https://doi.org/10.31925/farmacia.2025.6.22 **ORIGINAL ARTICLE**

COSMECEUTICAL POTENTIAL OF *CODIUM IYENGARII* EXTRACT LOADED NANOEMULSION

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Abstract

The study was designed to evaluate the cosmeceutical potential of ethanolic extract of *Codium iyengarii* extract loaded nanoemulsion. The chemical profile of extract revealed 40 bioactive compounds with total phenolic content (TPC) of 7.61 ± 0.47 mg gallic acid equivalents (GAE) and total flavonoid content (TFC) of 19.89 ± 3.35 mg quercetin equivalents (QE) per gram of sample. A total of 7 formulations were prepared with low energy method using olive oil and Smix (Tween 80 and PEG 400) of 1:1 ratio, according to the compositions generated by design expert. F6 formulation was selected for *in vitro*, *in vivo* and clinical evaluation, based on its droplet size (183.67 ± 28.17 nm), along with acceptable values for PDI (0.46), viscosity (296.67 ± 6.67 mPa.S) and pH (5.92 ± 0.01) for topical application. F6 was found to be stable, safe and showed good spreadability (33.48 ± 2.25 g.cm/s), photoprotective activity (SPF 2.23) and tyrosinase inhibition (52%). Clinical evaluation indicated a significant reduction in transepidermal water loss (-37.47%) along with an increase in skin hydration level (29.47%) and oiliness (27%) after 4 hours of application, compared to baseline. Overall, the *C. iyengarii* extract loaded nanoemulsion showed potential for cosmeceutical applications.

Rezumat

Studiul a evaluat potențialul cosmeceutic al extractului etanolic încărcat cu nanoemulsie de extract de *Codium iyengarii*. Profilul chimic al extractului a relevat 40 de compuși bioactivi cu un conținut total de compuși fenolici (TPC) de 7,61 ± 0,47 mg echivalenți de acid galic (GAE) și un conținut total de flavonoide (TFC) de 19,89 ± 3,35 mg echivalenți de quercetină (QE) *per* gram de probă. Au fost preparate 7 formulări prin metoda cu consum redus de energie folosind ulei de măsline și Smix (Tween 80 și PEG 400) în raport 1:1. Formula F6 a fost selectată pentru evaluarea *in vitro*, *in vivo* și clinică, pe baza dimensiunii picăturilor (183,67 ± 28,17 nm), împreună cu valorile acceptabile pentru PDI (0,46), vâscozitate (296,67 ± 6,67 mPa.S) și pH (5,92 ± 0,01) pentru aplicare topică. S-a constatat că F6 este stabil, sigur și a prezentat o bună distribuire (33,48 ± 2,25 g.cm/s), activitate fotoprotectoare (SPF 2,23) și inhibare a tirozinazei (52%). Evaluarea clinică a indicat o reducere semnificativă a pierderii transepidermice de apă (-37,47%), împreună cu o creștere a nivelului de hidratare a pielii (29,47%) și a aspectului uleios (27%) după 4 ore de aplicare, comparativ cu valorile inițiale. Nanoemulsia cu extract de *C. iyengarii* a demonstrat potențial pentru integrarea sa în aplicații cosmeceutice.

Keywords: seaweed, nanoemulsion, cosmeceutical, skin moisturizer

Introduction

Consumer interest in green and eco-friendly products has surged in recent years, particularly in the cosmetic market. This trend increasingly demands novel skin care products with natural, safe and effective ingredients to meet the global market demand [1-3].

Cosmeceuticals refer to the active and safe products developed by the cosmetics industry to improve skin appearance and cope with different dermatologic conditions, extending beyond cosmetics to promote skin functioning, such as anti-wrinkle moisturizers, antioxidant serum, skin lightening agents and anti-hair fall products are included in this category [4, 5]. It acts as bridge between drug and beauty care products exhibiting skin beneficial effects due to presence of several bioactive compounds for instance, vitamins, enzymes, essential oils and phytochemicals, available as creams, lotions, ointment and facial masks [1]. The cosmetic science has achieved further advancement with incorporation of nanocarrier system, resulting in increased consumer demand throughout the world [6]. Thus, nanocosmeceuticals have also been highly exploited for designing anti-aging products and extensively harnessed for the skin care, hair care and nail care formulations, aiming to promote their growth, protection and hydration power, with enhanced effectiveness as cosmetic products [7].

Nanocosmeceuticals, especially, nanoemulsion has been gaining attention from researchers due to adoption of latest technologies and the integration for innovative and sustainable products [8]. Nanoemulsions are divided into oil-in-water (O/W) and water-in-oil (W/O) emulsions, with droplet sizes typically ranges from 20 to 200 nm, can influence stratum corneum structure and increase hydration. The nanoemulsion systems offer superior cosmetic bases due to smaller droplets size, providing large surface area for uniform and efficient delivery of active ingredients through the skin and improved stability with a pleasant aesthetic character and skin feel [9, 10].

Marine resources, particularly seaweed (macroalgae), present a sustainable, renewable and promising resource for cosmetics, cosmeceuticals and nutricosmetics, attributed to their potential skincare properties. Seaweed components such as carbohydrates, lipids, proteins, vitamins, carotenoids and polyphenols have commercial applications in cosmetics and personal care products. These bioactive compounds offer a wide spectrum of skin beneficial effects including antioxidant, anti-aging, antimicrobial, anti-inflammatory, soothing, photoprotective, sensitizing, conditioning, whitening and moisturizing actions, contributing to their increased use in cosmeceuticals [2, 3, 11]. Seaweeds are eukaryotic and photosynthetic organisms, thriving in aquatic environment along with coastlines and in seawater. Three different types of seaweed are documented based on their pigments including *Chlorophyta* (green algae), *Rhodophyta* (red algae) and *Phaeophyta* (brown algae) [2, 10].

The green seaweeds are predominantly contain some important constituents with potential skin beneficial effects such as the carbohydrate content in this group surpassing those found in brown and red algae [12]. Additionally, presence of bioactive compounds like polysaccharides, sterols, fatty acids, pigments, vitamins, terpenes and polyphenolics, demonstrated potential cosmeceutical applications attributed to their antioxidant, anti-inflammatory, antimicrobial, anti-melanogenic, photoprotective, anti-wrinkle and moisturizing effects [13, 14].

The genus *Codium* of *Chlorophyta* class have been recognized for its notable pharmacological potential [15]. Among its species, *Codium iyengarii* (*Codiaceae*) is one of the widely distributed green seaweed along the Coastlines of Karachi, Pakistan.

The coastlines of Karachi, Pakistan, characterized by nutrient-rich waters, support a diverse range of potentially valuable algal species [16, 17]. Thus, the current study is the first to evaluate the cosmeceutical potential of *Codium iyengarii* extract with recent technology of nanoemulsion formulation to address global skin related challenges, offering ecofriendly promising solution for skin health.

Materials and Methods

Materials

Olive oil (Sasso, Italy) and virgin coconut oil were procured from local supplier in Karachi, Pakistan. Kojic acid, tyrosinase, nitric acid, Tween 80 and PEG 400 (Sigma-Aldrich, USA) and ethanol (Merck) were used in the study. Atomic absorption spectrophotometer standard (AAS) grade stock standards were procured from Fisher Scientific UK.

C. iyengarii sample was collected from the coastal area of Buleji, Karachi, Pakistan. The sample was authenticated by Professor Dr. Mohtasheemul Hassan, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Pakistan, with the herbarium number CI-02-20.

Ethical approval

The study protocol was approved by the Institutional Bioethics Committee (IBC), University of Karachi, Karachi, Pakistan, under the reference number IBCKU-141/2020/2024 for research involving animals and human. The study followed the guidelines outlined in the Declaration of Helsinki.

Preparation of seaweed extract

Approximately 100 g of seaweed sample was powdered and macerated with 500 mL of ethanol (95%) for 7 days and at room temperature. The mixture was shaken manually every 12 hours. The material was then filtered and concentrated using rotary vacuum evaporator (BUCHI, Switzerland). The resulting crude extract was stored in a glass container at 4°C for further analysis [18].

Estimation of total phenolic content (TPC) and total flavonoid content (TFC)

The flavonoid content (TFC) and phenolic content (TPC) were determined, following the aluminium chloride colorimetric method and Folin-Ciocâlteu method respectively, as followed earlier [19]. For TFC, extract sample (0.5 mL) of varying concentrations ranging from 1000 to 2000 µg/mL was added in 95% methanol (1.5 mL) and 0.1 mL of potassium acetate solution (1 M) along with $AlCl_3$ (10%), and 2.8 mL of distilled water. The mixture was then incubated for 30 min. After incubation, the absorbance was noted at 415 nm using Perkin Elmer SP-UV-500 Series UV-Vis Spectrophotometer. Same procedure was followed for Quercetin (0.625 µg/mL to 100 µg/mL), used as standard. For TPC, extract sample of 0. 5 mL (1000 µg/mL to 2000 µg/mL) was treated with 2.5 mL of sodium carbonate solution (7.5%) and 2.5 mL of Folin-Ciocâlteu reagent (10%). Following the incubation period of 30 to 40 minutes, the absorbance was measured at 765 nm. A calibration curve of gallic acid of concentration range from 6.25 µg/mL to100 µg/mL was utilized to quantify phenolic content in extract. The TFC was calculated following the equation derived from the standard quercetin curve:

$$\text{Absorbance} = 0.0068\ [\text{Quercetin (ug)}] + 0.012,$$

(R^2 = 0.9973). Whereas the TPC was calculated by following the equation derived from the standard gallic acid curve:

Absorbance = 0.0079 [GA (µg)] + 0.0146,

(R^2 = 0.9958).

Gas chromatography mass spectrometry (GC-MS) analysis

The study aimed to investigate the chemical profile of *C. iyengarii* ethanolic extract using GCMS analysis by following the earlier method with slight modification [20]. A SHIMADZU Nexis series GCMS-TQ8040 NX System, which includes auto sampler AOC-20i and SHIMADZU gas chromatograph GC-2030 coupled with triple quadrupole mass spectrometer was used. For Chromatographic separation, capillary column ZB-5 (30 m * 0.32 * 0.25 µm film thickness) was used. The Electron ionization (EI) system of the mass spectrometer operated in scan mode with an ionization energy of 70 eV. Helium gas (99.99%) was used as carrier gas with flow rate of 1 m/minute and 1 µL of the sample was injected. The oven startup temperature was programmed at 50°C for 5 minutes, then increased to 200°C with a rate of 5°C *per* minute for 10 minutes. Subsequently, the temperature was programmed to increase to 300°C with a rate of 8°C *per* minute for 10 minutes. The time for total run was 51 minutes whereas the mass spectrometer transfer line temperature was set at 260°C and the injector temperature was maintained at 250°C. Prior to the injection, the solvent cut time was set at 3 minutes. The Electron ionization (EI) system of the mass spectrometer operated in full scan mode with ionization energy of 70 eV, including the fragments ranging from 20 to 600 *m/Z* was employed. The ion source temperature was set at 200°C. The GCMS Real Time Analysis software database of the National Institute of Standards and Technology (NIST) identified the compounds spectrums.

Nanoemulsion components

To select the oil for nanoemulsion, the solubility of *C. iyengarii* extract in two commonly used vegetable oils, virgin coconut oil and olive oil were evaluated [21-23]. The variable amount of extract in 5 mL of oil (0.1 to 1%) was sonicated for 15 minutes at 37ºC followed by homogenization for 1 hour on magnetic stirrer (78-1, China). The subsequent mixture was vortexed for 5 minutes using a vortex mixer (Whirl Mixer Lab, FSA Supplier, England) and then centrifuged at 4000 rpm for 15 minutes using Heraeus Biofuge Primo R Centrifuge, Thermo Electron Corporation. After that, the solution was visually observed to identify any residue [22, 24]. The oil displaying the highest capacity to dissolve the algal extract was chosen [22]. Moreover, a UV spectrophotometer was utilized to evaluate the solubility of the extract in aforementioned oils. The absorbance was noted at various wavelengths varied between 400 nm to 800 nm [23, 25]. Nonionic surfactant (Tween 80) and co surfactant (PEG 400) were selected because of their minimal toxicity, biocompatibility with biological systems and widespread application in pharmaceutical products [9, 26, 27].

Pseudoternary phase diagram

Different ratios of surfactant with co surfactant (1:1, 2:1 and 3:1) with selected oil (olive oil) were used to identify the maximum nanoemulsion region by constructing a pseudo-ternary phase diagram utilizing the water titration method [23, 26]. Each Smix was mixed with olive oil with a weight ratio of 1:9 to 9:1. The mixture was gradually diluted with distilled water while constantly stirring until a stable state was achieved. The resultant sample was then observed visually to categorize as nanoemulsion, gel or emulsion. The CHEMIX ternary plot software (Chemix School 9) was employed to construct phase diagrams.

Preparation of C. iyengarii extract loaded nanoemulsions

Based on the pseudo-ternary phase diagrams, the largest area (1:1 Smix) was chosen as the optimal composition for nanoemulsions formulation development by employing a Simplex lattice design experiment (Design expert software Version 7.0) [28]. The nanoemulsion loaded with extract was prepared utilizing the low energy emulsification method. Extract loaded olive oil (0.2%) was homogenized with Smix for 5 minutes using magnetic stirrer. Under continuous stirring, the aqueous phase was introduced drop wise into the oil and Smix blend [26, 27]. The subsequent mixture was stirred for 1 hour until a clear mixture was attained. The nanoemulsions formulations were set aside in a refrigerator at 4°C in a well-closed container for further use.

Characterization of nanoemulsions

Droplet size and PDI (polydispersity index) of the nanoemulsion formulations were evaluated utilizing the DLS (Dynamic Light Scattering) technique (Laser spectroscatter-201, Xtal Consepts, Germany). The sample was diluted with deionized water (1:10) and analysis was executed at 25 ± 1°C with a fixed scattering angle of 90°. For the data analysis, Xtal Concepts software (Xtal Concepts, Germany) was utilized [27]. For pH measurement, 1 g of each formulation was diluted with distilled water (10 mL) and the pH of the resultant solution was noted at room temperature (25 ± 2°C) utilizing a pH meter (Mettler MP-220, Schwerzenbach, Switzerland) [29]. The Viscosity of the formulations was determined using a Brookfield viscometer **(**Haake-19, Karlsruhe, Spain) equipped with an L3 spindle at a speed of 60 rpm at controlled temperature (25 ± 2°C) [30]. Moreover, the electrical conductivity of the nanoemulsion formulations was measured at room temperature (25 ± 2°C) *via* a Conductometer (WPA-CMD-500, Cambridge, UK) [22, 31].

Stability studies

All formulations were observed for physical stability over a 24-hours period. The formulation chosen based

on the droplet size was further subjected to stability studies for a period of three months at 4 ± 2°C and 25 ± 2°C. The physical appearance including phase separation, colour change, clarity, texture and pH was evaluated. Additionally, droplet size and polydispersity index were determined during the storage period [22, 26, 32, 33].

FTIR analysis

Fourier Transform Infrared Spectroscopy (FT-IR) was employed to identify functional groups for the corresponding bioactive compounds and to investigate potential interactions between the components of the nanoemulsion and the extract. Approximately 20 mg of each sample (extract, olive oil, Tween 80, PEG 400 and nanoemulsion) were analysed using a FT-IR Spectrophotometer (Nicolet Avatar 330; Thermo Electron Co., USA) equipped with a diamond attenuated total reflectance (ATR) accessory. The spectra were noted within a wave number range between 400 cm^{-1} and 4000 cm^{-1} [23].

Spreadability

The spreadability of nanoemulsion was determined by "Drag & Slip" method with slight modification [34]. Two glass slides (each 7.5 cm long) were placed parallel to each other. One slide was set as movable while the other one was in a fixed position. The fixed glass slide was connected to a pulley. Nanoemulsion of 1 g was sandwiched between the slides. A 500 g weight was put on the slide for 5 minutes, followed by a 50 g weight connected to the movable side via a string, passing over the pulley. The time taken for the separation of slides was noted. Spreadability was calculated following the equation below, where S represents the spreadability of the formulation, M is the weight (g), L is the length of the upper slide (cm) and T denotes the time (sec) taken to separate the two glass slides from each other.

$$S = (M \times L)/T.$$

Toxicity assessments

In vitro toxicity study (heavy metals detection)

In vitro toxicity assessment of heavy metals (mercury, lead, arsenic and cadmium) was evaluated following the previously reported method with slight modification [35]. Prepared stock solutions of AAS Grade standards (1000 ppm/1000 mg/L) for cadmium, arsenic lead and mercury were diluted to 0.5 to 5 ppb to attain calibration curves. Wet digestion method was employed for the preparation of sample. Nanoemulsion (2 g) was transferred to 100 mL conical flask followed by adding 10 mL of concentrated nitric acid into the flask. The mixture was left overnight for better digestion and recovery of heavy metals. The subsequent mixture was heated on hot plate at 250ºC for 2 hours with addition of H_2O_2 (2 mL) until the solution became colourless. After cooling, 10 - 15 mL of distilled water was added to the digest. The sample was then shaken and filtered through a Whatman grade 42 filter paper. The volume was adjusted to 25 mL with distilled water. For arsenic determination, 2.5 mL of 20% potassium iodide and 5 mL of concentrated hydrochloric acid were introduced into the sample mixture. The same respective procedure was followed for the preparation of blank solution. The whole experiment was performed in triplicate. Heavy metals were estimated using Atomic Absorption Spectrophotometer (Hitachi Z-8000, Japan) equipped with graphite furnace, using argon supply used for lead and cadmium, hydride formation system for arsenic and cold vapor system for estimation of mercury.

In vivo toxicity study (acute dermal irritation test)

An acute dermal irritation study was conducted on healthy male Albino Wistar rats of weight ranged from 150 - 250 g. The animals were procured from the animal house of the Department of Pharmacology, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Pakistan. The animals were housed in a controlled environment with a temperature of 22 ± 2°C, relative humidity of 50 - 60 ± 0.2%, and a 12-hour light/dark cycle. Standard diets (*ad libitum*) and water were supplied to the rats. The dorsal skin of rats was shaved 24 hours prior to formulation application. Animals were divided into 3 groups (n = 6; in each group). Group I served as the control (untreated), while Group II received a commercially available 2% diclofenac gel. Group III was treated with *C. iyengarii* extract loaded nanoemulsion. Around 200 mg of respective samples were applied to Group II and Group III. After 24 hours of application, the rats' skin was examined for signs of rash, oedema and erythema using a 4-point severity scale (0 = no signs, 1 = slight redness/swelling, 2 = well-defined redness/swelling, 3 = moderate redness/swelling and 4 = scar formation). The Primary irritability index (PII) was calculated based on Draize dermal scores [23]. Data was analysed to compare skin irritation between the experimental groups.

Determination of SPF value

The sun protective factor (SPF) value was determined by UV-Vis spectrophotometer based on previously followed method with slight modification [36]. Around 1 g of nanoemulsion was transferred to 100 volumetric flask and volume was diluted to 100 mL with 96% methanol. A 5 mL volume of the resultant solution was transferred into a 25 mL volumetric flask, and the volume was then adjusted with methanol. A commercially available formulation was diluted to 200 µg/mL, served as positive control. The absorption of both samples was recorded at wavelength range between 290 nm and 320 nm with an interval of 5 nm and using blank (96% methanol). The SPF value was calculated for both test and positive control using the following equation:

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

where, EE(λ) = erythemal effect spectrum; I(λ) = solar intensity spectrum; Abs(λ) = absorbance of sample; CF = correction factor (10).

Tyrosinase inhibition

Tyrosinase inhibition was determined by following the previously described method with slight modification [33]. Nanoemulsion was diluted to 10 mg/mL and 5 mg/mL with DMSO, whereas Kojic acid was diluted to 0.5 mg/mL, served as positive control. Tyrosinase enzyme (150 µL; 333 unit/mL in 50 mM phosphate buffer with pH 7) was added into the 350 µL of sample solution. The mixture was then incubated at 37ºC for 15 minutes. A volume of 550 µL of substrate (L-tyrosine 2 mM in purified water) was introduced into the mixture, followed by incubation at 37ºC for 25 minutes. Absorbance was noted spectrophotometrically at 280 nm. The same procedure was followed for standard (kojic acid). The percent inhibition of tyrosinase activity was calculated according to the formula below:

$$\% \text{ inhibition of tyrosinase} = \frac{[(A-B)-(C-D)] \times 100}{(A-B)},$$

where, A = Absorbance of control with enzyme and substrate; B = Absorbance of control without enzyme; C = Absorbance of formulation with enzyme and substrate; D = Absorbance of formulation without enzyme.

The study aimed to evaluate and compare the effects of *C. iyengarii* extract loaded nanoemulsion on transepidermal water loss (TEWL), skin hydration and skin oiliness in healthy subjects compared with the control site (untreated site) [37].

Inclusion and exclusion criteria

A total of 11 volunteers of 24 to 45 years of age with normal skin were selected after taking their consents. Volunteers with history of known hyper-sensitivity, reaction to any product, presence of any type of dermatitis and/or scratches or wounds on study forearm, volunteers who were not physically fit or taking any topical and systemic treatment were excluded from the study. The selected participants were asked not to use any dermal products for the period of two weeks before and on the study day, except soap and other cleansing products. Forearms of subjects were selected for study, and they were requested not to expose the selected area of forearm directly to the sunlight [31, 32].

Transepidermal water loss, skin hydration and oiliness evaluation

An acclimatization period of 30 minutes was set, before the clinical evaluation. The right and left forearms of each participant serve as study areas for test and control respectively. Baseline was recorded for TEWL by using evaporimeter (Dermalab® Single, Cortex, Denmerk). While skin hydration and oiliness were evaluated through V-care Skin Analyzer. Approximately 50 mg of sample was applied on specified area in circular motion for 10 seconds [31, 32].

Transepidermal water loss was evaluated after the application of formulation, at 30 min, 1, 2, 3 and 4 hours for treated and control sites. Measurements were recorded in triplicate at each time interval. Percentage reduction in TEWL was calculated using base line readings [32, 37].

The pre-conditioning of test sites for skin hydration and oiliness were the same as that described for the measurement of TEWL. Skin hydration and oiliness were examined after 30 min, 1, 2, 3 and 4 hours on the skin surface of both treated and control sites. The measuring time was 30 s for each point. Percentage increase, in skin hydration and oiliness, was calculated using base line readings [31, 37].

Statistical analysis

Statistical analysis was performed by executing SPSS (version 27). For clinical trials, the variables were expressed as mean ± SD. The results of treated sites were compared with control for TEWL, skin hydration and oiliness values for 4 hours. Mann-Whitney *U* test was used to compare the data of treated site with untreated site after 4 hours. Whereas Wilcoxon test was employed for comparing results of treated site with baseline at each time point.

Results and Discussion

Quantitative analysis of flavonoids and phenolics

The quantitative analysis of *C. iyengarii* ethanolic extract revealed higher TFC of 19.89 ± 3.35 mg quercetin equivalents (QE)/g than TPC of 7.61 ± 0.47 mg gallic acid equivalents (GAE)/g of sample. This trend of higher TFC than TPC was also reported in previous studies of *C. fragilis* [38] and *C. tomentosum* [19]. An earlier study reported TFC of *Codium* species (*C. fragile*) 36.78 ± 1.08 µg QE/mg of dried ethanolic sample [38]. Whereas methanolic extract of another *Codium* species (*C. tomentosum*) showed 154.28 ± 0.003 mg of quercetin equivalent (QE)/g of sample [19]. The variable range of *C. fragile* was also reported such as 0.99 ± 0.1 – 17.27 ± 0.06 µg GAE/mg sample by Heffernan *et al.* [39], while Surget *et al.* [40] reported slightly higher TPC of 2.202 ± 0.103 µg GAE/mg sample of ethanolic extract. The methanolic extract of *C. tomentosum* resulted higher TPC of 138.97 ± 0.007 mg of GAE/g of sample [19]. The variability in phenolic and flavonoid contents can be attributed to several factors like environmental conditions, geographical origin, solvent type and extraction methods, as well as species [41, 42].

GC-MS analysis

GC-MS analysis revealed a spectrum of 40 compounds in the *C. iyengarii* ethanolic extract including hydrocarbons, ketones, aldehyde, saturated and unsaturated fatty acids, fatty acid derivatives, terpenes and sterols (Table I).

Table I

Compounds identified in gas chromatography mass spectrometry analysis

Peak#	Retention time	Retention Index	Area	Area %	Compound Name	Molecular weight	Molecular formula
1	21.683	1400	36409614	0.42	Tetradecane	198	$C_{14}H_{30}$
2	22.147	1403	36891490	0.43	(-)-Aristolene	204	$C_{15}H_{24}$
3	22.212	1440	15891108	0.19	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	190	$C_{13}H_{18}O$
4	22.887	1440	24776092	0.29	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	190	$C_{13}H_{18}O$
5	24.413	1600	133003600	1.55	Hexadecane	226	$C_{16}H_{34}$
6	25.890	1701	17528532	0.20	Pentadecanal	226	$C_{15}H_{30}O$
7	25.958	2574	53235704	0.62	Tricosanoic acid, methyl ester	368	$C_{24}H_{48}O_2$
8	26.829	1846	143863925	1.68	Octadecane, 5-methyl	268	$C_{19}H_{40}$
9	27.261	2045	789238923	9.19	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$
10	27.324	1754	94416883	1.10	Hexahydrofarnesyl acetone	268	$C_{18}H_{36}O$
11	27.529	3759	682575861	7.95	Phytyl stearate	562	$C_{38}H_{74}O_2$
12	27.748	1774	855023263	9.96	Neophytadiene	278	$C_{20}H_{38}$
13	27.962	2101	132636453	1.54	Linolenic acid, methyl ester	292	$C_{19}H_{32}O_2$
14	28.016	1886	56116176	0.65	Palmitoleic acid, methyl ester	268	$C_{17}H_{32}O_2$
15	28.243	3567	607968860	7.08	Octadecanoic acid, 3-hydroxy-2-tetradecyl-, methyl ester	510	$C_{33}H_{66}O_3$
16	28.456	1899	58677024	0.68	Isophytol	296	$C_{20}H_{40}O$
17	28.624	1968	162161420	1.89	Palmitic acid	256	$C_{16}H_{32}O_2$
18	29.007	2000	88502425	1.03	Eicosane	282	$C_{20}H_{42}$
19	29.640	2045	104473610	1.22	Phytol	296	$C_{20}H_{40}O$
20	29.834	2101	46975747	0.55	Methyl gamma linolenate	292	$C_{19}H_{32}O_2$
21	29.893	1563	183304277	2.13	Hexa-hydro-farnesol	228	$C_{15}H_{32}O$
22	29.999	2093	99025442	1.15	Linoleic acid, methyl ester	294	$C_{19}H_{34}O_2$
23	30.064	2085	410045586	4.78	Elaidic acid, methyl ester	296	$C_{19}H_{36}O_2$
24	30.165	1886	482068311	5.61	Oleic acid, methyl ester	268	$C_{17}H_{32}O_2$
25	30.295	2077	75761473	0.88	Stearic acid, methyl ester	298	$C_{19}H_{38}O_2$
26	30.449	2175	251411828	2.93	Oleic Acid	282	$C_{18}H_{34}O_2$
27	30.648	2220	39296435	0.46	Stearic acid	283	$C_{18}H_{36}O_2$
28	30.704	2185	32381586	0.38	Oleic acid, ethyl ester	310	$C_{20}H_{38}O_2$
29	30.991	2100	34050072	0.40	Heneicosane	296	$C_{21}H_{44}$
30	31.492	1924	215965916	2.52	Palmitoyl chloride	274	$C_{16}H_{31}ClO$
31	31.589	2308	44117384	0.51	Arachidonic acid, methyl ester	318	$C_{21}H_{34}O_2$
32	31.788	1842	59639525	0.69	Tetradecanoic acid, 3-hydroxy-, methyl ester	258	$C_{15}H_{30}O_3$
33	33.094	2131	174780971	2.04	Oleic acid chloride	300	$C_{18}H_{33}ClO$
34	33.646	2451	211920172	2.47	Behenic alcohol	326	$C_{22}H_{46}O$
35	33.927	2475	89601791	1.04	Behenic acid, methyl ester	354	$C_{23}H_{46}O_2$
36	35.561	2276	61122026	0.71	Arachidic acid, methyl ester	326	$C_{21}H_{42}O_2$
37	36.311	2914	33794738	0.39	Squalene	410	$C_{30}H_{50}$
38	37.433	2390	74198432	0.86	Cholesterilene	368	$C_{27}H_{44}$
39	39.573	2879	1304973626	15.20	Stigmasteryl acetate	454	$C_{31}H_{50}O_2$
40	43.217	2263	568290466	6.62	Chalinasterol	398	$C_{28}H_{46}O$
			8586116767	100.00			

The notified hydrocarbons were tetradecane, hexadecane, octadicane, 5-methyl, heneicosane and eicosane. Notably, two ketones, identified first time in *Codium iyengarii* included 4-(2,4,4-trimethyl-cyclo-hexa-1,5-dienyl)-but-3-en-2-one and 4-(2,6,6-trimethyl-cyclohexa-1,3-dienyl)-but-3-en-2-one). An aldehyde (pentadecanal) was also identified in the extract. The fatty acid profile is comprised of saturated fatty acids (palmitic acid, stearic acid) and unsaturated fatty acid (oleic acid,). Fatty acids ester profile included saturated fatty acid ester (tricosanoid acid, methyl ester; stearic acid, methyl ester; arachidic acid, methyl ester; behenic acid, methyl ester) and unsaturated fatty acids ester (linoleic acid, methyl ester; palmitoleic acid, methyl ester; linolenic acid, methyl ester; arachidonic acid, methyl ester; methyl gamma linolenate, elaidic acid, methyl ester; oleic acid, methyl ester; oleic acid, ethyl ester; arachidonic acid, methyl ester). Hydroxy methyl ester derivative of fatty acid (tetradecanoic acid, 3-hydroxy-, methyl ester), fatty acid alcohol (behinic alcohol) and fatty acid chloride (palmitoyl chloride, oleic acid chloride) were also recognized in the spectrum.

Terpenes like sesquiterpene (-)-aristolene), sesquiterpene alcohol (hexa-hydrofarnesol), diterpene (neophytadiene), triterpene (squalene), diterpene alcohols (3,7,11,15-tetramethyl-1-2-hexadecen-1-ol, phytol, isophytol and its derivative of phytol like phytyl stearate), sesquiterpene ketone (hexa hydrofarnesyl acetone) were noticed. Additionally, sterol (chalinasterol, stigmasteryl acetate and cholesterilene) were also identified.

Codium genus contains diverse fatty acid contents, that are influenced by various factors, such as species, growth stage, nutrient, temperature, season, salinity, location and depth. In this study varied types of fatty acids were also noted, which is in accordance with previous literature [15, 43]. Among these, palmitic acid and oleic acid are most common fatty acids in *Codium species* [15], reported to inhibit melanin synthesis by tyrosinase inhibition, showing skin whitening potential [44]. These fatty acids and their methyl ester identified in *Codium tomentosum,* also reported with antioxidant effect [19]. In addition, poly-unsaturated fatty acids such as linoleic acid, linolenic acid and arachidonic acid and the esters of fatty acids were also found in the *Codium species*, highlighted in the previous review [15], can support skin barrier protection and enhance other biological functions [1]. Linolenic acid methyl ester was reported with anti-cancer, antihistamine, antieczemic, antiproliferative and antifungal activities [45]. Moreover, the identified fatty acids such as palmitic acid, stearic acid, oleic acid, linoleic acid and esters of fatty acids have potential antimicrobial activity [46, 47]. Fatty acids are known to hinder microbial growth by disrupting the integrity of their cell membranes [47]. Behenic alcohol identified in the GC-MS data, was reported as antiviral agent and Docosanol 1% cream has been used for the treatment of herpes labialis [46]. The beneficial effects of fatty acids in topical applications include reducing trans-epidermal water loss, making them potential ingredients in moisturizers. Terpenes derived from seaweed extracts also possess skin care potential showing antibacterial and antifungal activities [1], such as Aristolene exhibited antibacterial effect with antioxidant activity [48]. While Neophytadiene demonstrated powerful antioxidant and anti-inflammatory activities [43]. The squalane, a triterpene is reported as photoprotective, anti-inflammatory and antioxidant agent [49]. Palaniyappan *et al*. [20] reported antifungal effect of 3,7,11,15-tetramethyl1-2-hexadecen-1-ol, a diterpene alcohol. Another diterpene alcohol, phytol, was reported to have antioxidant, anticancer, anti-inflammatory, antimicrobial and antispasmodic activity [45, 50]. Phytyl sterate, also showed cosmetic potential found in green seaweed *Chaetomorpha linum* [51]. Phytosterols have a wide range of pharmacological activities, notably anti-microbial activities, effective in combating various human pathogens such as bacteria, fungi, viruses and protozoa.

Seaweed-derived sterols such as β-sitosterol, campesterol, stigmasterol and epicoprostanol demonstrated significant antibacterial activity against various bacterial strains [52]. Sterols, chalionasterol, colesterilene, stigmasteryl acetate were identified in the study, responsible to regulate membrane fluidity [1]. Previously Nazarudin *et al*. [43] reported antioxidant activity of sterols derived from *Codium* Sp.

Overall, the marine bioactive compounds have potential in combating skin aging by influencing gene expression, supporting cell survival and metabolism, modulating inflammation, enhancing extracellular matrix production and regulating melanogenesis as well as keratinocyte functions such as proliferation, migration and differentiation [1].

The variation in chemical composition of *C. iyengarii* extract is found with literature, due to genetic variation, geographic location and environmental conditions, like salinity, temperature, luminosity and growth habitats [53].

Components of nanoemulsion

In the current study, two commonly used vegetable oils for skin care formulations were evaluated to determine the highest extract solubilizing potential. Subsequently, olive oil loaded with *C. iyengarii* extract was selected to formulate topical nanoemulsion due to its higher extract solubilizing potential (0.2%). In addition, UV-visible spectral analysis showed greater absorbance than coconut oil, signifying the better encapsulation of the extract in olive oil. This could be due to the presence of unsaturated fatty acids such as oleic acid.

Olive oil is commonly used in skincare formulations, and its incorporation into a topical nanoemulsion demonstrated enhanced skincare effects [21, 23, 54]. While selecting the nonionic surfactant and co-surfactant for the nanoemulsion formulation as a preferable choice is beneficial for topical products due to their reduced pH sensitivity and lower toxicity [24, 31]. An earlier study employed the combination of Tween-80 and PEG-400 as surfactant and co-surfactant, leading to the formation of a transparent nanoemulsion [55], which also demonstrated good compatibility with olive oil [54].

Pseudo ternary phase diagram and formulations design

Phase diagrams serve as an important tool to determine the composition of Smix by identifying a maximum nanoemulsion region [26]. The best composition of surfactant with cosurfactant was suggested by pseudo ternary phase diagrams was 1:1 (Figure 1). The coloured areas of phase diagrams present nanoemulsion regions, whereas the colourless area shows emulsion or gel formation.

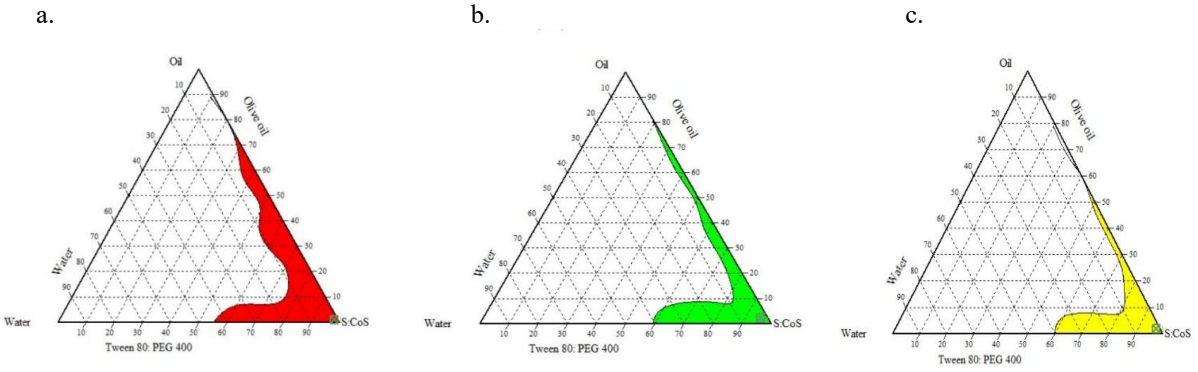

Figure 1.
Phase diagrams of olive oil, water with surfactant (Tween 80) and cosurfactant (PEG 400) at ratio of (a) 1:1, (b) 2:1 and (c) 3:1

Preparation and characterization of nanoemulsion formulations

Based on the selected composition of Smix (1:1), design expert generated 7 formulations. Smix (Tween 80 and PEG 400) is hydrophilic, though both components exhibit amphipathic characteristics, have both hydrophilic and lipophilic regions. This dual nature enables them to interact with oil and water phases, reducing the interfacial tension and facilitating spontaneous nano-emulsion formation [56]. In this study, water was gradually added to oil loaded with extract and Smix blend under vigorous stirring. After the equilibrium state, formulations were visually stable for 24 hours and found to be clear nanoemulsions as shown in Figure 2. A Similar method was reported by various research studies [26, 29, 57]. Table II presented the droplet size of nanoemulsions ranges from 11.34 ± 1.8 to 488 ± 80 nm (below 500 nm) [58]. PDI values varied from 0.28 to 0.6 presented acceptable range for topical application (0.05 to 0.7) [59]. The nano sized droplets provide a greater surface area, long-term stability, enhance the absorption of drugs and improve their bioavailability [21, 31]. The pH of all formulations was found to be in the range of 5.54 ± 0.11 to 5.92 ± 0.01, compatible with the skin pH ranges from 4.5 to 6 [34]. All nanoemulsions, except F2, categorized as O/W nanoemulsion because their conductivity values were higher than 10 µs/cm [22]. Viscosity is an important parameter that influences the flow characteristics and stability of the formulation. In this study, viscosity was measured at 60 rpm, indicating a moderate shear rate that prevents excessive stress, which might disturb the internal structure of nano-emulsion. A previous study measured the viscosity at 50 rpm to obtain consistent results [29]. The viscosity of formulations varied between 173.33 ± 8.82 to 726.67 ± 6.67 mPa.S.

Table II
Composition and characterization of *C. iyengarii* extract nanoemulsions computed by simplex lattice design

Formulations	Oil (%) X1	Smix (%) X2	Water (%) X3	Droplet size (nm)	PDI	Viscosity (mPa.S)	Conductivity (µS/cm)	pH
F1	13.33	73.33	13.33	396 ± 50.34	0.55	480 ± 5.77	13.86 ± 0.17	5.75 ± 0.05
F2	15	75	10	402 ± 63.2	0.56	473.33 ± 8.82	8.86 ± 0.32	5.62 ± 0.07
F3	15	65	20	488 ± 80	0.65	726.67 ± 6.67	20.26 ± 0.53	5.54 ± 0.11
F4	5	75	20	11.34 ± 1.8	0.28	173.33 ± 8.82	20.90 ± 0.30	5.65 ± 0.04
F5	13.33	68.33	18.33	353.4 ± 50	0.63	526.67 ± 6.67	16.98 ± 0.26	5.87 ± 0.07
F6	8.33	73.33	18.33	183.67 ± 28.17	0.46	296.67 ± 6.67	20.87 ± 0.03	5.92 ± 0.01
F7	11.67	71.67	16.67	324.5 ± 41.13	0.57	516.67 ± 3.33	19.56 ± 0.38	5.71 ± 0.01

The literature suggested that the nanoemulsion with droplet size 20 - 200 nm are preferable for topical application [34]. Thus, based on the characterization, F6 (CI-NE) with its optimal droplet size 183.67 ± 28.17 nm and PDI value of 0.46, was selected for further *in vitro*, *in vivo* and clinical evaluation. The small droplets with uniform size distribution contribute to greater surface area, enhanced transport through skin and bioavailability of active compounds for desired cosmeceutical effects [9, 10, 59]. Moreover, olive oil loaded with *C. iyengarii* extract constitutes 8.333% of CI-NE, further aids in the penetration of small droplets to ensure the desired effects [23]. A previous study incorporated curcumin extract with 5% olive oil in nanoemulsion formulation that effectively produced antibacterial effect [60]. Another investigation revealed that nanoemulsion formulated with 6.401% *Moringa oleifera* seed oil along with Tween 80 and PEG 400 exhibited anti-alopecia effect. This formulation had a slightly larger droplet size of 237.06 nm and a poly-dispersity index (PDI) of 0.467 comparable to the current study, displaying effective carrier system for

delivering active ingredients [55]. Recently, algal extract based nanoemulsions containing 1% of *Sargassum cymosum* and *Dictyopteris justii* extracts with droplet size of approximately 130 nm and low polydispersity value (< 0.3) showed significant photoprotective effect [59]. Formulation F4 was not selected, due to the least extract content and the highest Smix concentration among all formulations. Furthermore F4 presented droplet size below 20 nm, susceptible to cleared quickly from skin surface [61] or capable of diffusing into the viable epidermis without skin penetration [10]. Figure 4, illustrating the nanoemulsion formulations and subsequent selection of F6 formulation.

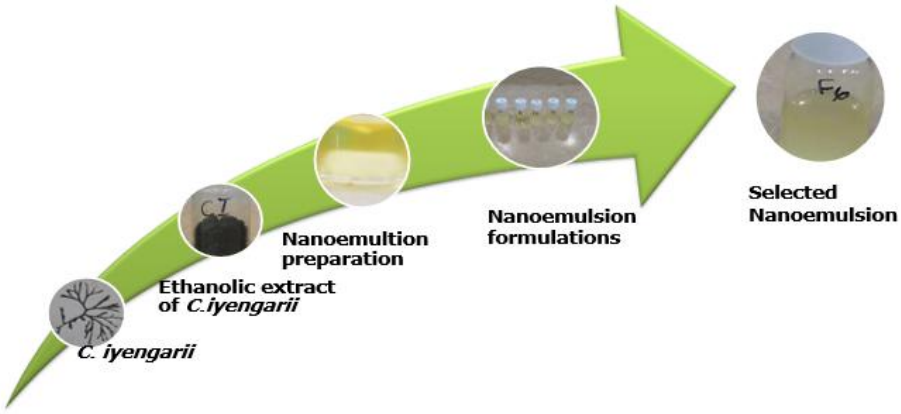

Figure 2.
Graphical presentation of *C. iyengarii* extract nanoemulsion formulations and selection of F6 formulation

Stability study

All the formulations were found to be physically stable for 24 hours. Extended stability study CI-NE formulation over the storage of three months was inspected visually and in terms of its droplet size and PDI. The formulation remained clear, with no significant change in pH (Table III). Moreover, no phase separation was observed all over the storage period. Table IV and Figure 3 show a slight change in the droplet size and PDI. The droplet size remained in the nanoemulsion range of 170.13 ± 24.28 nm to 350.5 ± 26.19 nm and PDI value slightly shift from 0.46 to 0.57. According to John *et al*. [27], both droplet size and PDI tend to increase over time. However, in this study, minimal changes in droplet size and PDI were noted, likely due to the stabilizing effect of olive oil [57].

Table III

Stability studies for *C. iyengarii* extract nanoemulsion at different storage temperatures

Parameters	At fresh state		After 1 month		After 2 months		After 3 months	
	Temperature							
	4 ± 2°C	25 ± 2°C	4 ± 2°C	25 ± 2°C	4 ± 2°C	25 ± 2°C	4 ± 2°C	25 ± 2°C
Colour	Y	Y	Y	Y	Y	Y	Y	Y
Clarity	C	C	C	C	C	C	C	C
Texture	NG	NG	NG	NG	NG	NG	NG	NG
Phase separation	-	-	-	-	-	-	-	-
pH	5.92 ± 0.010	5.92 ± 0.010	6.00 ± 0.019	5.90 ± 0.012	6.00 ± 0.038	6.02 ± 0.029	5.69 ± 0.034	5.52 ± 0.020

Y = Yellow; C = Clear; No phase separation (-); NG = non greasy

Table IV

Droplet size and PDI of *C. iyengarii* extract loaded nanoemulsion at different time intervals

Variables	Time intervals			
	1st day	3rd day	15th day	90th day
Droplet size (nm)	170.13 ± 24.28 - 197.21 ± 32.06	180.30 ± 20.92 - 189.45 ± 25.17	202.16 ± 26.61	247.9 ± 21.50 - 350.5 ± 26.19
PDI	0.46	0.51	0.57	0.55

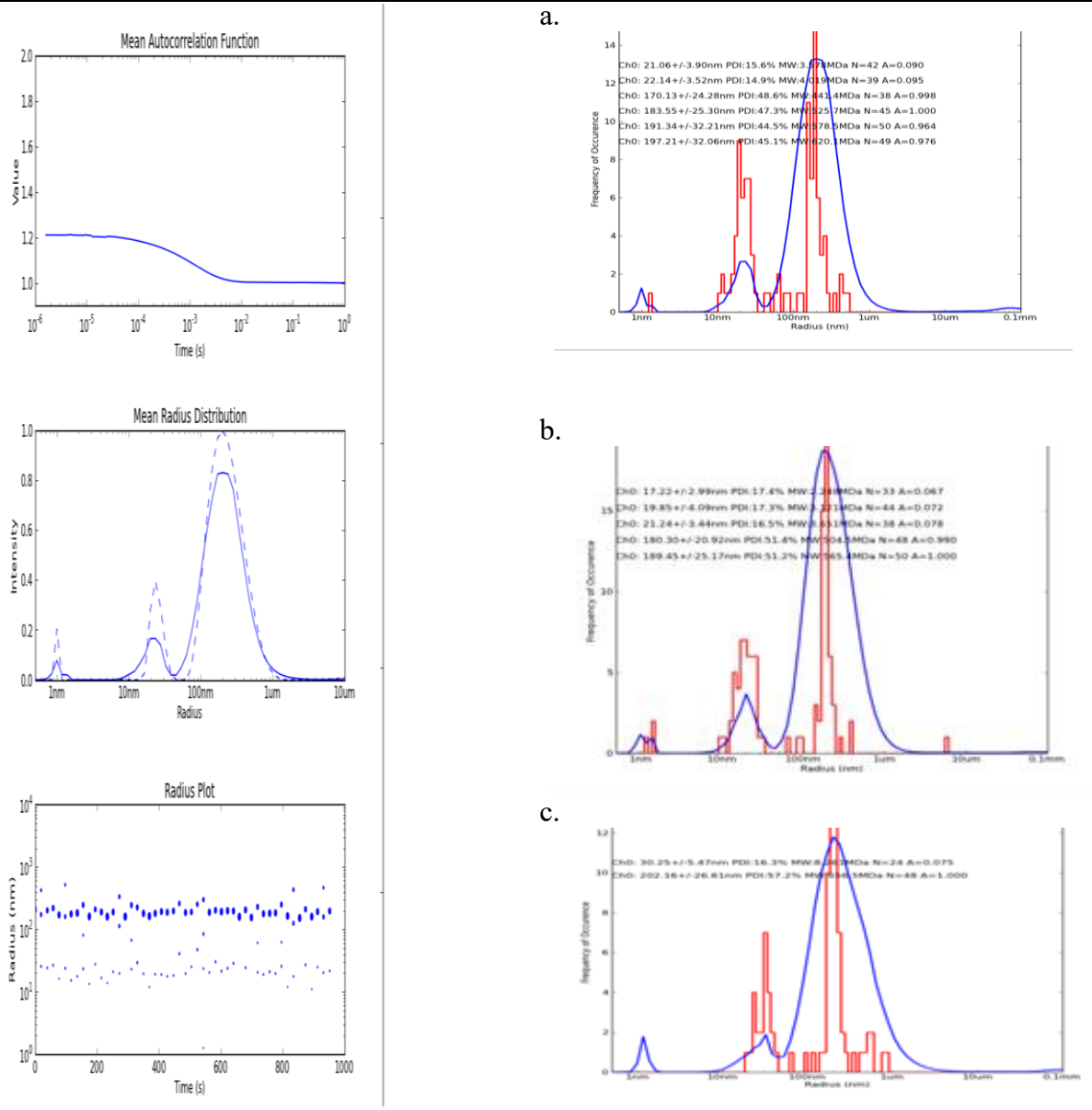

Figure 3.

Measurement of the Droplet size of *C. iyengarii*-based NE. (Left panel) DLS results of NE presenting the experimental parameters *i.e.*, the mean autocorrelation function, monodispersity and corresponding radius, respectively. (Right panel) Comparative corresponding droplet size of NE at various time intervals (from top to bottom panel, respectively) (a) Zero hour; (b) 48 hours; (c) 15 days. All experiments were performed with an auto-piloted run of 50 measurements at every 20 s, with a wait time between of 1 s (at 25 ± 2°C)

Spreadability evaluation

Spreadability of selected formulation (33.48 ± 2.25 g.cm/s) ensured its acceptability for better absorption of extract through skin, comparable with previous study [62]. This value indicates good spreadability, allowing for easy and even application on the skin [26].

FTIR analysis

FT-IR is a powerful analytical tool that extensively used for studying the possible interactions between drug and excipients used in the formulation. FT-IR characterization provides the detailed information of specific functional groups in bioactive compounds [23].

The extract's FT-IR spectrum revealed multiple absorption bands, suggesting the occurrence of various functional groups.

The absorption at 3349.62 cm^{-1} representing O-H stretching vibrations indicate the presences of the polysaccharide, flavonoids, phenolic compounds and terpenes [63-65]. while the peak 2916.16 cm^{-1} and 2851.11 cm^{-1} are associated with C-H stretching of alkane. The C=O stretching appeared at 1635.13 cm^{-1}. Whereas the peak of 1037.7 cm^{-1} is related to the C-O stretching [66]. Additionally, a peak at 1733.82 cm^{-1} reflects the presence of C=O stretching vibration associated with various functional groups, including

esters, carboxylic acids, ketones and aldehydes [26, 63]. The FT-IR findings of the present study were inconsistent with those reported for another *Codium* species, *Codium Codium bernabei*, which exhibited the presence of sulfated polysaccharides, in contrast to our results [67].

Notably, the FT-IR spectrum of the nanoemulsion of *C. iyengarii* extract exhibited similar functional groups with slight shifts in peak positions towards higher wavelengths. The peaks of O-H, C-H, COO, C=O and C-O stretching vibration slightly shifted to higher wavelength for instance, 3404.08 cm^{-1}, 2921.51 cm^{-1}, 1456.66 cm^{-1}, 1646.67 cm^{-1}, 1739.52 cm^{-1} and 1088.75 cm^{-1} respectively, demonstrating no chemical interaction in the extract excipient combination [26]. These findings indicated physisorption, in which the extract molecules stick to the nanoemulsion surface through weak Van der Waals forces, and didn't substantially alter the chemical structural integrity of the extract components [63].

In vitro toxicity assessment

Heavy metals are high molecular weight elements found naturally in the earth's crust including marine life, with a density of at least five times greater than that of water. Seaweeds, along with beneficial bioactive compounds, also accumulate naturally occurring harmful metals, which raises public health concerns. Arsenic, cadmium, lead and mercury are considered highly toxic to the skin, even in traces amounts [15, 68, 69]. According to WHO, the permissible limits of Cd, Pb and Hg are 0.3 µg/g, 10 µg/g and 1 µg/g respectively. Exceeding these concentration limits may lead to skin irritation and allergic dermatitis. Lead, being a neuro-toxin, is associated with cognitive, behavioural and verbal impairments, while mercury is associated with toxicity affecting the nervous, reproductive, immune and respiratory systems. Mercury is a component in thiomersal, a preservative that includes mercury, raises concerns about its use in cosmetic preparations. Cd in high concentration in skin and hair care products potentially absorbed through dermal rout, stored in liver and kidney and it is recognized to be carcinogenic to humans [70].

So, it is important to check the allowable limits of such heavy metals in the seaweed formulations. *C. iyengarii* has potential to accumulate heavy metals due to the presence of carboxylic and sulfonate groups of polysaccharides which play an important role in biosorption to save marine life [17]. Nonetheless, in this study a smaller quantity of extract was utilized in nanoemulsion formulation which quantifies heavy metals concentration under limits. Furthermore, the results showed Hg, Pb and Cd concentrations of 0.017 ppb, 0.237 ppb and 0.05 ppb respectively which fell under the limits according to WHO, with no arsenic amount, suggested its safe use for topical applications [70].

In vivo dermal toxicity evaluation

Skin irritation was evaluated using Rat's skin with Draize dermal irritation scoring scale, varying from 0 (no irritation) to 4 (severe irritation) based on redness (erythema) and swelling (oedema). Figure 4 illustrated that the nanoemulsion formulation didn't show any signs of erythema or oedema following 24 hours of treatment. Thus, it was found to be non-irritating with a PII score of 0.00. While diclofenac gel induced mild/slight redness in one animal with no swelling (PII value; 0.17 ± 0.1). The results are indicative of an excellent safety profile of designed nanoemulsion for dermal applications.

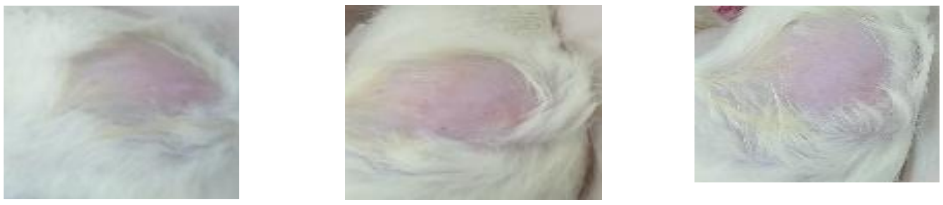

Figure 4.
Acute dermal toxicity assessment on rats' skin (a) Control; (b) Diclofenac gel; (c) CI-NE

Tyrosinase inhibition assessment

Fairer skin tone is one of the focusing trends of both man and women in all over the world particularly in Asian countries which has resulted profound increase demand of skin whitening products. Tyrosinase enzyme involved in synthesis of melanin, thereby inhibiting this enzyme various hyperpigmentary conditions like melasma can be managed [71].

In the current study, Kojic acid exhibited the highest percentage inhibition of tyrosinase at 0.5 mg/mL, being potent skin whitening agent, while formulated nanoemulsion presented dose dependent increase in tyrosinase inhibition of 49.2% and 52% at 5 mg/mL and 10 mg/mL respectively.

Presence of phytochemicals like flavonoids and phenolics previously showed antityrosinase activity [33, 72, 73]. Literature also suggested effect of polysaccharides against tyrosinase inhibition [74]. Fatty acids like oleic acid, linolenic acid and linoleic acids also demonstrated to reduce tyrosinase activity [44]. Additionally, olive oil as an oil phase itself incorporating its antityrosinase activity which could be due to the presence of 2-hydroxytyrosol, a natural substance in the olive oil, effectively inhibits mushroom

tyrosinase. Thus, these results also provide a better option to use algal extract in olive oil to further enhance anti-melanogenesis quality by nanoemulsion formulation [33].

Sun protective factor evaluation

The Sun Protection Factor value is the standard value for assessing a sunscreen's ability to protect skin from harmful UV rays [75]. Seaweeds enriched with bio-active compounds with potential sun protective effect for instance, phenol hydroquinone, flavonoids and tri-terpenoids and carotenoids expected to have photo-protective activity [65]. Our result showed a close value of sun protective factor (2.23) at relatively low extract concentration in nanoemulsion with similar emulsion concentration containing seaweed *Euheuma cottonii* extract [75], showing its sun protective effect. The positive control (labelled as SPF 15) used in this study, showed a lower SPF value of 10.42 than label claims. However, this value, falls within the range of 8 to 15, signifying effective sunscreen performance [76].

Transepidermal water loss, skin hydration and oiliness evaluation

Skin moisture content is an essential parameter for maintaining a normal skin physiology, preserving a healthy and intact skin barrier system. Thus, it is important for the management of various skin pathological conditions and in the cosmetic domain as well [37]. A significant (P = 0.0001; Mann-Whitney U test) reduction in transepidermal water loss (TEWL) was observed in the CI-NE-treated area after 4 hours, showing a 29.43% decrease compared to the control. Whereas TEWL values decreased significantly (P = 0.0001; Wilcoxon test) from the baseline at 0.5, 1, 2, 3 and 4 hours, recorded as -60.08%, -53.88%, -40.38%, 31.69% and -37.47% respectively. Figure 5 showed a comparatively higher reduction in TEWL than control.

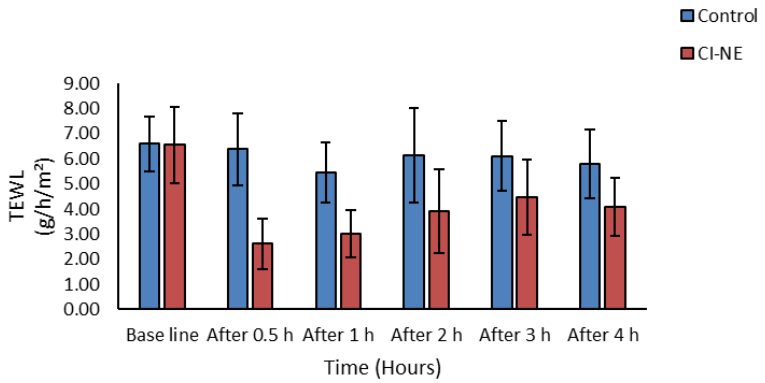

Figure 5.
Evolution of TEWL
Values are expressed as mean (SD); CI-NE *vs*. Control at 4 hours: P = 0.0001; Mann-Whitney U test

Moreover, significantly (P = 0.0001; Mann-Whitney U test) increased skin hydration (22.41%) in CI-NE treated site was observed after 4 hours in contrast to control. While significantly (P = 0.0001; Wilcoxon test) elevated skin hydration was noted at each time interval compared with base line value for instance 29.74%, 30.22%, 29.68% and 29.47% at 0.5, 1, 2, 3 and 4 hours, respectively (Figure 6). This study, consistent with previous research, focused on short-term evaluation (minutes to hours) of biophysical skin parameters [31, 32, 34].

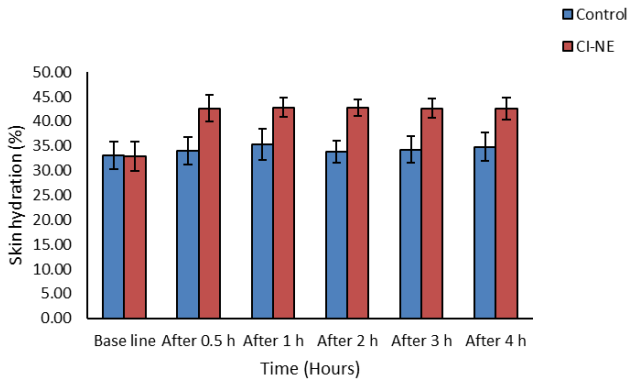

Figure 6.
Evaluation of skin hydration in percentage
Values are expressed as mean (SD); CI-NE *vs*. Control at 4 hours: P = 0.0001; Mann–Whitney U test

The current study revealed that CI-NE has good moisturizing and skin protective cosmeceutical property which is attributed to bioactive compounds in the *C. iyengarii* extract enhanced with olive oil and nano carrier system. It has been reported that seaweeds derived bioactive components, such as flavonoids, phenolics and polysaccharides have hyaluronidase inhibitory effects [77, 78]. HA is one of the principal constituents of skin, involved in tissue repair and water retention process in the skin. Hyaluronidase degrades HA in the extracellular matrix, causing reduced skin elasticity, wrinkle formation and overall skin aging [77, 79].

Free fatty acids and cholesterol represent the major bioactive lipids found in *Codium* species [15], also contribute as moisturizers by restoring deficient lipids in skin barrier dysfunctions such as eczema and psoriasis, thus participating in hydrating skin by mimicking the skin's own natural moisturizing systems [80].

Codium species are reported to have hyaluronidase inhibition activity such as *C. fragile*, *C. tenuifolium*, *Codium subtubulosum* Okamura [79]. The percentage of skin oil content significantly (P = 0.001; Mann-Whitney *U* test) increased, compared to control after 4 hours and it was 18.21% higher than control. The percentage of skin oil content in the treated site in comparison with baseline at each time was found to be significantly (P = 0.0001; Wilcoxon test) higher which was 31.31%, 31.58%, 30.26%, 29.62%, 27% at 0.5, 1, 2, 3 and 4 hours respectively (Figure 7).

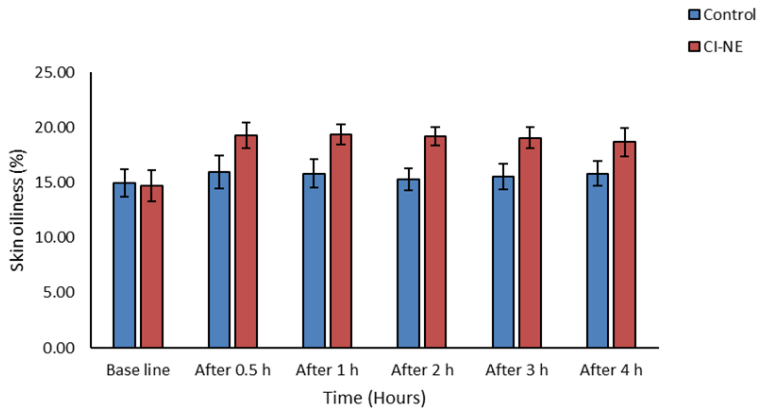

Figure 7.
Evaluation of skin oiliness in percentage
Values are expressed as mean (SD); CINE *vs*. Control at 4 hours: P = 0.001; Mann–Whitney U test

The increase in percentage of skin oiliness was also observed in previous study conducted up to 4 hours [31], ensuring extract loaded olive oil absorbed into skin for effective results. Olive oil is reported to have occlusive and emollient effects, causing reduction in transepidermal, demonstrated skin hydrating effects. Whereas the fatty acids in the extract also contributing to occlusive and emollient effect, preventing water loss, in addition to other skin care benefits such as anti-allergic and anti-inflammatory activities [3, 44]. Incorporating the olive oil loaded with *C. iyengarii* extract in nanoemulsion offered promising skin hydrating effect [81].

Conclusions

The formulation designed was found to be a promising and ecofriendly approach of nano cosmeceutical. *C. iyengarii* extract-loaded nanoemulsion showed excellent stability with optimal droplet size and narrow distribution. Higher Smix concentration produces more homogenous system, providing greater surface area to stabilize the oil-water interface and form smaller droplets. Furthermore, incorporation of olive oil ensured effective penetration for topical delivery. The developed *C. iyengarii* loaded nanoemulsion demonstrated several skin beneficial effects, including a reduction in trans-epidermal water loss, increased skin hydration and oiliness, along with notable sun-protective and anti-melanogenic activities. Although low concentration of extract, the presence of wide spectrum of bioactive compounds such as phenolic compounds, flavonoids, fatty acids, terpenes and sterols are associated with these effects. The current study offers foundational insights that align with established methods. However, further research is necessary to evaluate the formulation optimization parameters and clinical trials with extended timeframes to achieve a more comprehensive understanding of the sustained effects.

Conflict of interest

The authors declare no conflict of interest.

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