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RESEARCH ARTICLE

Phytochemical and cosmeceutical assessment of *Carissa carandas* L. extracts for skin lipoprotein protection

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Abstract

Oxidative damage to skin lipoproteins is a critical factor in ageing and dermatological disorders, yet natural interventions targeting this pathway remain insufficiently studied so plant-based cosmeceuticals of *Carissa carandas* L. extracts to protect skin lipoproteins from oxidative stress and ageing-related damage. Fully ripened fruits and leaves, dried in powder form, were subjected to ultrasound-assisted extraction with 50 % ethanol. Antioxidant and antimicrobial potential were determined. The yield of fruits and leaves extracts was obtained up to 43.30 and 25.00 %, respectively. Bioactives profiling was done by high-performance liquid chromatography (HPLC), quantify the presence of phenolic and flavonoids. Moreover, fruits extract displayed significant potential for total phenolic content, total flavonoid content (TFC) and total carotenoid content (TCC) compared to the leaves extract. Fruits formulation exhibited the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition (84.9 %) compared to the leaves formulation (76.5 %). In the ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay, fruit extract (76.90 %) and formulation (86.28 %) showed higher scavenging activity than leaves extract (60.38 %) and formulation (68.60 %). Fruits extract exhibited a larger antibacterial and antifungal zone of inhibition using *Staphylococcus aureus* & *Escherichia coli*, *Aspergillus niger* and *Aspergillus flavus* than the formulation. Antiaging activities were optimized by inhibition of antiaging enzymes, i.e. tyrosinase, collagenase and elastase. Fruits and leaves formulation inhibited all three enzymes, exhibiting the best anti-ageing effect. The findings suggested that *C. carandas* fruits and leaves extracts phenolic components may be used as an anti-ageing ingredient in cosmetics.

Keywords: anti-ageing; antibacterial; antioxidants; bioactive; *Carissa carandas* L.; HPLC; molecular docking

Introduction

Reactive oxygen species (ROS) induced lipid peroxidation is acknowledged as a primary mechanism behind skin barrier disruption, inflammation and dermatological pathologies. Molecular dynamics simulations of oxidized stratum corneum lipid bilayers (composed of ceramide, cholesterol and fatty acids) reveal that peroxidation leads to disrupted membrane structure characterized by increased area per lipid, reduced thickness and enhanced permeability, which allows more ROS to transverse the barrier and induce apoptosis (1). Complementary clinical and experimental data demonstrate that lipid peroxidation products such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) accumulate in conditions like atopic dermatitis and psoriasis, triggering keratinocyte apoptosis, immune activation and chronic inflammation (2, 3). These biochemical disruptions undermine the stratum corneum's ability to regulate Tran's transepidermal water loss, maintain elasticity and resist microbial infiltration. Moreover, oxidative damage exacerbates skin ageing by fragmenting collagen and elastin fibres and driving inflammatory cytokine release via NF-κB pathways (4). Consequently, preventing or reversing lipoprotein oxidation is central to strategies aimed at preserving skin health, hydration and barrier function, especially in pathological contexts influenced by environmental stressors and ageing (5).

Advances in skin science underscore that plant-derived phytochemicals, especially flavonoids, phenolic acids and terpenoids can inhibit ROS-mediated mechanisms implicated in lipid barrier degradation (6). These natural bio-actives augment endogenous antioxidant defence systems such as superoxide dismutase (SOD), catalase and glutathione peroxidase, while protecting against lipid peroxidation and restoring barrier integrity (7). In addition to scavenging free radicals, many compounds modulate inflammatory pathways by downregulating NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), IL-6 (interleukin-6) and TNF-α (tumor necrosis factor-alpha) and promoting collagen/elastin biosynthesis, key processes underlying skin repair and anti-ageing (7, 8). Formulations delivering these bio-actives via nanotechnology (liposomes, ethosomes, microneedles and hydrogels) have been shown to enhance skin absorption and sustained release, resulting in superior therapeutic outcomes compared to conventional agents (9). With the rising demand for

effective, safe and sustainable skincare, such multifunctional cosmeceuticals are gaining prominence as alternatives to synthetic compounds that can cause irritation or environmental concerns (10, 11).

Carissa carandas (karonda) is a tropical shrub with extensive ethnomedical use across South and Southeast Asia (12). Phytochemical profiling reveals a dense assembly of therapeutic constituents, including flavonoids (quercetin, rutin), phenolic acids, anthocyanins and pentacyclic triterpenoids such as ursolic acid, oleanolic acid and novel compounds like carissin and carandinol (11). Extracts from leaves and fruits, especially in ethyl acetate or methanol, have demonstrated significant antioxidant capacity, inhibition of hyaluronidase, elastase, tyrosinase, collagenase and matrix metalloproteinases (MMP-1, MMP-2, MMP-9), as well as anti-inflammatory effects through NF-κB, IL-6 and TNF-α suppression (13). Notably, ursolic acid content reaches approximately 412mg/g extract in leaves ethyl acetate fractions, correlating strongly with these biological activities. Together, these findings highlight *C. carandas* as a rich source of bioactives with desirable properties for inclusion in cosmeceutical formulations targeting skin health.

Despite compelling biochemical evidence, no studies to date have specifically evaluated *C. carandas* bio-actives against dermal lipoprotein oxidation, an essential pathway in skin barrier impairment. Foundational work using ethyl acetate leaves extract rich in ursolic acid (~412mg/g) showed strong inhibition of MMP-1, NF-κB activation, IL-6 and TNF-α secretion, as well as tyrosinase and elastase activity, biological markers central to skin ageing and inflammation. Furthermore, isolated triterpenoids (e.g., ursolic acid derivatives, carandinol, uvaol) inhibit ROS generation in immune cells and support cytoprotective responses. Therefore, the present study was designed to evaluate the cosmeceutical potential of *C. carandas* fruits and leaves extracts by assessing their antioxidant, antibacterial, antifungal and anti-ageing enzyme inhibitory activities. Additionally, the extracts were formulated into topical preparations and compared to their crude forms to determine enhancement in activity. Phytochemical profiling through high-performance liquid chromatography (HPLC) and molecular docking was conducted to identify key bioactives and explore their interactions with ageing-related enzymes. We hypothesized that *C. carandas* extracts, especially when formulated, possess potent antioxidant, antimicrobial and anti-ageing properties due to their rich phenolic and flavonoid content, justifying their use in natural skincare applications.

Materials and Methods

Preparation of plant extract

Carissa carandas fruits and leaves were collected at the ripened stage from the botanical garden of Government College University, Faisalabad. Samples were washed, shade-dried, ground into a fine powder and extracted separately using ethanol and distilled water (1:5 w/v) for 72 hr. The mixtures were sonicated (30 min total, 10 min intervals), filtered and the filtrates were concentrated using a rotary evaporator at 50 °C under reduced pressure (0.07 MPa). Extracts were stored at 4 °C for further analysis. The percentage yield was calculated using Equation 1:

$$\% \text{ Yield} = \frac{W_1 - W_2}{W} \times 100 \qquad \text{(Eqn. 1)}$$

Where, W_1 represents the weight of the container with extract after evaporation, W_2 represents the weight of the empty container and W represents the weight of the dried plant material used for extraction.

Proximate analysis

Determination of moisture content

Moisture content was determined by oven-drying 2 g of each powdered sample at 105 °C until a constant weight was achieved.

Determination of total ash content

Ash content was measured following the Association of Official Analytical Chemists (AOAC) standard procedures. A 2 g portion of each dried sample was placed in a pre-weighed porcelain crucible and incinerated in a muffle furnace at 600 °C for 4-6 hr until complete combustion was achieved and a constant grayish -white ash was obtained. Crucibles were cooled in a desiccator to room temperature and reweighed (14). Ash content was calculated using Equation 2.

$$\text{Ash content } (\%) = \frac{W_1 - W_2}{W_3} \times 100 \qquad \text{(Eqn. 2)}$$

Where, W_1 represents the weight of the crucible with ash, W_2 represents the weight of the empty crucible and W_3 represents the weight of the dried sample taken for analysis.

Determination of crude protein content

Crude protein content was determined (15), which involves the conversion of organic nitrogen into ammonium sulfate through acid digestion, followed by distillation and titration. A known weight (1 g) of the dried sample was digested with concentrated sulfuric acid in the presence of a catalyst mixture. The digest was diluted and the ammonia released upon alkalinization was distilled and captured in a boric acid solution. The trapped ammonia was then titrated with standard 0.5 N hydrochloric acid. Crude protein content was calculated using the following Equation 3.

$$\text{Crude protein } (\%) = \frac{N \times T \times 10 \times 14 \times 6.25}{1000} \qquad \text{(Eqn. 3)}$$

Where, N represents the normality of the acid used in titration and T represents the titre value.

Total fat content determination

Total fat content was estimated using the Soxhlet extraction method. A 2 g of dried sample was placed in a pre-weighed extraction thimble and subjected to continuous extraction using petroleum ether (boiling range 55-80 °C) for 6-8 hr (16). Following extraction, the thimble was oven-dried at 100 °C for 1 hr, cooled in a desiccator and reweighed. The fat content was calculated using the following Equation 4.

$$\text{Fat content } \% = \frac{W_2 - W_1}{W_S} \times 100 \qquad \text{(Eqn. 4)}$$

Where, W_2 represents the weight of the extraction thimble with fat after extraction, W_1 represents the weight of the empty thimble before extraction and W_S is the weight of the dried sample used for fat determination.

Total carbohydrate % determination

The total contents of protein, fat, moisture and ash were subtracted from 100 % to determine the amount of carbohydrates present. The following calculation was used to get the total carbohydrate content (17):

Carbohydrate content (%) =

$$100 - (\text{Moisture \%} + \text{Protein \%} + \text{Fat \%} + \text{Ash \%}) \quad \text{(Eqn. 5)}$$

HPLC analysis of *C. carandas* extracts

The potent analytical method known as HPLC, is used to separate, identify and quantify the constituents of a mixture. A mobile phase was prepared and degassed, the analytical column was adjusted and the sample was meticulously prepared and filtered. Following sample injection into the HPLC system, the constituents were identified by an appropriate detector and separated according to how they interact with the column. The sample components were identified and quantified by comparing the resultant chromatogram to a calibration curve. Reliable and accurate findings were ensured by an appropriate upkeep and secure practices (18).

Preparation of anti-ageing formulation

Anti-ageing formulations were prepared using *C. carandas* fruits and leaves extracts. Oil and aqueous phases were measured according to standardized ratios (Table 1) and heated separately in a water bath at 75 °C. Once fully liquefied, the phases were combined and homogenized on a hot plate, followed by gradual cooling to 40-45 °C. Sodium benzoate was incorporated as a preservative and the mixture was stirred until a uniform creamy consistency was achieved. Finally, 1-2 drops of natural fragrance were added and the formulation was mixed thoroughly to ensure complete homogenization. Prepared formulations were transferred to sterile containers and stored at ambient conditions for further evaluation (19).

Evaluation of parameters for formulations

The product's physical and chemical integrity and packaging stability were evaluated by the following tests:

Organoleptic characteristics

Physico-chemical testing of the dermato-cosmetic formulas was performed to evaluate their physical properties, colour, texture, phase separation, homogeneity and fragrance (20). The consistency and presence of coarse particles were checked by pressing a small amount of cream between the thumb and index finger. In addition, phase separation, immediate skin feel and skin absorption were also evaluated. These parameters of both creams were monitored for one month (21).

Table 1. Composition for making anti-aging formulations

	Composition	
Oil phase	**Formulation 1**	**Formulation 2**
Olive oil	1 mL	1 mL
Stearic acid	6.25 g	6.25 g
Cetyl alcohol	0.79-1 g	0.80-1g
Liquid phase	N/A	N/A
Disodium EDTA (ethylene diamine tetraacetic acid)	0.025 g	0.022 g
Glycerin	2 mL	2 mL
Polyethylene glycol	1.50 mL	1.50 mL
TED (tri-ethanolamine)	0.50 mL	0.50 mL
Tween 80	2.50 mL	2.50 mL
Rose water	3.50 mL	3.50 mL
Distilled water	3 mL	4 mL
Sodium benzoate (add after mixing 2 phases)	0.25-0.30 g	0.25-0.30 g
C. carandas Linn. fruits and leaves extracts separately (same protocol	1 mL	3 mL

Freeze-thaw cycling stability test

Freeze-thaw cycling one-month stability test of cream was performed using a standard procedure with modifications (22). Formulated creams (2 g) were subjected to thermal cycling by sequential storage at -10 °C, room temperature and 45 °C, each for 24 hr. Phase separation was recorded and homogeneity was evaluated through visual inspection and tactile assessment (23).

Irritancy test

The irritancy test was done by using standard procedures. Briefly, the cream was applied on a one square centimeter area on the left dorsal layer of skin and time was recorded and irritability was observed, erythema and oedema for periodic intervals up to 24 hr, recorded if any.

Spreadability

Spreadability of the formulations was evaluated using the parallel plate method. Approximately 2-3 g of cream was placed between two glass slides, with the lower slide fixed and the upper slide movable. A 100 g weight was applied for 5 min to ensure uniform thickness and excess cream was removed. A 10 g weight was then used to pull the upper slide across a distance of 10 cm. The time taken to separate the slides was recorded (24). Spreadability (S) was calculated using Equation 6 :

$$S = \frac{m \times l}{t} \quad \text{(Eqn. 6)}$$

Where, S represents spreadability, m represents the weight applied to the upper slide (g), l represents the length or distance moved by the slide (cm), and t represents the time taken to move the slide.

Emolliency

A certain amount of cream was applied to the skin and its slipperiness and the quantity of residues left or not after applying the creams were checked (25).

Dilution test

Emulsion type was assessed using the dilution test. Stability upon dilution with water confirmed an oil-in-water (o/w) emulsion, indicating water as the continuous phase (25).

Type of smear

The skin was treated with creams before the test and a non-greasy smear developed (25).

The cream's pH

A pH meter was used to measure the pH of the antiaging formulations. 50 mL of water was measured in a beaker and 5 g of each cream was dissolved in a beaker containing distilled water. The pH of the cream was investigated and the reading was recorded (26).

Evaluation of the nutraceutical capacities of extracts and formulations

Determination of total phenolic content

Total phenolic content was quantified using the Folin-Ciocalteu assay. Briefly, 100 µL of sample, 100 µL of Folin-Ciocalteu reagent and 900 µL of distilled water were mixed in test tubes and incubated for 5 min. Subsequently, 1 mL of 7 % Na_2CO_3 (sodium carbonate) solution was added and the mixture was incubated in

the dark for 30 min. Absorbance was measured at 750 nm using a microplate reader. Absolute ethanol was used as the blank. The procedure was performed for both the plant extracts and the anti -ageing formulations (27).

Total flavonoid content determination

The total flavonoid contents of all experimental samples were determined by using the aluminium chloride colourimetric test (28). In each test tube, 4 mL of distilled water, 300 µL of $NaNO_2$ (sodium nitrite) solution and 1 mL of ethanol-diluted extract (20 mg/mL) were mixed. Absolute ethanol served as the blank. After incubation for 5-10 min, 300 µL of $AlCl_3$ (aluminum chloride) and 2 mL of NaOH (sodium hydroxide) were added, followed by dilution with distilled water to a final volume of 10 mL. After thorough mixing, 150 µL of each reaction mixture was transferred into a 96-well microplate in triplicate. Absorbance was recorded at 510 nm using an enzyme-linked immunosorbent assay (ELISA) microplate reader (29).

Total carotenoid content determination

Total carotenoid content was measured by diluting *C. carandas* fruits and leaves extracts (20 mg/mL in ethanol). For each extract, 10 mL of distilled water and 1 mL of the diluted extract were added to test tubes in triplicate. The same procedure was applied to the anti-ageing formulations. A 150 µL aliquot from each mixture was transferred to a microplate and absorbance was recorded at 450 nm using an ELISA microplate reader. Total carotenoid content was calculated using the following Equation 7.

$$\text{Total carotenoids} = \frac{V(A - 0.0051)}{1.75 \times FW} \quad \text{(Eqn. 7)}$$

Where V is the extract's final volume, A is the diluted extraction's absorbance value at 450 nm and FW is the fresh weight (g).

Determination of antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Antioxidant activity of *C. carandas* extracts and their formulations was assessed using the DPPH radical scavenging method. A 0.005 g DPPH solution was prepared in 25 mL of absolute ethanol. Samples (10 mg/mL) were mixed with DPPH solution in varying concentrations, incubated in the dark at room temperature for 30 min and analysed in triplicate (30) (Table 2). Absorbance was measured at 517 nm using a microplate reader. Ethanol served as the blank and the DPPH solution without a sample was used as the control. The percentage inhibition of DPPH radicals was calculated using Equation 8:

$$\text{Inhibition (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \quad \text{(Eqn. 8)}$$

Where, A_0 represents the absorbance of the DPPH control solution (without extract), and A_1 represents the absorbance of the solution containing the extract or formulation.

2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The ABTS radical scavenging activity of *C. carandas* extracts and their formulations was evaluated using a standard method with some modifications (31). $ABTS^+$ radicals were generated by mixing 0.0384 g ABTS with 10 mL distilled water and adding 6.6 mg potassium persulfate. The solution was incubated in the dark for 6-17 hr. Extracts and formulations (15 µg/mL in ethanol) were mixed with the $ABTS^+$ solution and incubated for 30 min at room temperature. Absorbance was measured at 734 nm using a microplate reader with 150 µL of each mixture loaded into wells. Ascorbic acid served as the standard. The percentage inhibition was calculated using Equation 8.

Table 2. Proximate analysis of *C. carandas* fruits and leaves

Parameters	Percentage (%)	
	Fruits	Leaves
Moisture	16.0 ± 0.49	7.48 ± 0.03
Ash	4.86 ± 0.08	8.49 ± 0.01
Protein	2.23 ± 0.06	1.06 ± 0.03
Fat	8.44 ± 0.29	2.21 ± 0.06
Carbohydrates	66.52 ± 0.53	79.67 ± 0.49

Antibacterial activity

Antibacterial activity of *C. carandas* extracts was evaluated against *E. coli* and *S. aureus*. Bacterial strains were maintained on nutrient agar slants at 4 °C. Luria-Bertani broth (4 g/200 mL) and agar media (3.5 g/150 mL) were prepared, dispensed in sterile containers and autoclaved at 121 °C for 15 min. Inoculum was prepared by transferring a loopful of bacterial culture into 5 mL of nutrient broth and incubating at 37 °C for 24 hr. Subculturing was performed on nutrient agar plates to obtain pure colonies. These were used to inoculate fresh broth cultures, which served as the bacterial suspension for the disc diffusion assay. Extracts were tested for antibacterial activity by measuring zones of inhibition after 24 hr incubation at 37 °C (32).

Minimum inhibition concentration (MIC) determination

Extracts (20 mg/mL) were tested using the disc diffusion method. Sterile nutrient agar plates were inoculated with *E. coli* or *S. aureus and* sterile filter paper discs impregnated with test samples were placed at the centre. Plates were incubated at 37 °C for 24 hr and zones of inhibition were measured to assess antibacterial activity (33).

Antifungal activity

The following fungus strains were used to determine antifungal activity: *A. niger and A. flavus.* Antifungal activity was evaluated against *A. niger* and *A. flavus*. Potato dextrose agar (4.5 g) and agar (3.5 g) were dissolved in 100 mL of distilled water, dispensed in flasks and sterilized by autoclaving at 121 °C for 15 min. Fungal strains were revitalized and subcultured on PDA plates at 37 °C for 24 hr to obtain pure cultures. Inocula were prepared by transferring fungal spores into sterile nutrient broth and incubating at 37 °C for 12 hr. These suspensions were used for antifungal testing (34).

Minimum inhibition concentration (MIC) determination

Extracts and formulations (20 mg/mL in ethanol) were evaluated. Sterilized PDA was poured into Petri plates and allowed to solidify. Plates were inoculated with *A. niger* or *A. flavus* and left at room temperature for 20-30 min. Wells were aseptically created and 100 µL of each sample was added. Plates were sealed with Parafilm and incubated at 37 °C for 24 hr. Antifungal activity was assessed by measuring the diameter of the inhibition zones (35).

Evaluation of anti-ageing potential

Tyrosinase inhibition activity

Tyrosinase inhibitory activity was evaluated using L-tyrosine as the substrate. Fruits and leaves extract formulations were diluted

to 1 mg/mL in ethanol. Each reaction mixture contained 400 µL tyrosinase enzyme, 100 µL sample and 700 µL L-tyrosine. Samples were incubated at 37 °C for 20 min and absorbance was measured at 475 nm using a spectrophotometer (36).

Collagenase inhibitory activity

Collagenase inhibitory activity was assessed using a modified spectrophotometric method. Collagenase (0.8 U/mL) was dissolved in 50 mM tricine buffer (pH 7.5) and the 2-furanacryloyl-L-leucylglycyl-L-prolyl-L-alanine substrate (FALGPA) (0.5 mM) was prepared in the same buffer. The reaction mixture contained 20 µL of *C. carandas* fruits or leaves extract formulation, 20 µL of enzyme solution and 200 µL of substrate. Absorbance was recorded kinetically at 340 nm for 1 min. Enzyme inhibition was calculated as:

$$\text{Inhibition (\%)} = \left(\frac{A_C - A_S}{A_C}\right) \times 100 \qquad \text{(Eqn. 9)}$$

Where, A_c= activity of control and A_s= activity in the presence of inhibitor.

Elastase inhibitory activity

Elastase inhibitory activity was evaluated using a modified spectrophotometric method. Elastase (2 U/mL) was dissolved in 1 M tris-HCl buffer (pH 8) and the substrate N-succinyl-Ala-Ala-Ala -p-nitroanilide (1.6 mM) was prepared in the same buffer. The reaction mixture consisted of 40 µL of sample, 20 µL of enzyme solution and 540 µL of substrate. Absorbance was measured at 410 nm over 5 min. Inhibition was calculated using equation (37).

$$\text{\% Inhibition} = [(V\,\text{max control} - V\,\text{max sample})/V\,\text{max control}] \times 100 \quad \text{(Eqn. 10)}$$

Molecular docking studies

To support our tyrosinase activity results, phytochemicals that are identified during HPLC are used for molecular docking studies. Molecular docking is a computational technique used to predict the interaction between a receptor and potential ligands by simulating their binding affinity and orientation. The three-dimensional structure of tyrosinase (PDB ID: 8HPI) was obtained from the Protein Data Bank and Chain A was selected for molecular docking. The compounds identified as peaks in the HPLC analysis were selected as ligands and their structural data are included. Structure Data Format (SDF) format was retrieved from PubChem (38). To enhance the quality of the protein structure, structure minimization was performed using the GalaxyRefine online server (39). The active site of the protein was identified using DogSiteScorer. The active site includes Arg6, Arg70, Gln81, Tyr177, Trp238, Ser239, Val240, Trp241, Gln242, Ile243, His245, Arg246, Gln248, Asn249, Tyr250, Met277, Asn278, His279, Arg280, Lys 281, Tyr284, Val285, Tyr286 and And Glu289. Molecular docking was carried out using PyRx software, following the methodology (40). The grid box dimensions for the centre were set at (x = 20.71, y = 24.54 and z = 29.29) and for the size were set at (x = 19.50 Å, y = 28.92 Å and z = 25 Å) using a default exhaustiveness value of eight to maximise the receptor-ligand binding interaction examination.

Statistical analysis

All experiments were conducted in triplicate (n = 3) to ensure reproducibility and reliability of results. Data were presented as mean±standard deviation (SD). Triplicate measurements were performed for each experimental parameter, including physicochemical evaluations (pH, spreadability, emolliency, smear type and stability), proximate composition (moisture, ash, protein, fat and carbohydrate), phytochemical content (total phenolics, flavonoids and carotenoids), antioxidant activity (DPPH and ABTS assays), antibacterial and antifungal activity (zone of inhibition) and enzyme inhibition assays (tyrosinase, collagenase and elastase). Statistical significance between sample groups and formulations was determined using paired and unpaired t-tests, as appropriate. A *p*-value less than 0.05 was considered statistically significant. The statistical analysis was performed using the SPSS v. 22.0 program.

Results

Extraction, phytochemical constituents and biological activities of ethanolic extract of *C. carandas*

The 50 % ethanolic extract was prepared from the *C. carandas* fruits. The obtained percentage yield of fruits extract is 43.1 ± 1.15 and the leaves extract is 25 %.

Proximate analysis of *C. carandas* fruits and leaves

The proximate composition of *C. carandas* fruits and leaves is summarised in Table 2. Notable differences were observed in moisture, fat and carbohydrate contents between the two samples. Fruits samples exhibited higher moisture (16.5 %) and fat (8.90 %) contents, whereas leaves samples showed a higher carbohydrate (80.61 %) and ash (8.5 %) composition.

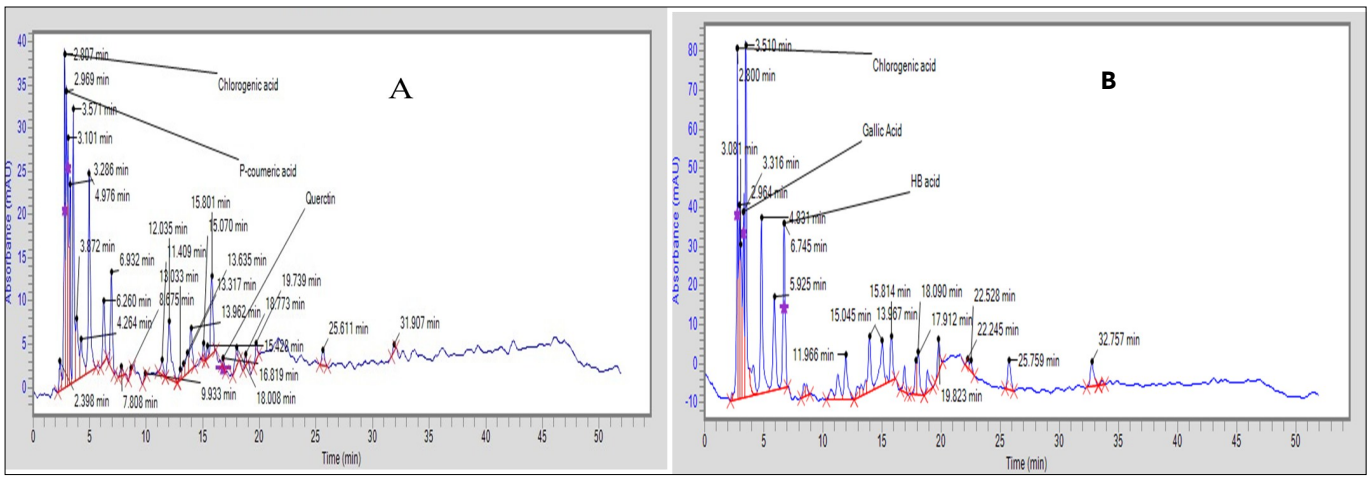

Fig. 1. HPLC results of *C. carandas* 50 % ethanolic extract (A: leaves and B: fruits).

Table 3. Physical parameters of fruits and leaves extract formulation (cream)

Physical parameters of antiaging formulations	Results	
	Fruits	Leaves
Appearance	Smooth	Smooth
Color	Pink white	Pink white
Aroma	Pleasant	Pleasant
Texture	Creamy	Creamy
State	Semisolid	Semisolid
Grittiness	No grittiness	No grittiness
Homogeneity	Good	Good
Type of smear	Non-greasy	Non-greasy
Emolliency	Smoothly mixed no residues left	Smoothly mixed no residues left
Dilution test	o/w type	o/w type
Irritancy	Nil	Nil
pH	5.42	6.3
Removal	Easy	Easy
Spreadability	10 sec	13 sec
Freeze-thaw cycling stability	Stable in all temp. / no phase separation	Stable in all temp. /no phase separation

High performance liquid chromatography

High performance liquid chromatography analysis of ethanol extracts from *C. carandas* fruits and leaves revealed the presence of several prominent phenolic and flavonoid compounds (Fig. 1). Major constituents identified included kaempferol, quercetin, coumarin, rutin and phenolic acids such as gallic, ferulic, benzoic, chlorogenic, sinapic and p-hydroxybenzoic acid (HB acid).

Physical parameters of *C. carandas* fruits and leaves formulation

Formulations prepared from *C. carandas* fruits and leaves extracts exhibited a uniform, semisolid consistency with a pinkish-white appearance and a smooth, non-greasy texture. The pH ranged from 5.42 to 6.3, indicating skin compatibility with no signs of irritation. All formulations showed good homogeneity, spreadability and ease of removal. Freeze-thaw and dilution tests confirmed product stability, while emolliency and smear characteristics were favourable. Evaluation results are summarised in Table 3.

Evaluation of the nutraceutical capacities of extracts and formulations

Determination of total phenolic content (TPC), total flavonoid content (TFC) and total carotenoid content (TCC)

The TPC, TFC and TCC of fruits and leaves extracts, and their formulations were determined. All samples exhibited notable levels of bioactive compounds, with formulations showing significantly higher values than crude extracts. The fruits based formulation recorded the highest TPC, TFC and TCC values shown in Table 4.

Determination of antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

Fig. 2 shows the DPPH radical scavenging activity of *C. carandas* fruits and leaves extracts and their formulations at varying concentrations. Antioxidant activity increased with concentration, reaching maximum inhibition at 100 µL/mL. Leaves and fruits formulations showed the highest % inhibition (84.9 % and 76.5 %, respectively), outperforming their crude extracts. This suggests enhanced efficacy of the formulations compared to raw extracts.

2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging activity

Fig. 3 shows the ABTS radical scavenging activity of *C. carandas* fruits and leaves extracts and their formulations. Antioxidant

Table 4. Total phenolic content (TPC), total flavonoid content (TFC) and total carotenoid content (TCC) of *C. carandas* (fruits and leaves extracts and formulations)

S. No.	Samples	Total phenolic content (mg/g)	Total flavonoid content (mg/g)	Carotenoid (mg/g)
1.	Fruits extract	48.61 ± 0.36	59.98 ± 0.67	0.043 ± 0.0012
2.	Fruits formulation	89.18 ± 0.23	124 ± 0.10	0.098 ± 0.0010
3.	Leaves extract	47.16 ± 0.02	54 ± 1.24	0.03 ± 0.0013
4.	Leaves formulation	68.32 ± 0.12	70.09 ± 0.67	0.084 ± 0.0011

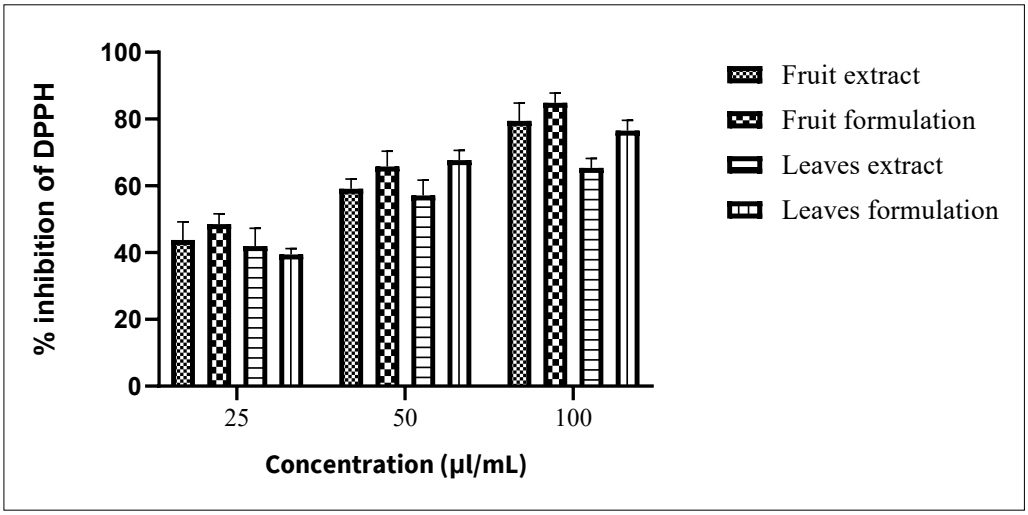

Fig. 2. DPPH radical scavenging activity of *C. carandas* (fruits and leaves extracts and formulations).

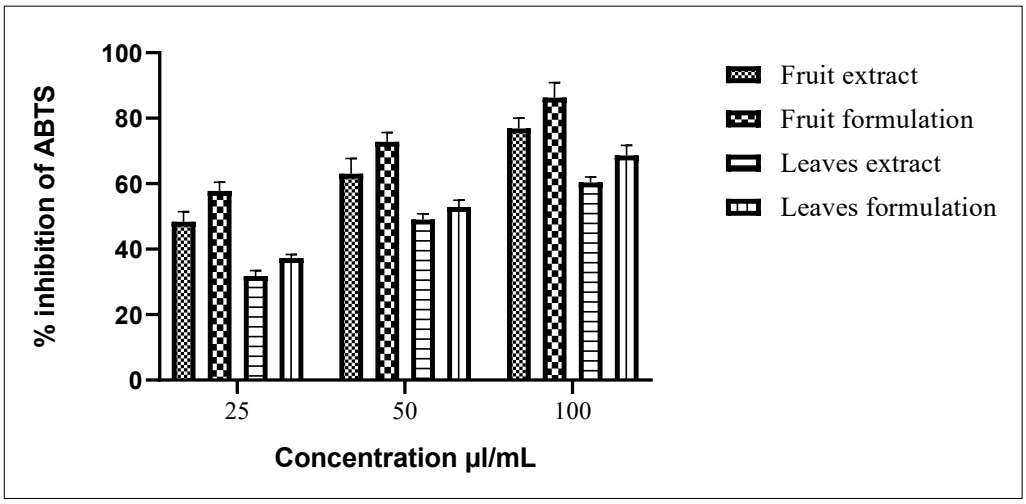

Fig. 3. ABTS radical scavenging activity of *C. carandas* (fruits and leaves extracts and formulations).

Fig. 4. Percent inhibition of tyrosinase, collagenase and elastase of *C. carandas* (fruits and leaves formulation).

Table 5. Antimicrobial activity of *C. carandas* (fruits and leaves extracts and formulations)

Antibacterial activities of samples						
Bacterial strains	**Fruits extract**	**Fruits formulation**	**Ciprofloxacin**	**Leaves extract**	**Leaves formulation**	**Control**
Staphylococcus aureus	16.67 ± 0.58	9.67 ± 0.57	23.33 ± 2.08	15.66 ± 0.33	12.66 ± 0.33	25 ± 0.5
Escherichia coli	15.33 ± 1.53	7.67 ± 0.57	21.33 ± 1.52	20.00 ± 0.57	9.33 ± 0.33	25 ± 0.5
Antifungal activities of samples						
Fungus strains	**Fruits extract**	**Fruits formulation**	**Fluconazole**	**Leaves extract**	**Leaves formulation**	**Control**
Aspergillus niger	27.0 ± 1.0	21.0 ± 1.0	30.0 ± 1.0	17.33 ± 0.33	22.00 ± 0.57	33.00 ± 0.5
Aspergillus flavus	29.33 ± 0.58	26.0 ± 1.0	33.0 ± 1.0	16.33 ± 0.33	26.00 ± 0.57	33.00 ± 0.5

activity increased with concentration, with the fruits formulation exhibiting the highest inhibition (86.28 %) at 100 µL/mL. Overall, both formulations showed stronger ABTS inhibition than the crude extracts.

Evaluation of anti-ageing potential

Tyrosinase, collagenase and elastase enzyme inhibition activity

The anti-ageing activity of *C. carandas* fruits and leaves formulations is based on their inhibition of tyrosinase, collagenase and elastase enzymes. Both formulations showed significant enzyme inhibition, with collagenase inhibition being the highest (74.8 % for fruits and 74.16 % for leaves). Tyrosinase and elastase were also effectively inhibited, indicating the strong anti-aging potential of both formulations in Fig. 4.

Evaluation of the therapeutic potential of extracts and formulations

Antibacterial and antifungal activity

Table 5 presents the antibacterial and antifungal activities of *C. carandas* fruits and leaves extracts and their formulations. The fruits and leaves extracts showed stronger antibacterial activity against *S. aureus* and *E. coli* compared to their formulations, with

Table 6. Docking complex binding energies

S. No.	Ligand name	Binding score (Kcal/mol)	Interacting residues in the active pocket of tyrosinase
1	Rutin	-6.6	Arg6, Glu77, Gln81, Arg280, Lys281 and Gly283
2	Chlorogenic acid	-6.3	Arg246, Glu274, Met277, Asn278 and Lys281
3	Kaempferol	-6.8	Asn239, Arg246 and Met277
4	Salicylic acid	-5.2	Gln242 and His 245
5	P-coumaric acid	-5.5	Gln242, Ile243, Arg246 and Gln248

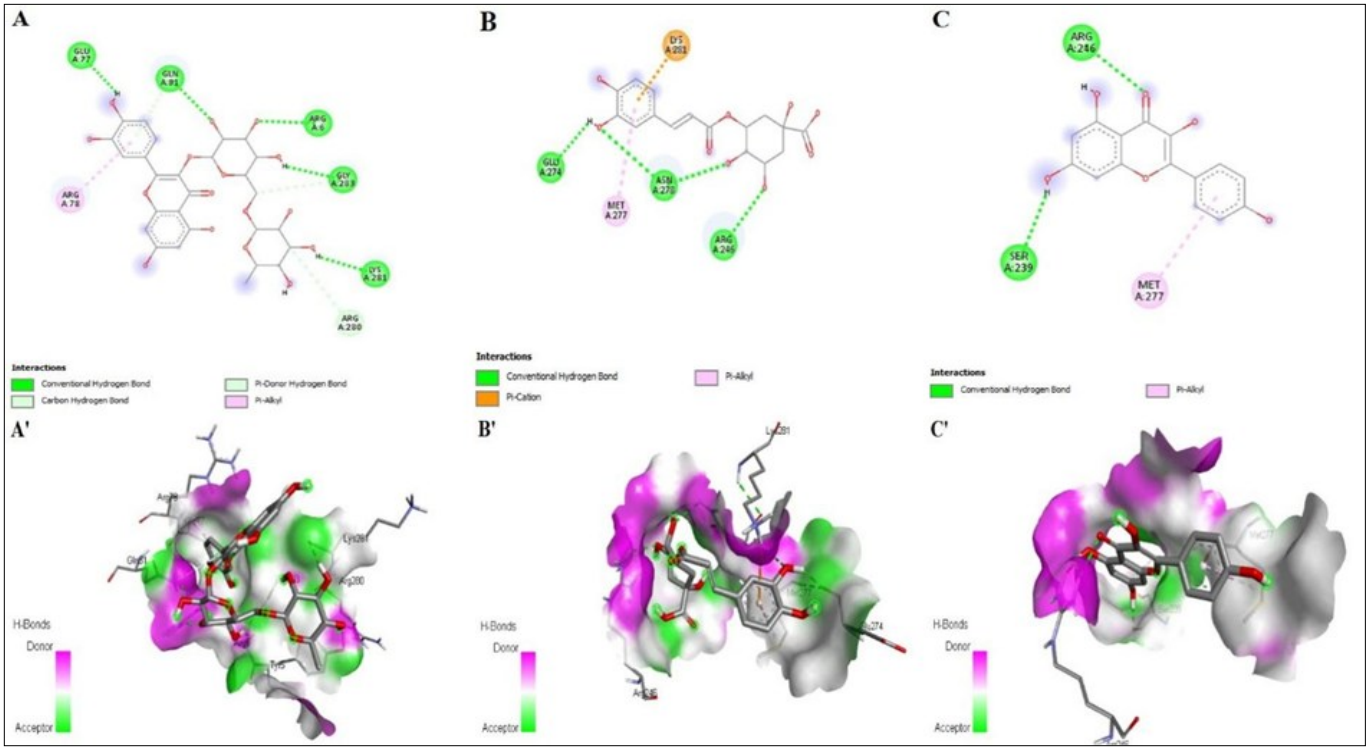

Fig. 5. Molecular docking results of several compounds within the active pocket of tyrosinase. **A-C:** Ligand interactions, **A'-C':** Binding patterns. **A-A':** The Rutin ligand and binding interaction, **B-B':** Chlorogenic acid ligand and binding interaction, **C-C':** Kaempferol ligand and binding interaction.

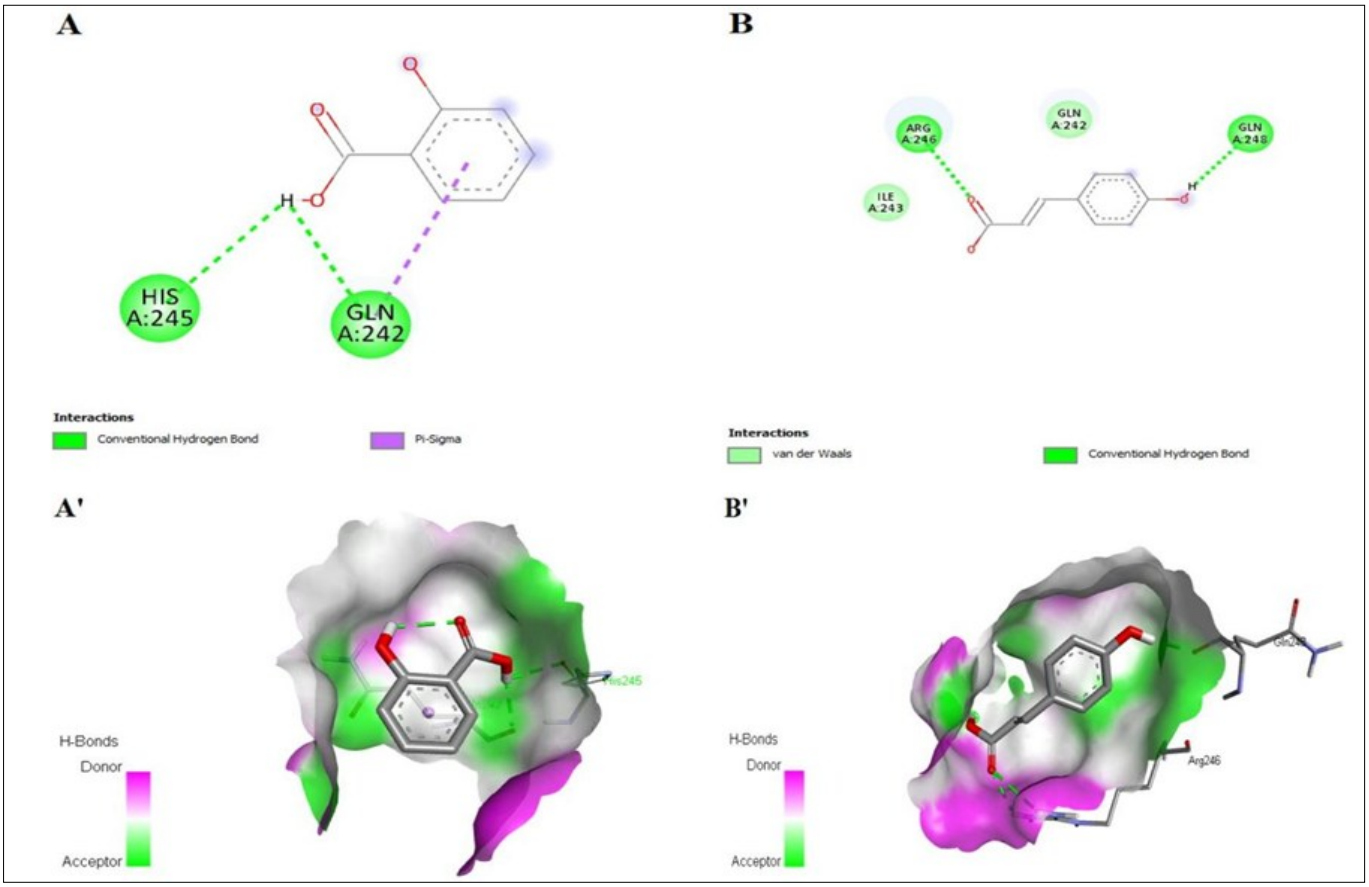

Fig. 6. Molecular docking results of several compounds within the active pocket of tyrosinase **A-B:** Ligand interactions, **A-B':** Binding patterns, **A-A':** Salicylic acid ligand and binding interaction, **B-B':** P-coumaric acid ligand and binding interaction.

the fruits extract exhibiting the highest inhibition zone against *E. coli*. Ciprofloxacin served as the positive control. For antifungal activity, the fruits extract demonstrated significant inhibition against *A. niger* and *A. flavus,* while leaves formulations also showed notable activity, particularly against *A. flavus*. Fluconazole was used as the standard for antifungal testing.

Molecular docking interaction analysis

Table 6 presents the molecular docking results of five bioactive compounds identified through HPLC analysis, evaluated for their interaction with the active site of the tyrosinase enzyme. Binding scores ranged from -6.8 to -5.2 kcal/mol, with kaempferol showing the strongest binding affinity (-6.8 kcal/mol), followed closely by rutin (-6.6 kcal/mol) and chlorogenic acid (-6.3 kcal/mol). These compounds interacted with key active site residues such as Arg246, Lys281 and Met277, indicating their potential as effective tyrosinase inhibitors. Salicylic acid and p-coumaric acid exhibited lower binding affinities (-5.2 and -5.5 kcal/mol, respectively) but still formed relevant interactions, suggesting moderate inhibitory activity in Fig. 5 and 6. The results support the potential biological relevance of these compounds in inhibiting tyrosinase, aligning with their roles in skin-lightening and anti-melanogenic applications.

Discussion

The fruits of the *C. carandas* are rich in biological and therapeutic qualities. The safety and effectiveness of the fruits extract used in emulgel for cosmetic reasons as an anti-ageing element (41). The fruits of *C. carandas* is high in minerals, phenolic compounds, natural antioxidants and energy. *Carissa carandas* is a disease- and pest-free plant that is simple to grow. Phenolic acids, steroids, flavonoids, carotenoids and terpenoids are found in fruits, leaves and roots and are responsible for various pharmacological actions. This plant has been a key source of remedies for millennia, used to cure diarrhoea, anaemia, dysentery and to prevent illness. Isolating certain components that are in charge of various biological processes is necessary. The development of *C. carandas* fruits processing technologies is also necessary to provide various commercial goods. One of the top research priorities is to optimize and scale *C. carandas'* goods for both domestic and foreign markets (42). Moreover, the safety and effectiveness of the *C. carandas* fruits and leaves formulation were also evaluated in relation to its anti-ageing properties.

Carissa carandas extracts were assessed to investigate their biological characteristics and potential uses in cosmetics. This study was designed for the preparation of formulations from 50 % ethanolic fruits and leaves extract of *C. carandas* separately. *In vitro* analysis was performed on both fruits and leaves extracts and formulation to assess their antioxidant, antibacterial, antifungal and anti-ageing enzyme activities. HPLC, TPC, TFC and TCC were performed to assess the phytochemicals present in the fruits and leaves extract and formulation. DPPH and ABTS were used to assess the antioxidant activity. Anti-bacterial and anti-fungal activity was assessed on *S. aureus, E. coli, A. flavus and A. niger*. Anti-tyrosinase, anti-collagenase and anti-elastase assays were performed to assess the anti-ageing effect of the fruits and leaves formulation.

The percentage yield of *C. carandas* fruits extract was 43.1 ± 1.15 and that of leaves was 25 %. Previous research reported the percentage yield of *C. carandas* ethanolic fruits extract as 35.27 ± 0.53 and 38.59 %. Research indicates similar observations from the research trial (13, 43). It is observed that TPC, TFC and TCC are present in *C. carandas* fruits and leaves extract and formulation. Gallic acid was used as a standard for the determination of TPC and quercetin for TFC. Ethanolic fruits extract has shown significantly higher values for TPC, TFC and TCC of the extract and that of the leaves extract (44). While formulation of fruits and leaves has shown more significant results than the extract for TPC, TFC and TCC, respectively.

The antioxidant activity of *C. carandas* fruits and leaves extract and formulation was assessed by DPPH and ABTS assays. The *C. carandas* fruits extract exhibited more potential for DPPH and ABTS radical scavenging activity. Both DPPH and ABTS assays for antioxidant activities showed significantly higher results and agreed with the previous results (45). The results have shown that the IC_{50} values 52.12 % for DPPH varied by degree, ranging from the fruits formulation exhibited the highest DPPH inhibition (84.9 %) compared to the leaf formulation (76.5 %). In the ABTS assay, fruits extracts (76.90 %) and formulation (86.28 %) showed higher scavenging activity than leaves extracts (60.38 %) and formulation (68.60 %). Fruits extract exhibited a larger antibacterial and antifungal zone of inhibition using *S. aureus* & *E. coli*. and *A. niger* and *A. flavus* than formulation. The standard ascorbic acid was also used in this assay.

The antioxidant activities for the formulation of *C. carandas* fruits and leaves were evaluated and showed significantly higher results than the fruits and leaves extracts. This demonstrated that the anti-ageing formulation of fruits extract has significant antioxidant activities. This means that the antiaging formulation of *C. carandas* fruits extract has the highest potential to protect the body from the harmful effects of free radicals (13). Antimicrobial peptides are effective at eliminating skin pathogens due to their strong antimicrobial properties. Additionally, they enhance the host's immune response, providing a dual action against skin infections (46)

The antibacterial and antifungal activity of *C. carandas* fruits and leaves extract and their formation were evaluated against both *E. coli*. and *S. aureus* bacteria and *A. flavus* and *A. niger* fungus, respectively. Results indicate that the values were significant and the fruits extract showed the highest antibacterial potential than its formulation. The standard used for antibacterial and antifungal activity was ciprofloxacin and fluconazole, respectively. Research indicates that significant results were achieved from the different trials (43). The phytochemical components found actively in the ethanolic extract of *C. carandas* fruits and leaves, such as saponin, alkaloids, tannins, glycosides, terpenoids and flavonoids, can be used as active components for anti-acne skincare products (31).

The anti-ageing activities of the fruits and leaves formulation of *C. carandas* were studied on three ageing enzymes: tyrosinase, collagenase and elastase. Fruits formulation inhibited all three enzymes and exhibited the best anti-ageing effect. The % inhibition of tyrosinase, collagenase and elastase of fruits formulation was 59.44 ± 1.18, 74.8 ± 1.15 and 50.87 ± 0.93, which showed the strong inhibition of ageing enzymes and prevention of fast ageing. Research indicates that significant inhibition was comparable with the previous results (13). The tyrosinase inhibition activity is the process that prevents melanogenesis, reduces

melanin production and is a powerful agent for skin lightening. The discovery of elastase enzyme inhibitors may be beneficial in the fight against ageing, as it can avert the thinning and sagging of skin (47). This biological activity may be closely linked to the presence of specific bioactive compounds such as kaempferol and rutin, which are well-known for their anti-ageing and antimicrobial mechanisms.

The formulated creams were evaluated for their topical suitability and physicochemical stability. Both formulations exhibited a smooth, homogeneous and non-greasy texture, with a pinkish-white colour. The pH ranged between 5.42 and 6.3, which is within the range compatible with human skin (48). Freeze-thaw and dilution tests confirmed the stability of the formulations, with no phase separation or loss of consistency. The creams were easily spreadable and non-irritant upon application, meeting essential criteria for cosmetic acceptability. Research indicates that similar stability parameters have been reported for polyherbal cosmetic formulations, validating the current preparation strategy (49).

Enzyme inhibition studies were conducted to evaluate the anti-ageing potential of the extracts and formulations. Both samples inhibited the activities of tyrosinase, collagenase and elastase, with the fruits formulation showing superior results. These enzymes are involved in melanin production and the degradation of collagen and elastin, respectively. Therefore, their inhibition implies potential anti-melanogenic and anti-wrinkle effects. Kaempferol and chlorogenic acid, in particular, are recognized in the literature for their ability to inhibit these enzymes and protect against oxidative degradation of skin components (50, 51). Research indicates that significant enzyme inhibition occurs with similar bioactives extracted from traditional medicinal plants (52, 53).

To further elucidate the mechanism of enzyme inhibition, molecular docking studies were performed. Kaempferol, rutin and chlorogenic acid showed strong binding affinities with tyrosinase, with docking scores of -6.8, -6.6 and -6.3 kcal/mol, respectively. These compounds formed interactions with active site residues such as Arg246, Lys 281 and Met 277, indicating stable and biologically relevant binding. Salicylic acid and p-coumaric acid, though lower in binding affinity, also contributed to moderate inhibition. The docking data support the *in vitro* enzyme inhibition results, demonstrating a strong correlation between binding affinity and bioactivity. This mechanistic validation enhances the credibility of using *C. carandas* phytoconstituents as effective tyrosinase inhibitors. Research indicates that similar binding affinities and reliability of interactions in predicting biological function (54-62).

The overall results demonstrate that *C. carandas* holds considerable promise as a source of multifunctional bioactive agents for cosmeceutical applications. The high levels of antioxidants, combined with effective inhibition of ageing-related enzymes and antimicrobial potential, position this plant as a candidate for inclusion in herbal skincare products. Importantly, the enhanced activity observed in the formulated products suggests that formulation not only stabilizes the active components but also improves their delivery and bio-efficacy. Research indicates that botanical-based cosmeceuticals often rely on optimised formulation for maximum skin benefit (62).

Despite these promising findings, the study has several limitations. It was confined to *in vitro* and *in silico* analyses. No *in vivo* assessments or clinical studies were performed to validate the skin efficacy or safety in human models. Moreover, the use of ethanol as an extraction solvent, while effective, limits the spectrum of extracted compounds.

Conclusion

This study demonstrated the significant cosmeceutical potential of *C. carandas* fruits and leaves extracts through a comprehensive assessment of their phytochemical content, antioxidant capacity, antimicrobial efficacy and anti-ageing enzyme inhibition. Enhanced biological activity was observed in the formulated creams, which exhibited skin-compatible pH, stability and favourable application characteristics. The presence of key bioactives such as kaempferol, rutin and chlorogenic acid was validated through HPLC and supported by molecular docking analyses, confirming their mechanistic role in tyrosinase inhibition. Overall, the findings establish *C. carandas* as a promising source of multifunctional phytoconstituents for natural skincare applications. Moreover, isolation of individual phytoconstituents and mechanistic exploration of their bioactivities will be essential to validate and optimize the cosmeceutical potential of *C. carandas* for sustainable dermatological applications.

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Authors' contributions

SAB was responsible for investigation, resources, data curation, supervision and project administration. FA conceptualised, wrote the original draft, reviewed and edited the manuscript. TN contributed to methodology, validation, formal analysis and writing of the original draft. AS contributed to methodology, validation, formal analysis and writing of the original draft. FT did editing, visualisation and formal analysis. RR was involved in editing, visualisation and formal analysis. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflicts of interest to declare.

Ethical issues: None

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