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Yield and potency of solid extract from unripe *Carissa carandas* fruit on collagen synthesis and stretch-mark treatment

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ABSTRACT

The extracellular matrices (ECM) of dermis layers of the skin are mainly composed of collagen fibers. Quick rupture and fragmentation of the fibers, such as due to pregnancy and sunburn, may result in stretch marks. In this research, the unripe fruit of *Carissa carandas* was extracted using 95% ethanol, and a solid substance of 2% by weight was obtained after cooling the extract at 6°C overnight. The solid extract was assayed to present ascorbic acid of 85% equivalent as well as polyphenols of 105 mg gallic acid equivalent. The antioxidation property of the extract was revealed regarding the DPPH scavenging antioxidant assay equivalent to 175 mg Trolox per gram extract. A fibroblast cell line, L929, was cultured and treated with 20 µg/mL of the extract or ascorbic acid standard for 20 hours. A decrease of *mmp1* and *mmp9* gene transcription and an increase of *col1a* gene transcription and translation were found after treatments. This used concentration caused 20% cell death so it was the IC_{20}. Light cream containing 1% extract was prepared. Under sensational analysis, the cream product was spreadable and adsorbable, whereas an ordinary fruit smell remained on the skin. Each 0.5 g of the formulated cream and the placebo was separately applied on half of an abdomen of 12 female subjects twice daily for 3 and 6 weeks. No allergic signs were recorded for such use. Improved appearance of indented stretches, regarding skin fineness and cell orientation, by 2 and 3 subjects was demonstrated, which was of 16% and 25% effectiveness, respectively. The results of *in vitro* RNA and protein analyses and clinical evaluation are complementary, implicating the developing of a new and efficient cosmetic product for stretch-mark treatment. Trials using larger groups of volunteers are important to be carried out for endorsing the cream's efficiency on addressing stretch marks.

Keywords: *Carissa carandas*, *col1a* gene, collagen, natural ascorbic acid, polyphenols, antioxidation, anti-inflammation, stretch marks.

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INTRODUCTION

The skin is the outermost body surface of two layers and is confronted with various environmental insults during the day. The highly cellular epidermal outer layer provides the barrier function, and the inner dermal layer ensures strength and elasticity and gives nutritional support to the epidermis. High concentrations of ascorbic acid (so-called vitamin C) are present in normal and healthy skin and are indicated to assist collagen synthesis and antioxidant protection against UV-induced photodamage. This has been a rationale for adding ascorbic acid to

cosmetic products (Khalid et al., 2024; DePhillipo et al., 2018), although the applied efficiency cannot be certain at present.

Cells of the human body have evolved to effectively utilize nutrients from natural sources. In particular, the body cannot produce ascorbic acid by itself, indicating that external sources must be used to supply it. So far, natural ascorbic acid is known to be complex with elements of its nature while working synergistically to provide skin health and brightening. However, such elements are lacking in regard to synthetic vitamin C (Thiel, 2000). The top 10 high vitamin C fruits include guava, orange, strawberries, kiwis, lychees, ripe papaya, pomelo, rambutan, pomegranate, and Indian gooseberry. All are commercially valuable in recent dates. Notably, *Carissa carandas* can flourish well in regions with high temperatures, including Thailand, and few economic values of the plant's fruit have been reported. Several compounds are present in *C. carandas* fruit, and high amounts of ascorbic acid are found in the unripe fruit (Tettevi et al., 2022; Dhatwalia et al., 2021). The fruit has been used in ancient Indian medicine, called Ayurvedic, to treat indigestion, fresh and infected wounds, skin diseases, diabetic ulcers and urinary disorders (Saeed et al., 2024).

Antioxidant activity screening based on the DPPH assay has revealed the IC_{50} of 195.8–259.5 µg/mL and 27.4 µg/mL when ethanol and methanol have been used for extracting the fruit, respectively. There has been ~7-9.5 times increased antioxidant activity by using methanol. The polarity of this solvent might be in good matching to the hydrophilicity of antioxidant compounds therein has been implicated (Ounis et al., 2023; Mishra et al., 2017; Sarma et al., 2015). There are strict regulations applied for cosmetic products when methanol is used in particular steps of the production (https://www.formulationbio.com/methanol-test-for-cosmetics.html) because even the residual solvent can contribute some problems to the central nervous system, the respiratory tract, and the skin. In accordance, ethanol was used as a solvent to extract the unripe fruit of *C. carandas* in this study. This was followed by precipitation induction at 6°C overnight. Crystallized compounds that were obtained majorly consisted of ascorbic acid and polyphenols. Evaluations of anti-oxidant and anti-inflammatory activities were performed by using the DPPH assay and real-time PCR method on L929 cell culture, respectively. Upon incubation with 20 µg/mL extract for 20 hours, transcription and translation of *mmp1*, *mmp9*, and *col1a* genes were investigated for the treated cells. Improved collagen synthesis was apparent for the extract compared to using vitamin C as standard. This was concomitant with reduced *mmp9* transcription. Light cream of 1% extract was prepared, and the benefit in reducing stretch marks on female abdomens was evaluated. This effect was superior for the prepared cream compared to the control counterpart. There was a simple, environmentally friendly and cost-effective protocol developed for obtaining a high yield of active extract. Designing large-scale extraction method for *C. carandas* unripe fruit would be possible in the future.

MATERIALS AND METHODS

Chemicals, reagents and instruments

Ethanol (95% and absolute), gallic acid monohydrate, Folin & Ciocalteu's phenol reagent (2N), Trolox, and a reagent set for the DPPH scavenging antioxidant assay were purchased from Sigma-Aldrich (MA, USA). Standard ascorbic acid was bought from Fluka (NC, USA). Chemicals for iodometric titration (such as sodium carbonate (RANKEM), potassium iodate, potassium iodide (ANaPURE) and starch (UNIVAR)) and compounds for cream preparation (such as glycerin, octyl palmitate, mineral oil, Span60 and Tween60) were ordered from standard manufacturers. Reagents for real-time PCR method including Trizol® reagent, SuperScript™ III RT (200 U/µL), Brilliant II SYBR Green QPCR master mix (Agilent Technologies, USA), and oligonucleotide primers for *gapdh*, *mmp1*, *mmp9*, and *col1a* genes were ordered from international agencies. Chemicals for SDS-PAGE and Western blotting such as acrylamide, bis-acrylamide, Tris-base, Tris-HCl, sodium dodecyl sulfate, guanidine hydrochloride, urea, Tween 20, PVDF membrane, Col I primary (1^o) antibody, HRP-conjugated secondary (2^o) antibody, and BM Chemiluminescence Reagent Kit were bought from Merch (MA, USA).

Plant material

Ten kilograms of *C. carandas* unripe fruit were bought from a certified garden in Nonthaburi province, Thailand. The pulp was separated from the fruit, cut into thin pieces and dried in an oven at 50°C to 95% dryness. The dried plant material was ground to a fine powder using a blender and kept in an air-tight container at 6°C until use.

Extraction procedures

The pulp powder was weighed and macerated in 95% ethanol using a 1:3 weight-to-volume ratio for 24 hours, protected from light. The supernatant was separated by centrifugation at 3000 x *g* for 15 min. This operation was one cycle. For a sample, three consecutive cycles were carried out. The supernatants were pooled, and the solvent was evaporated at 45°C for 90 min using a rotary evaporator (Buchi 23012A100). The concentrated extract

was kept at 6°C overnight. The precipitate in the bottom was collected by filtering through a Büchner funnel, dried under vacuum, and kept at 6°C with light protection until use.

Yield and ascorbic acid content

The solid extract was weighed. The extraction yield (%) was calculated based on 1 g of the plant material used. The assay of ascorbic acid was in accord with the iodometric titration method (https://www.scribd.com/document/643037030/Determination-of-Ascorbic-Acid-by-Titration-Method-pdf). Iodine solution is the titrant. Iodide anion and dehydroascorbic acid are the reaction products between iodine and ascorbic acid. Starch TS is the indicator, indicating an intense dark blue-violet color at the titration endpoint. The titrant's volume was recorded at the endpoint. An accurately weighed ascorbic acid was titrated in parallel, and the data were used to calculate the ascorbic acid equivalent in the sample.

Polyphenol content and DPPH antioxidant activity

Five concentration levels of the extract and gallic acid in ethanol were prepared (15, 30, 60, 120 and 240 µg/mL). Total phenolic content was determined according to the Folin-Ciocalteu method (Kalpoutzakis et al., 2023). Each solution of 0.1 mL was mixed with 0.5 mL of Folin-Ciocalteu reagent and 0.4 mL of sodium carbonate (75g/L). After 1 hour of incubation at room temperature, the OD_{765} was measured against the blank using a microplate reader. The results were expressed as milligrams of gallic acid equivalent per gram of the extract by comparing with the calibration curve. The radical scavenging activity of the extract against the stable DPPH was determined as follows. Trolox solutions of 0-300 µg/mL concentration range were prepared. Either sample or Trolox solution of 10 µL was added into a tube containing 190 µL of DPPH solution (315 µM in ethanol), mixed and incubated for 30 min at 37°C. A blank sample was prepared by replacing the extract with ethanol. The OD_{515} was measured by using a microplate reader. The DPPH antioxidant activity was calculated and expressed as milligrams of Trolox equivalent per gram of the extract by comparing it with the calibration curve.

Cell culture

L929 fibroblast cell line of ATCC was routinely maintained in Minimum Essential Medium Eagle (MEM) completed with 10% fetal bovine serum, 200 mM L-glutamine, and 1% antibiotics-antimycotic at 37°C in a 5%-CO_2 incubator. The cells were sub-cultured every 3 days.

The IC_{20} of the extract and ascorbic acid standard

The MTT method was used for standardizing the extract according to the growth-promoting potential (or viability) compared to the ascorbic acid standard. Sample and standard solutions of concentrations ranging between 0 and 300 µg/mL in completed MEM were prepared and filtered through 0.22-µM membrane filters before use. In a well of 96-well plates, cells of 95% confluency were incubated with each prepared solution for 20 hours in an incubator at 37°C and removed. MTT solution (2 mg/mL in serum-free MEM) of 100 µL was added and further incubated for 4 hours. Excess MTT solution was removed. DMSO of 100 µL was added for dissolving formazan crystals formed in live cells. The OD_{570} was measured by using a microplate reader. Data of samples and standards were recorded. The % viability was calculated, and 100% viability was considered for the untreated control cells. The concentration of the extract and the standard causing cell death by 20% (IC_{20}) was used for treating cells in the next experiments.

Transcription of *mmp1*, *mmp9* and *col1a* genes

Extraction of RNAs

Cells of 95% confluency in a well of 6-wells plates were incubated with a test sample or the standard at each corresponding IC_{20} for 20 hours in an incubator. The supernatant was removed. Trizol® reagent of 0.5 mL was added per well, incubated for 5 min, scraped using sterile micropipette tips, and then obtained a cell lysate. The lysate was transferred to a new 1.5 mL tube. Chloroform of 0.1 mL was added, mixed by vigorous shaking, and incubated for 2–3 min. Centrifugation at 12,000×g for 15 min at 4°C was carried out. The upper aqueous phase was transferred to a new tube. Isopropanol of 0.25 mL was added, incubated for 10 min at 4°C, and centrifuged at 12,000×g for 10 min at 4°C. The supernatant was carefully discarded. Ethanol (75%) of 0.5 mL was added to suspend the pellet in the bottom using a vortex. Centrifugation at 7500×g for 5 min at 4°C was performed. The supernatant was discarded. The pellet was air-dried for 5–10 min, dissolved using RNase/DNase treated water, incubated at 55-60°C for 10-15 min, and then resulting in total RNA. The RNA concentration was quantified using a Qubit™ RNA High Sensitivity (HS) reagent kit. It was stored at -70°C until use.

cDNA synthesis

Of a 20 µL reaction in a 200 µL PCR tube, it contained 500 pg of total RNA, 1 µL of dNTP (40 mM), 1 µL oligo$(dT)_{20}$ primer (50 µM), 2 µL of RT buffer (10x), 1 µL

of SuperScript™ III RT reagent, 4 µL of $MgCl_2$ (25 mM), 2 µL of DTT (0.1 M), 1 µL of RNaseOUT™, and water to 20 µL. Thermal cycles were set at 50°C for 50 min and 85°C for 5 min.

Quantitative PCR (qPCR)

For a 25 µL reaction in a MIC tube, it contained 2 µL of cDNA, 12.5 µL of 2× Brilliant II SYBR Green QPCR master mix, 2 µL of forward primer, 2 µL of reverse primer, i.e., the final concentration of each primer was 200 nM (Table 1), and water to 25 µL. The reaction was operated using a two-step thermal cycling: 95°C, 10 min; 40 cycles of 95°C, 30 sec, and 60°C, 1 min. MIC PCR Cycler (Bio Molecular Systems, Australia) was utilized for the operation. Data of the cycle threshold (Ct) were recorded. The Ct of *gapdh* gene was used for transcription normalization. To report relative gene expression in folds changed, the normalized Ct of a treated sample for a gene was divided by that of the untreated control.

Table 1. Sequences of oligonucleotide primers of genes in qPCR experiment.

Gene name	Sequence in 5′ to 3′ direction
gapdh	F: CAT CAC TGC CAC CCA GAA GAC TG R: ATG CCA GTG AGC TTC CCG TTC AG
mmp1	F: AGG AAG GCG ATA TTG TGC TCT CC R: TGG CTG GAA AGT GTG AGC AAG C
mmp9	F: GCT GAC TAC GAT AAG GAC GGC A R: TAG TGG TGC AGG CAG AGT AGG A
Col1a	F: CCT CAG GGT ATT GCT GGA CAA R: CAG AAG GAC CTT GTT TGC CAG G

Analysis of protein expression

Sample protein concentration

Lysis buffer, i.e., RIPA buffer containing protease inhibitor cocktail, of 0.5 mL was added in a well of the treated cells in 6-well plates. After incubation on ice for 10 min, the cells were scraped on ice using micropipette tips, and then a lysate was obtained. The protein concentration of the lysate was assayed using a Pierce™ BCA Protein Assay kit. Standard protein or a sample of 25 µL was added in a well of 96-well plate containing 200 µL of BCA working reagent, mixed by pipetting, and incubated at 37°C for 30 min. The OD_{562} was measured within 10 min by using a microplate reader. To calculate sample protein concentration, the sample OD_{562} was compared to that of the standard.

SDS-PAGE and Western Blotting

A sample of 100 µg protein was separated on 10% SDS-PAGE. Protein bands on the SDS-gel were transferred to a PVDF membrane (Millipore, Germany) using a wet-transfer apparatus (Cytiva, USA). The membrane was blocked by incubation in TBS-tween for 4 hours and then incubated with β-actin and Col I 1° antibodies (1:1000 dilution in TBS-tween) at 4°C overnight. Excess 1° antibodies were washed away using TBS-tween. The membrane was next incubated with HRP-linked 2° antibody (1:2000) for 1 hour at room temperature and removed. Excess 2° antibody was washed away by using TBS-tween. A chemiluminescent signal was generated using an enhanced chemiluminescence kit (Millipore). Any existing bands on the membrane were recorded by using a Luminescent Image Analyzer (GE Healthcare, Sweden). Band density was calculated using ImageQuant™ TL 10.2 analysis software.

Preparation of 1% cream

To prepare 100 g of 1% cream, 80 g of water, 5.5 g of glycerin, 3 g of mineral oil, 6 g of octyl palmitate, 2.5 g of Span60, 2 g of Tween60, and 1 g of the extract were accurately weighed. A glycerin solution in water was first prepared and used to dissolve the extract. The water phase and oil phase were separately heated up to 65°C for 30 min in a water bath. The oil phase was poured into the water phase while stirring and the mixture was stirred until cooled to room temperature. The resulting cream was subjected to sensory tests, e.g., spreadability, etc., or clinically applied on the abdomens of female volunteers. A placebo was formulated using ingredients similar to the sample, excluding the extract. Some fragrant was added to the placebo to imitate the fruit's ordinary smell, which was beneficial for subject blinding.

Product safety and clinical investigation

Experiments concerning human volunteers were ethically approved by the Faculty of Traditional Thai Medicine, Prince of Songkla University (EC.62/TTM.01-003). A purposive sampling technique was used to select a specific group of individuals. There were inclusion criteria such as gender (female), age (20-45 years old), having stretch marks on her abdomen, and not using corticosteroids in the previous 2 months before enrolling. Exclusion criteria included having allergic response to a prepared cream, taking some medications during the enrollment, and discontinuing the examination herself. Twelve female volunteers were enrolled. Evaluation of the cream's safety was done on the per-protocol population with n = 6. The enrolled subjects were double-blinded to receive both the sample cream and the placebo. A 0.5-g each was applied on half of the abdomen, 2 times daily in the morning and at night, for 3 and 6 weeks. In this circumstance, no other cosmetics were applied to the abdomen. Parameters of skin fineness and cell orientation were recorded and analyzed using I-Scope USB 2.0 (MORITEX Corporation, Kanagawa, Japan) at the beginning and end of the experiments.

Data analysis

The parametric statistics were calculated and reported as the mean ± standard deviation (S.D.) using Paired T-Test at 95% confidence (p-value < 0.05). For non-parametric statistics, an average of the quotations obtained from each volunteer was applied.

RESULTS

Yield and beneficial properties of the extract

A yield of approximately 2% by weight was obtained from the extraction using 50 g of the plant material and 400 mL total volume of 95% ethanol. Afterward concentrated using a rotary evaporator at 45°C for 90 min and stored at 6°C overnight, the solidified compound was left in the bottom. The solid extract was examined to consist of 85% ascorbic acid equivalent and polyphenols of 105 mg gallic acid equivalent per gram sample. An antioxidant activity of 174.73 mg Trolox equivalent per gram sample was observed in the DPPH assay.

The IC_{20}

The viability of L929 cells after incubation with the extract or ascorbic acid standard of 0-300 µg/mL concentrations for 20 hours was accounted for using MTT assay. At 20 µg/mL final concentration of these two compounds, the cells were induced to die by 20%, suggesting the IC_{20} of both.

Transcriptions of *col1a, mmp1, and mmp9* genes

By mediating mRNAs transcription, the genes *mmp1, mmp9*, and *col1a* are encoded for metalloproteinase 1 (MMP1), metalloproteinase 9 (MMP9), and type I collagen (Col I), respectively. Recently, changes in transcriptional activities of L929 fibroblasts after being treated with the extract or ascorbic acid standard at each corresponding IC_{20} were measured using the qPCR technique. Lipopolysaccharides (LPS) were used for positive inflammation induction. The results of Figure 1 show that fold changes of *mmp1* and *mmp9* gene transcriptions before and after treatments were less than 1. The *mmp1* transcription of LPS-induced cells was intensely suppressed by the vitamin C standard, but this was activated by the extract (Figure 1a). It was noted that the *mmp9* gene transcription of other inflammation-induced cells was decreased after treatments using the extract and the standard (Figure 1b). Of both treatments, increased transcription of the *col1a* gene was observed for normal (or LPS-untreated) cells. But this was decreased as the cells had inflammation. The great motivation of *col1a* gene transcription was determined for the extract compared to the vitamin C standard (Figure 1c).

Expression of Col I protein

The expressed Col I and Col III proteins of L929 cells were examined using the Western blotting technique. The results of Figure 2a show that the Col III protein was intrinsically expressed in this fibroblastic cell line. Increased synthesis of Col I protein was apparent for treatments using the extract compared to the vitamin C standard (Figure 2b). Both the extract and the vitamin C standard indicated to have an inductive effect on Col I production.

Effects of the prepared cream on volunteers

Safety

The product safety was evaluated on 6 volunteers. A test sample cream was placed on the inner side of the upper arm for 24 hours. The degree of skin irritation was recorded through the numeric scale, i.e., 0 – none, 1 – erythema, 2 – oedema, 3 – desquamation, and 4 – vesicles. Since the average irritant score of 0 was accounted, all volunteers were well tolerant to the tested

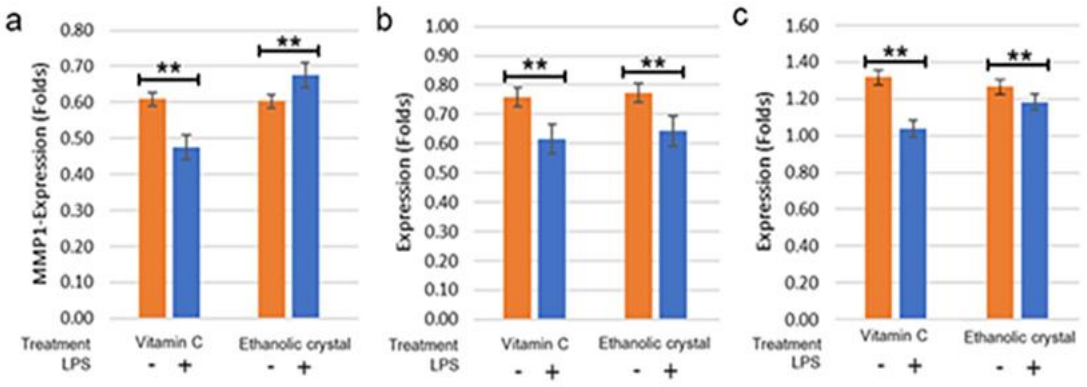

Figure 1. Transcriptional activity in folds change of *mmp1* (a), *mmp9* (b), and *col1a* (c) genes for L929 cells after treated with the extract and vitamin C standard with (+) or without (-) LPS induction, determined by qPCR technique; **, $p < 0.01$.

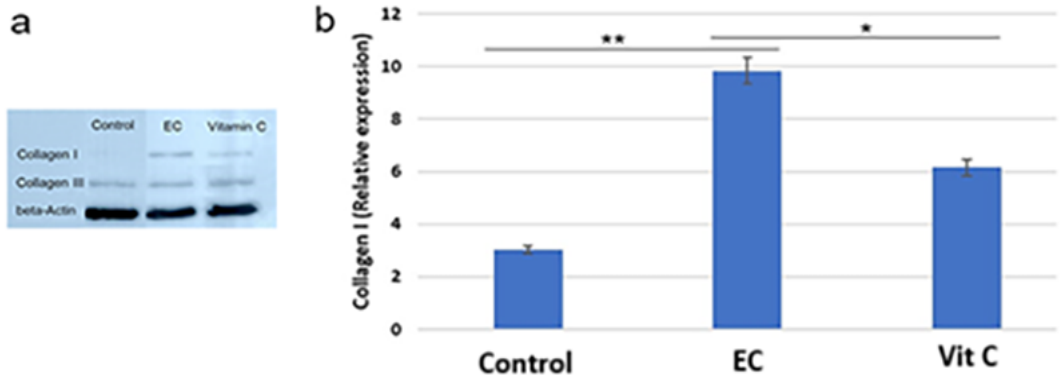

Figure 2. (a) Bands of the expressed Collagen I, Collagen III, and β-Actin proteins of control cells and those of the extract (EC) and vitamin C standard treatments, determined by Western blotting technique; (b) The analysis of bands' density on the membrane of (a) by using ImageQuant™ TL 10.2 software; **, $p < 0.01$; *, $p < 0.05$.

creams. None of the enrolled subjects developed allergies after 6 weeks of exposure.

Sensational determination

Sensory tests have been a valuable tool for qualifying consumers' perceptions of cosmetic products. Recently, an individual of 6 subjects evaluated the odor, spreadability, stickiness, oiliness, adsorption, and after-feeling of cream samples by taking questionnaires of the 1-5 scale into consideration, i.e., 1 = minimum and 5 = maximum). Data show that the prepared cream was not sticky, spreadable, and well adsorbable, but it presented a moderately pleasant smell. The after-feeling was not sufficiently good and oily. Especially, the after-feeling of subjects who had dry or mostly dry skin was better than those having oily skin.

Clinical results

Chronic stretch marks were identified on the subjects' abdomens according to Ud-Din et al. (2016), because faded-, hypopigmented-, atrophic-, and wrinkled appearances were observed. Representative photographs of an abdomen before and after application using 0.5 g of the cream sample or the placebo, 2 times per day for 3 and 6 weeks, were shown in Figure 3. The stretch marks faded with shallower grooves when using 1% cream for 6 weeks compared to the placebo. These effects were less for the shorter use of 3 weeks. A specific equipment, I-Scope USB 2.0, was utilized to evaluate skin fineness and cell orientation to avoid subject biases, and the results were summarized in Table 2. Improved skin fineness and cell orientation were revealed after 3 weeks of using the prepared cream. In comparison, such changes were not significantly affected after 6 weeks of application.

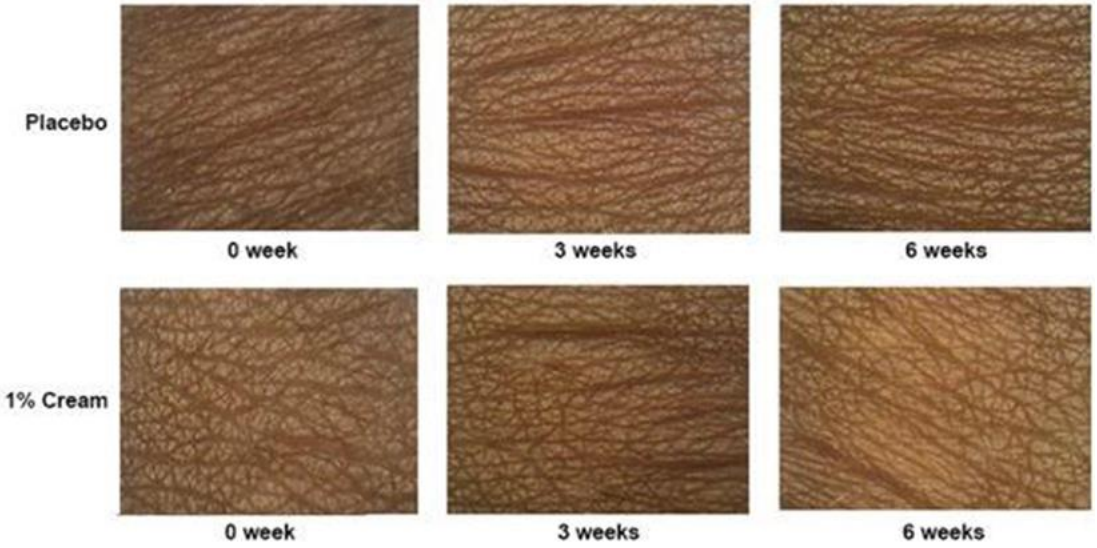

Figure 3. Representative photographs of half a subject's abdomen smeared with the placebo or the sample (1% cream) for 3 and 6 weeks consecutively, recorded using the I-Scope USB 2.0.

Table 2. Results of skin fineness and cell orientation from 12 subjects, measured using the I-Scope USB 2.0[a].

Texture criterion	Placebo			1% Cream		
	0 week	3 weeks	6 weeks	0 week	3 weeks	6 weeks
Fineness						
High					2(16.67)	3(25.04)
Medium	7(58.33)	10(83.33)	10(83.33)	7(58.33)	5(41.67)	8(66.67)
Low	5(41.67)	2(16.67)	2(16.67)	5(41.67)	5(41.67)	1(8.33)
Cell Orientation						
High	12(100)	12(100)	11(91.67)	11(91.67)	12(100)	11(91.67)
Medium						1(8.33)
Low			1(8.33)	1(8.33)		

[a] the number in front of the bracket indicated the number of volunteers being categorized, and the number in the bracket was the mean in % calculated for 12 subjects.

DISCUSSION

Stretch marks are common skin lesions which can be developed by rapid changes in the orientations of collagen and elastin fibers of the dermis layers (Shen et al., 2022; Schuck et al., 2020). Although this skin lesion is not an illness, people with it may develop negative self-images. In accordance, treating stretch marks is of considerable concern for improving the quality of life. The most conversant treatment strategy is using topical cosmetics. This is because the efficacy reported to date has been just improved appearances without size reduction or elimination of stretch marks (Shu et al., 2023). Finding new compounds which have the potential to remove stretch marks is a current research trend.

Natural ascorbic acid has been present in citrus fruits and vegetables, such as cruciferous vegetables. This substance is advantageous for the skin's health according to its properties like antioxidation, anti-dark spots, freckles, wrinkles, and anti-inflammation (Fam et al., 2022; Prieto, 2023). Although synthetic and naturally derived ascorbic acid are identical in chemical structure, data from various animal studies have shown high differences in bioavailability between the two compounds. So far, the presentation of other phytochemicals in the natural extracts has been suggested to implicate such increasing bioavailability (Doseděl et al., 2021).

In this research, ethanol was used for extracting the unripe fruit of *C. carandas*, followed by a clinical trial using the acquired extract to prepare 1% light cream for treatment of stretch marks. The extraction method developed was simple, environmentally friendly and cost-effective since at least 2% extraction yield was obtained, depending on the sources of the plant material. According to the recent findings, the extract contained ascorbic acid of 85% equivalent as well as polyphenols of

105 mg gallic acid equivalent per gram extract. The DPPH antioxidant activity was 174.73 mg Trolox equivalent per gram extract. High content of the naturally derived ascorbic acid was notably obtained from the pulps of unripe fruit of *C. carandas* when the developed method for extraction was applied according to previous literature (Ghazzawia et al., 2021; Um et al., 2020).

Fibroblast cells of the dermis layers are responsible for producing proteins essential for maintaining the strength and tenderness of the skin. Tensile stresses that are put on the collagen fibers by a stretching force during pregnancy or due to excessive exposure to UV light and pollutants are common causes of stretch marks found in early-aged people. Different inflammation processes are subsequently triggered, resulting in functional impairment of skin cells. Decreased collagen synthesis accompanied by increased collagen degradation by collagenases, such as MMPs, have been the mechanisms implicated in the existence of stretch marks (Fisher et al., 2023). In this project, decreased transcription of *mmp1* and *mmp9* genes and increased transcription and translation of the *col1a* gene were observed after incubating L929 fibroblasts with 20 µg/mL of the extract or vitamin C standard. Notably, greater stimulation on collagen synthesis was apparent for the extract compared to the standard (Figure 1 and 2). The effect was beneficial in supporting the healthiness of skin cells. There was a rationale to prepare a cosmeceutical cream of which 1% extract was prior trialed for the treatment of stretch marks consequently (Ghahremani-Nasab et al., 2023; Gref et al., 2020).

Twelve healthy females who had indented stretch marks on the abdomens were enrolled for evaluation of the prepared cream product on sensational aspects and treatment efficiency. The cream was spreadable and adsorbable, but moderate perception was inquired by the subjects owning to the ordinary fruit smell and the oily-feeling texture. This preparation would be optimal for dry or mostly dry skin. Change of the cream's ingredients to others of increased hydrophilicity, such as Carbopol cream base, was impossible because the extract and the in-formular triethanolamine were incompatible due to the extract's acidity (Yarnykh et al., 2020; Begum et al., 2018). The female subjects were revealed to present faded-, hypopigmented-, atrophic-, and wrinkled-lesions on their abdomens. These signs were chronic stretch marks that are slightly difficult or impossible to cure (Huang et al., 2022; Ud-Din et al., 2016). Interestingly, 16% and 25% of these subjects revealed improvements in skin fineness and cell orientation after using the cream for 3 and 6 weeks (Figure 3 and Table 2).

CONCLUSION

Lately, many cosmetic products for the efficient eradication of stretch marks have been advertised. Such a claim is mostly ambiguous due to the lack of clinical information to support effective use. The experimental and clinical data from this project were primary, concerning solid extract of *C. carissa* unripe fruit. The benefit of using 1% cream for treating stretch marks was found but it was inadequate. Several topics associated with new cosmeceutical formulations, expanded volunteer numbers for other clinical trials, and combined use or preparation with other active substances or means to maximize treatment efficiency and minimize possible risks are being researched (Määttä, 2023; Wollina and Goldman, 2020).

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DISCLOSURE

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