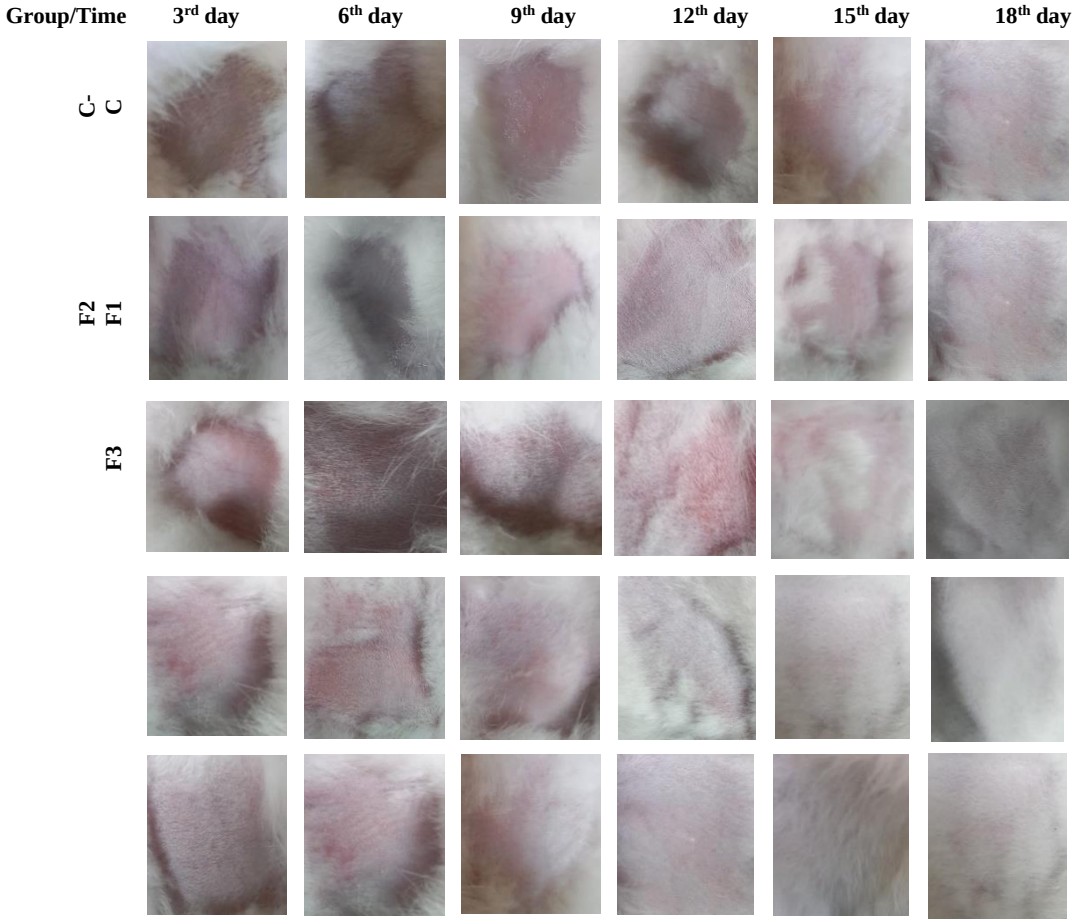

Figure 7: Photographic images of rabbits' skin for visual assessment of changes in hair shaft elongation over time (hair growth stimulation activity). Photos were captured with a standard smartphone camera with 2x magnification.

This indicates that the combination of celery extract and candlenut oil in formulas F1 - F3 is comparable to the effectiveness of minoxidil, but with a much lower potential for side effects. On the third day, formulas F1, F2, and F3 each showed hair lengths of 2.58 mm, 2.63 mm, and 2.72 mm, respectively, while the C− group only showed 0.57 mm and C showed 0.37 mm. This trend continued to increase until day 18, where F1 reached a length of 11.36 mm, F2 11.41 mm, and F3 the highest at 11.47 mm. On the other hand, C− only reached 2.46 mm and C 0.96 mm. The average daily growth rate in F1 and F2 was 0.63 mm/day, and F3 was 0.64 mm/day, much higher compared to C− (0.14 mm/day) and C (0.05 mm/day) (Table 9). Compared to the literature, celery leaf extract alone is reported to produce hair growth rate of about 0.25 mm/day,[5] and candlenut oil 0.32 mm/day.[38] For comparison, 2% minoxidil, which is the standard medical therapy for alopecia has been shown to exhibit hair growth rate of 0.65 mm/day.[39] This indicates that the combination of celery extract and candlenut oil in formulas F1 - F3 is comparable to the effectiveness of minoxidil, but with a much lower potential for side effects. The hair growth stimulation effect of this formula is suspected to originate from the synergism of bioactive compounds such as flavonoids and phenolics from celery leaf extract, which act as antioxidants and antiandrogens, as well as essential fatty acids from candlenut oil that support hair follicle health. This combination is believed to extend the anagen phase, suppress hair growth-inhibiting hormones such as DHT and cortisol, and enhance the expression of growth factors such as VEGF and IGF-1.[31,32,41] Thus, this combination of natural ingredients could become an effective and safe alternative for herbal-based hair loss treatments.

Conclusion

The findings from this study have demonstrated that the nanoemulsion-based spray formulation, which is composed of celery leaf extract and candlenut oil, is safe for topical use and has a pH value that is suitable for the skin. It does not induce irritation. The hair growth rate achieved by formulations F1–F3 was 0.63–0.64 mm/day, which is comparable to the efficacy of 2% minoxidil, but with fewer adverse effects. The synergistic action of flavonoids, phenolics, and essential fatty acids in

this product reinforces its efficacy in stimulating hair follicles. The potential for the development of this formulation into a useful, environmentally sustainable, and safe herbal product is substantial. This formula has the potential to be further developed for clinical trials and commercialization as an alternative hair loss therapy that is based on natural ingredients.

Conflict of interest

The author reports no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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