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Original Article

CHARACTERIZATION, EVALUATION OF CYTOTOXIC ACTIVITY ASSAY AND OPTIMIZATION OF NANOEMULSION DELIVERY SYSTEM FORMULAS OF TOMATO LYCOPENE (*SOLANUM LYCOPERSICUM.* L)

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ABSTRACT

Objective: This study aimed to evaluate the cytotoxic effects of tomato lycopene, quantify its bioactive fraction, and formulate tomato lycopene into nanoemulsions.

Methods: Lycopene was fractionated using chloroform and purified using methanol. Tomato lycopene was identified using liquid chromatography-mass spectrometry and ultraviolet-visible spectrophotometry, with the content determined using thin-layer chromatography-densitometry. Cytotoxicity was assessed in T47d, DU145, and HeLa cells using the MTT assay. The composition of the oil phase (Virgin Coconut Oil), surfactant (Tween 80), cosurfactant (PEG 400), and the aqueous phase of the nanoemulsion base was determined using a pseudo ternary phase diagram. Tomato lycopene was added to the oil phase and mixed with other components by spontaneous titration. The nanoemulsions were characterized by determining droplet size, zeta potential, Poly Dispersity Index (PDI), transmittance, pH, density, and morphology using transmission electron microscopy.

Results: Needle-shaped crystals were obtained, with a retention factor of 17.32 min, m/z 535.4316 (calculated for $C_{40}H_{56}$), and maximum wavelengths of 457, 484, and 517 nm. The bioactive fraction (chloroform) comprised 866.68 mcg/ml lycopene. Increasing lycopene concentration was inversely proportional to T47d, DU145, and HeLa cell viability after 96 h of incubation. Six of the 54 base formulations produced transparent solutions (droplet size: 14.10–500.50 nm). Incorporating 0.1% tomato lycopene into the base generated physically stable nanoemulsions with spherical droplets exhibiting the following features: particle size, 13.37–82.52 nm; zeta potential, (-12.4)–(-5.66) mV; PDI, 0.0813–0.4247; transmittance, 96.18–99.14%; pH, 5.49–6.40; relative density, 1.049–1.067.

Conclusion: Tomato lycopene showed weak citotoxic on T47d, DU145, and HeLa cell line. Six nanoemulsions with good physicochemical properties were obtained as transparent yellow solutions. The optimized lycopene nanoemulsion formulation (TLN6) was confirmed at the compisition of 0,1% lycopene, 7% VCO, 50,4% Tween 80 and 12,6% PEG 400.

Keywords: Tomato, Lycopene, Nanoemulsion, Cell viability, Physicochemical properties

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INTRODUCTION

Lycopene, a bright red pigment, is a phytochemical compound found in tomatoes and other red fruits. This compound has been extensively studied and reportedly exhibits a strong antioxidant capacity [1]. Elevated blood lycopene levels were found to be inversely associated with cardiovascular diseases, diabetes, and cancers [2]. In a meta-analysis assessing 72 epidemiological studies, 57 found that consumption of lycopene-rich food or supplementation was associated with a reduced risk of cancer, and 35 studies reported a statistically significant correlation. In addition to the antioxidant activity, the biological effects of lycopene can be attributed to other mechanisms, such as regulation of antioxidant response elements, modulation of growth factor signaling pathways, and inhibition of the cancer life cycle [3].

The chemical structure of lycopene comprises an unsaturated chain with a hydrocarbon straight chain consisting of thirteen double bonds; eleven of these double bonds are conjugated, whereas the remaining two are unconjugated. Pure lycopene ($C_{40}H_{56}$) has a molecular weight of 536.88, needle-like crystals, and is brown in color with a melting range of 172–175 °C. In terms of solubility, pure lycopene is insoluble in methanol and ethanol, soluble in chloroform and benzene, very soluble in hexane and ether. Lycopene is strongly hydrophobic and undergoes degradation through isomerization and oxidation reactions due to light, oxygen, high temperatures, drying techniques, peeling processes, storage, and acids [4].

Following oral consumption, only 7–10% of lycopene is absorbed, with 50% undergoing fecal and urinary elimination [1]. Poor lycopene absorption results in low blood levels of lycopene, resulting in poor bioavailability. This is a common phenomenon observed with active substances from natural ingredients, which show substantially effects *in vitro* but exert poor effects when tested *in vivo*, or vice versa. Low bioavailability could be attributed to factors such as decomposition in the stomach or extensive intestinal metabolism [5]. Therefore, it is necessary to establish a delivery system to protect lycopene and obtain a high blood levels, thereby eliciting improved efficacy.

Nanoemulsions are kinetically stable dispersed systems comprising two immiscible phases–an oil phase and an aqueous phase–stabilized by surfactant and cosurfactant. Hydrophobic drugs are trapped in nanosized vesicles (20–200 nm) that afford protection and allow delivery across the cell membrane. Li *et al.* [6] studied an oil-in-water lycopene nanoemulsion using Octenyl Succinic Anhydride (OSA)-modified starch as an emulsifier and medium-chain triglycerides as a carrier oil, which was fabricated using a high-speed homogenizer (18,000 rpm for 4 min). Lycopene molecules were loaded into the hydrophobic core of nanoemulsion droplets at low concentrations and encapsulated into an interfacial layer at higher concentrations. The author concluded that the location of lycopene in the vesicles primarily impacted its stability, suggesting that the oil-in-water nanosuspension was a potential lycopene delivery system for functional foods.

In the present study, we aimed to standardize the chloroform fraction of tomato lycopene, evaluate its cytotoxicity, and formulate

a Tomato Lycopene Nanoemulsion (TLN) using Virgin Coconut Oil (VCO; oil phase), Tween 80 (surfactant), and Poly Ethylene Glycol 400 (PEG 400; cosurfactant) using the spontaneous aqueous titration method. Subsequently, we evaluated the physicochemical properties of TLN.

MATERIALS AND METHODS

Materials

Ripe tomatoes were obtained from a tomato farm in Alahan Panjang, Solok, West Sumatera, Tomatoes were confirmed as *Solanum lycopersicum* L. by the Herbarium of ANDA, Andalas University (Document no. 033/K-ID/ANDA/II/2016). Chloroform (Merck), ethyl acetate (Merck), hexane (Merck), dichloromethane (Merck), methanol (Merck), lycopene (Sigma-Aldrich, USA), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reagent were used. All solvents used were of analytical grade. The T47d and HeLa cell lines were obtained from the Biomedical Laboratory of the Faculty of Medicine, Universitas Andalas, Padang, Indonesia; the DU145 cell line was obtained from the Laboratory of Cell Culture and Cytogenetics, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. In addition, we used Camag Thin-Layer Chromatography (TLC) canner at 4 wavelenght of 560 nm.

Fractionation of tomato lycopene

Briefly, tomatoes were washed thoroughly, then mashed using a blender, and steamed for 5 min at 90–100°C After cooling, the steamed tomatoes were squeezed to separate the juice from the pulp. The pulp was then macerated with chloroform (1:4) in a dark-colored bottle for 3×24 h at room temperature with occasional stirring. The solvent was separated by filtration, and fresh solvent was added to the remaining solid, followed by maceration until a clear solution was obtained. Tomato juice fractionated using chloroform at a ratio of 1:2 until a clear fraction was obtained. The two fractions were combined, and the solvent was evaporated using a rotary evaporator until a thick extract was obtained. Methanol (an anti-solvent) was slowly added to the thick extract until a precipitate was formed. The mixture was left undisturbed for several hours and then filtered. The precipitate was purified by slowly adding methanol to obtain tomato lycopene; the presence of tomato lycopene was confirmed using UV-visible spectrophotometry and TLC (Shimadzu UV-1700®).

Characterization of tomato lycopene

TLC profile

The mobile phase comprised hexane: toluene (19:1). The fraction was dissolved in chloroform at a concentration of 300 µg/ml, then spotted at the start line using a glass capillary on a TLC plate (Silica gel 60 PF_{254}, Merck), and eluted to the boundary line. A solution of pure lycopene in chloroform was used as the reference. The plate was air-dried for 1-2 min until the appearance of new orange zones along the sample path.

Liquid chromatography-mass spectrometry (LC-MS/MS) profile

High-resolution mass spectrometry experiments were performed using an Ultra-Performance Liquid Chromatography (UPLC) unit (LC: ACQUITY UPLC® H-Class System, Waters, USA) and a mass spectrometer (Xevo G2-S QTof, Waters, USA). This involved using a C18 column (1.8 µm 2.1x100 mm, ACQUITY UPLC® HSS, Waters, USA) at temperatures of 50 °C (column) and 25 °C (room). The LC analysis used a mobile phase that was water+5 mmol ammonium formic (A) and acetonitrile+0.05% formic acid (B), with a flow rate of 0.2 ml/min (step gradient) running for 23 min (see slide moving phase) and an injection volume of 5 µl (initially filtered through a 0.2 µm syringe filter). The Mass Spectrometry (MS) analysis was conducted using Electrospray Ionization (ESI) in positive mode with a mass range of 50–1200 m/z and source and desolvation temperatures of 100 and 350 °C, respectively. Additionally, cone and desolvation gas flow rates of 0 l/h and 793 l/h were also used correspondingly, while the collision energy varied between 4 and 60 eV. Masslynx software version 4.1 was used for data acquisition and analysis as well as instrument control.

UV-visible spectrophotometry profile

The fraction was dissolved in chloroform at a concentration of 300 µg/ml and scanned using a UV-visible spectrophotometer (Shimadzu UV-1700®) at a wavelength of 400–600 nm.

Thin-layer chromatography (TLC)-densitometry

Densitometry was assessed as reported previously [7, 8]. The TLC plate comprised a 20×10 cm silica gel 60 F254, with a mobile phase comprising hexane: toluene (19:1). Chromatography was conducted in a saturated chamber for approximately 45 min. Next, the plate was dried and measured using a TLC-Camag Scanner 4 at a wavelength of 560 nm, and the results were analyzed using the win CATS application version 1.4.7, which generated a linear calibration plot based on the standard regression equation. The mobile phase was hexane/toluene in a ratio of 19:1. To validate the chloroform fraction of tomato, a linearity analysis was initially performed by eluting five concentrations of a standard solution (1000, 500, 250, 125, and 62.5 µg/ml) on to silica gel plates, followed by area measurement.

Evaluation of cytotoxic activity

T47d, DU145, or HeLa cell lines were subcultured and maintained at 37 °C under 5% CO_2 until 80% confluency was achieved. The cells were then treated with 100 µl tomato lycopene at concentrations 1000, 500, 250, and 125 µg/ml, respectively, in quadruplicate and incubated for 96 h. Untreated cells were used as a negative control; cells incubated with DMSO only (0.5%, v/v) were employed as the vehicle control. After the treatment, 25 µl of MTT solution (0.05 µg/ml) was added to each well, and the plates were incubated at 37 °C for 4–6 h. DMSO was added following the formation of formazan crystals, and the absorbance was measured at 595 nm using an ELISA Microplate Reader (xMark Microplate Reader). Data were analyzed using Microsoft Excel to determine the percentage of viable cells. Morphological changes in the cell lines were observed under an inverted microscope (Zeiss, Germany).

Optimization of nanoemulsion base using a pseudo ternary phase diagram

The proportions of the oil phase (VCO), surfactant (S)-cosurfactant (CoS) mixture (Tween 80–PEG 400), and aqueous phase were determined using a pseudo ternary phase diagram. The diagram was created using CHEMIX School version 3.60. The nanoemulsion bases were prepared by using the water titration method, which was performed at a stirring rate of 1400 rpm using a magnetic stirrer (IKA, Germany) for 60 min at 70 °C. The formation of a clear and transparent liquid characterized the formation of a nanoemulsion system. A 4:1 proportion of the Tween 80-PEG 400 (S: CoS) mixture resulted in a stable nanoemulsion. Furthermore, nanoemulsion formulations of tomato lycopene were prepared using Tween 80-PEG 400 at ratios of 4:1, which were mixed with VCO in ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 (table 1). Fifty-four formulas were generated based on the pseudo-ternary phase diagram. Tomato Lycopene Nanoemulsions (TLN1-TLN6) were prepared by incorporating 0.1% lycopene into the oily phase of the bases. Transparent nanoemulsion were evaluated. The TLN formulations comprised three components: first, tomato lycopene in VCO as the oil phase; second, Tween 80 as the main surfactant strengthened with PEG 400 as cosurfactant; third, water as the aqueous phase.

Evaluation of TLN

Relative density measurement

The relative density of the nanoemulsion was determined using a pycnometer at 25 °C. Relative density was calculated using the following equation:

$$\rho = (W_2 - W_0)$$

$$(W_1 - W_0)$$

Where: W_2 = the weight of the pycnometer filled with nanoemulsion

W_1 = the weight of the pycnometer filled with water

W_0 = the weight of an empty pycnometer

Viscosity

Measurements were carried out using Brookfield viscometer (Brookfield Engineering Laboratories), 50 g of the preparation is placed in a cup, and the viscosity measurement begins when the spindle needle moves and stabilizes at a speed of 30 rpm.

Physical stability

Physical stability was assessed using freeze-thaw cycling tests. The nanoemulsion was maintained at-5 °C for 24 h and subsequently at 25 °C for another 24 h; the test was performed in triplicate. The physical stability of the nanoemulsions was observed.

Measurement of pH

The pH of prepared nanoemulsions was measured using a pH meter (Hanna Instruments, Germany), previously calibrated with a standard buffer solution at pH 4 and 7. The electrode was immersed in the nanoemulsion, and the pH was measured. Measurements were performed once weekly during a six-week storage at room temperature.

Droplet size analysis

The droplet size distribution, polydispersity index (PDI), and zeta potential were measured using a particle size analyzer (Horiba SZ 100). Samples were diluted with distilled water and placed in electrophoretic cells.

Microscopic analysis using transmission electron microscopy (TEM)

The morphology of the TLN droplets was analyzed using a transmission electron microscope (JEOL JEM 1010, Japan) at 80,0 KV and 30,000× magnification. A 10 µl sample was dropped on a grid, jet-dyed with uranyl acetate, and dried. The observations were performed at room temperature.

Measurement of transmittance

The transmittance of the nanoemulsions was measured using a UV-visible spectrophotometer (SHIMADZU UV-1601, Japan) at a wavelength of 650 nm. A transmittance approaching 100% indicated the transparency of the liquid samples.

RESULTS AND DISCUSSION

Lycopene was extracted from heated tomato paste by simple liquid-liquid extraction using chloroform-water. A thick extract with a yield of 0.27% was purified by recrystallization using methanol as the antisolvent [9]. Needle-shaped crystals were obtained (fig. 1).

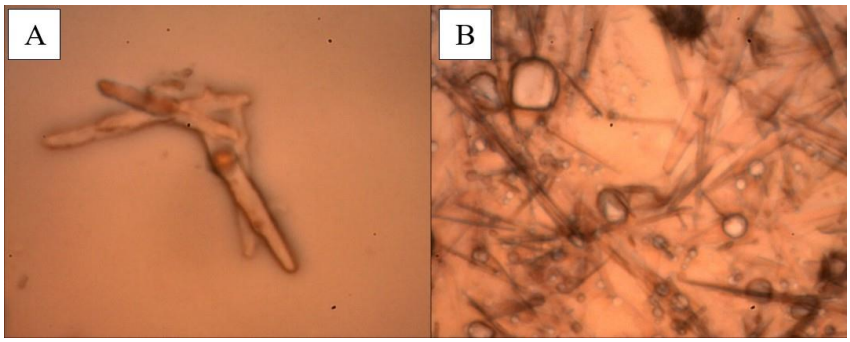

Fig. 1: Needle-shaped crystals of tomato lycopene captured by using microscope with a magnification of 400 (A) and 100 (B)

The TLC profile showed spots with a retention factor of 0.67, comparable to that of pure lycopene (fig. 2).

Fig. 2: The thin-layer chromatography (TLC) profile of the isolated tomato lycopene (K) in comparison with pure lycopene (Sigma) (P), mobile phase: Hexane-toluene (19:1)

The UV spectrum revealed maximum wavelengths at 457, 484, and 517 nm (fig. 3), similar to the UV-visible spectra of pure lycopene at 456, 484, and 517 nm under the same conditions. According to LC-MS/MS data, the isolated compound had a retention time of 17.32 min, and its molecular formula was m/z 535.4136 (calculated for $C_{40}H_{56}$, negative mode) (fig. 4A and 4B). Herein, we explored the potential of tomatoes as a source of lycopene and attempted to extend the shelf life of this raw material through cooking to obtain lycopene with improved bioavailability. Lycopene occurs naturally as an all-trans form, and its chain contains seven double bonds, which can be isomerized to mono-cis or poly-cis upon exposure to heat, light, oxygen, acids, catalysts, and metal ions [4]. In addition, a study using Caco-2 cells has revealed that the absorption of cis-lycopene was substantially greater than that of the all-trans isomer [10]. Thus, cis isomers have higher bioavailability than all-trans isomers.

According to a survey conducted at a tomato farm center in West Sumatra, Indonesia, approximately 9.734 and 3.617 tons of tomatoes were wasted and left unharvested by farmers in the Alahan Panjang and Tanah Datar districts in 2014, respectively [11]. Overproduction causes the price of tomatoes to drop; therefore, farmers allow tomatoes to rot on the stems or discard tomatoes that have already been harvested. No systematic efforts have been made to address this issue. Processing tomatoes during overproduction, for example, the production of tomato paste as a source of lycopene can reduce losses to farmers owing to decreasing prices. According to a recent study, lycopene from wasted tomatoes in two districts was utilized as poultry feed [11]. Therefore, a simple method is needed to process tomatoes to preserve the fruits and avoid losses to farmers.

Bioavailability lycopene increased after heating and cooking. Another study found that lycopene in tomato paste is up to four times more bioavailable than that in raw tomatoes. Upon heating, an

isomerization reaction converts the all-trans form in raw tomatoes to the cis form in tomato paste. Thus, processing tomatoes not only preserves the fruits but also increases the concentration of bioavailable lycopene [12]. In the present study, lycopene was isolated from tomato paste under conditions to yield cis-lycopene. Pure lycopene was obtained as needle-shaped crystals that were unstable at room temperature; this lack of stability complicates the determination of melting point, Fourier-Transform Infrared Spectroscopy (FTIR) analysis, and other physicochemical methods. Therefore, lycopene was identified using TLC, UV-visible spectrophotometry, and LC-MS/MS. The TLC profile and UV spectrum of tomato lycopene were similar to those of standard lycopene determined under identical conditions. Herein, the UV-visible spectra of lycopene displayed three peaks at 457, 484, and 517 nm, markedly similar to the maximum wavelengths reported by Chemat-Djenni (2010) at 457, 483, and 516 nm. The LC-MS/MS chromatograms showed a major peak with a fragmentation pattern, confirming that the fraction was lycopene [13].

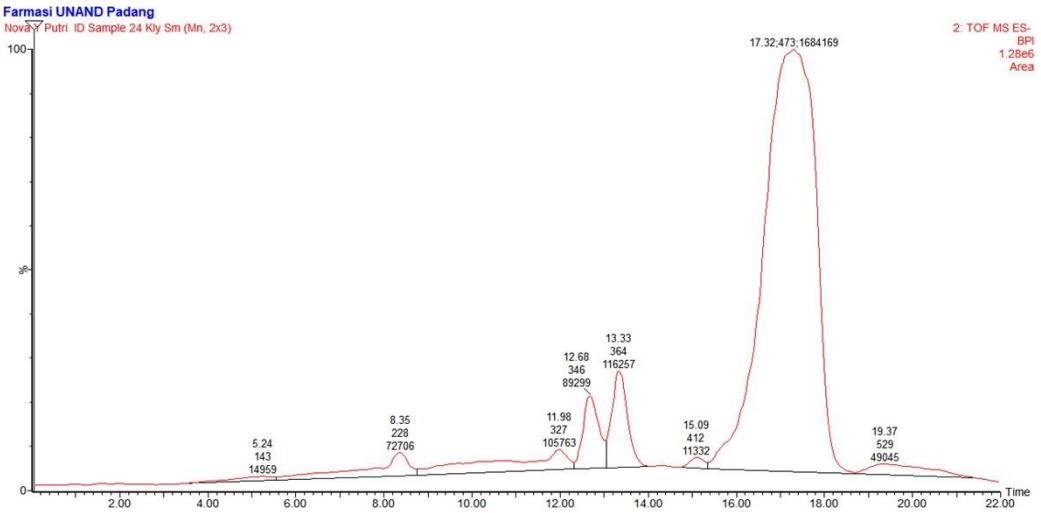

Fig. 3A: The liquid chromatography-mass spectrometry (LC-MS) profile of tomato lycopene

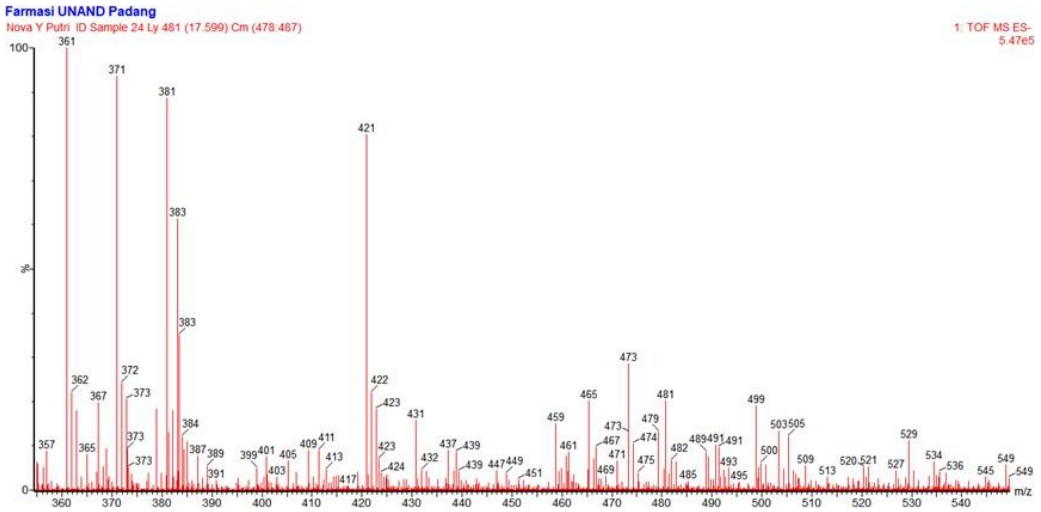

Fig. 3B: The mass spectrometry (MS) spectrum of tomato lycopene

Quantitative analysis is essential for providing information on the composition and concentration of secondary metabolites in natural materials responsible for specific pharmacological activities. Although spectrophotometry and high-performance liquid chromatography are the preferred analytical methods for lycopene quantification [14, 15]. TLC-densitometry is deemed a suitable alternative owing to its accuracy, lack of complexity, and ease of performance. The lycopene content was measured at a wavelength of 560 nm. The calculation resulted in the equation y = 14.462-406.74, with a correlation coefficient of 0.9987, as shown in fig. 5. In the current study, the limit of detection (LOD) and limit of quantification (LOQ) values were 0.0098 µg/spot and 0.0298 µg/spot, respectively. The accuracy of the study was analyzed based on the coefficient of variation (CV), which ranged from 1.49-3.66%. In previous reports, CV values ranging from 0.12-0.91% have been reported, falling within the required %CV value (i.e.,<5%). An accuracy test was used to determine the closeness of the percentage obtained from the analysis to the actual lycopene content[16]. The resulting value was 93.53% (w/b), which was within the required range of 80–120%. The lycopene content in the chloroform fraction was 866.68 µg/ml (8.6668%).

A previous report identified a substantial interaction between lycopene, as a Bcl2 inhibitor, and DNMT1 proteins *in silico* [17]. In the current study, we evaluated the cytotoxic activity of tomato lycopene at concentrations of 125–1000 µg/ml; this concentration is based on studies that tested the cytotoxicity of natural compounds with the MTT Assay. In this study, the concentration has not been used as a basis for calculating the dose of lycopene that will be used by humans, because it requires further research. Cytotoxic activity of tomato lycopene at concentrations of 125–1000 µg/ml revealing different effects on the viability of T47d (breast cancer), DU145

(prostate cancer), and HeLa (cervical cancer) cells. After 96 h of incubation, cells treated with 1000 µg/ml lycopene showed reduced viability when compared with controls, reaching 95.63, 71.42, and 85.42% reduction in the HeLa, DU145, and T-47d cell lines, respectively. The effect of tomato lycopene on cell viability was determined by subjecting the three cell lines to the MTT assay. The T47d cell line was used to observe activity in breast cancer, DU145 in prostate cancer, and HeLa in cervical cancer. The IC_{50} of lycopene was 316.00, 430.64, and 251.19 µg/ml against the HeLa, DU145, and T47d cell lines, respectively, thereby indicating weak cytotoxic activity. This finding is inconsistent with *in vitro*, *in vivo*, and epidemiological studies regarding the bioactivity of tomato lycopene in breast, prostate, and cervical cancer [18, 19]. Considerable accumulated evidence has suggested the robust cytotoxic activity of lycopene as an anticancer agent, owing to its antioxidant capacity and apoptotic mechanisms[3]. Wang (2012) questioned whether the observed bioactivity in various cellular functions and signaling pathways could be due to the direct action of lycopene itself or its metabolites[5]. Similar to other nutritional compounds, lycopene is extensively metabolized in the gut. Reportedly, lycopene metabolites, such as lycopenals, lycopenols, and lycopenoic acids, can exert anticancer effects in humans with a high dietary intake of tomatoes and tomato products [1, 20]. This inconsistency could be attributed to the knowledge gap and the evolving field of carotenoid research [20]. In the present study, the weak bioactivity of intact lycopene in the *in vitro* assays may be due to the lack of metabolites with markedly greater activity. Testing the activity of a compound *in vitro* does not always give the same results as if done *in vivo*.

The result of the cytotoxic test againts the fraction lycopene from tomato showed weak cytotoxic activity against T47d, DU145, and HeLa cell lines [21]. From previous studies, lycopene can penetrate the blood brain barrier and has good permeability in the intestine [17]. For this reason, a nanoemulsion formula was developed as a drug delivery system for lycopene. This system can improve unfavorable physicochemical properties and can deliver lycopene directly to the target. Nanoemulsion formulations are also able to protect lycopene against oxidation and enhance transport across biological membranes after transdermal, oral, or parenteral administration, which is beneficial for lycopene due to its lipophilic nature and chemical instability to light, heat, and oxygen. In this study, a cytotoxic test was not carried out on the nanoemulsion formula, but a stability test was carried out on the nanoemulsion formula.

The nanoemulsion formulations comprised three components: tomato lycopene in VCO as the oil phase, a mixture of Tween 80-PEG 400, and an aqueous phase. The proportions of the three components were plotted on a pseudo-ternary phase diagram to screen the composition of the formulation that produced the nanosized droplets (table 1). For an S-CoS (Smix) ratio of 4:1, only six formulas with (oil+Smix) to water ratios of 30:70, 40:60, 50:50, 60:40, 70:30, respectively, produced transparent nanoemulsion bases, as indicated by the red dot in fig. 4.

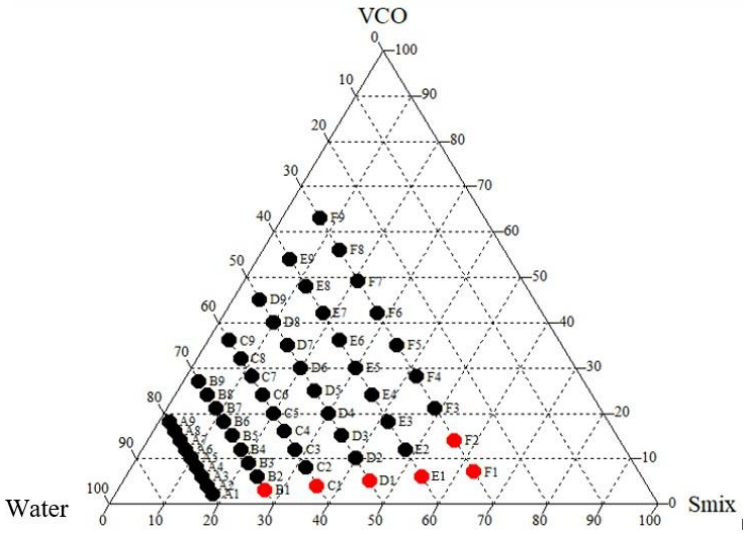

Fig. 4: A pseudo ternary phase diagram of nanoemulsion bases at various weight ratios of VCO (virgin coconut oil): Tween 80-PEG 400: Water

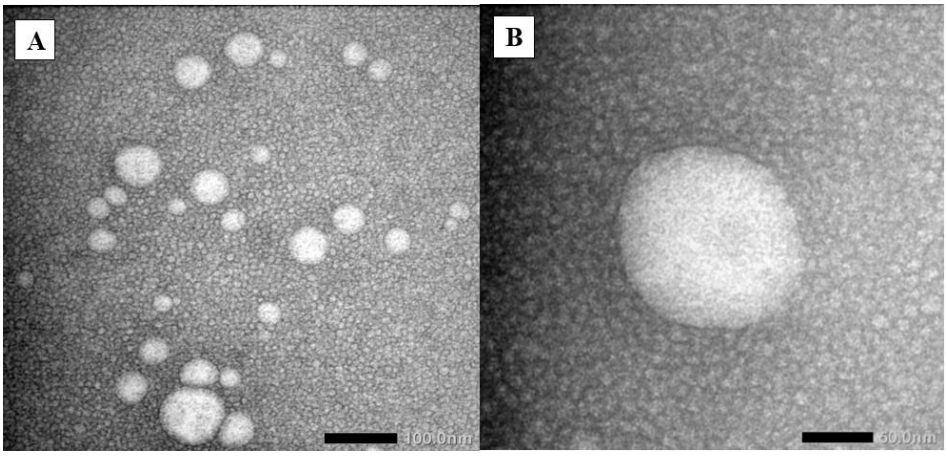

Fig. 5: Transmission electron microscopy image of TLN (Tomato lycopene nanoemulsions) 6 (scale bar A = 100.0 nm; B = 60.0 nm)

The nanoemulsion base formulas (B1, C1, D1, E1, F1, and F2) showed nanosized droplets (14.10–500.5 nm), with a PDI ranging between 0.096–0.594, and a zeta potential ranging between (-14.7)–(-7.43) mV (table 2). The basic nanoemulsion formula, which has a particle size below 100 nm was chosen to be made into tomato lycopene nanoemulsion because it is the optimal formula, Tomato Lycopene Nanoemulsions (TLN1-TLN6) were prepared by incorporating 0.1% lycopene into the oily phase of the bases (B1, C1, D1, E1, F1, and F2). The physicochemical properties of the TLNs are summarized in table 3. No changes in color, odor, or homogeneity were observed during the 8-week storage at room temperature. Physical stability was confirmed by performing three cycles of the freeze-thaw test. The droplet size ranged between 13.37–82.52 nm; the PDI ranged between 0.0813–0.4247; and the zeta potential ranged between (-14.90)-(-5.66) mV. The pH value was in the range of 5.49–6.40. The transmittance varied in the order of TLN2<TLN3<TLN4<TLN1<TLN5<TLN6. The viscosity ranged between 174–1220 cP, indicating a viscous liquid-to-semi-solid consistency. Morphological examination of TLN6 using TEM revealed unilamellar vesicles characterized by the formation of spherical globules (fig. 5).

The compositions of the three components were plotted on a pseudo-ternary phase diagram to screen the composition of an appropriate TLN formulation. The six nanoemulsion base formulas (B1, C1, D1, E1, F1, and F2) contained (VCO+S-CoS) ranging between 30–70% and an aqueous phase composition ranging between 30–70%, with a VCO: S-CoS ratio of 1: 9 (table 1). This means that nine parts of S-CoS are needed for one part of the oil, and the high concentration of S-CoS at the interface solubilized the globules in the continuous phase, resulting in a transparent, stable, and nanosized emulsion [22, 23]. The incorporation of lycopene into the optimum base formulation resulted in spherical vesicles surrounded by a thin membrane layer, indicating the presence of small unilamellar globules. According to the physicochemical properties, TLN exhibited a substantially smaller globule size than the optimum nanoemulsion base and met the nanoemulsion droplet size criteria (20–200 nm) [6]. The globules had a narrow size distribution, as indicated by the PDI. PDI is a measure of the broadness of the particle size distribution; a value between 0.10–0.20 is considered a relatively narrow distribution, while>0.5 is a very broad distribution. The zeta potential value ranged between (±5)–(±15) mV, indicating incipient instability to aggregation [24]. Moreover, the data revealed an increase in the relative density with an increase in the amount of oil and S-CoS. The viscosity increased with increasing Tween 80 concentration. The pH was in the weakly acidic range, i. e., the physiological pH range, and can be favorable for topical or transdermal product formulations [25]. The transmittance of the TLN varied, with high transmittance values indicating the clarity of the solution. The transmittance of all TLN formulations was close to 100%, indicating transparent solutions, which remained stable after storage. An increase in the transmittance value indicated an increase in the clarity and homogeneity of the formulation [26]. Importantly, nanoemulsion formulations have been found to improve the stability of unstable drugs and the permeability of hydrophilic or lipophilic active pharmaceutical ingredients, thereby indicating their therapeutic potenatial. TLN formulations are capable of protecting lycopene against oxidation and improving transport across biological membranes after transdermal, oral, or parenteral administration, which is beneficial for lycopene owing to its lipophilic nature and chemical instability to light, heat, and oxygen, thereby limiting its use as a therapeutic agent. TLN can reach sufficient concentrations in the blood, thereby increasing its efficacy in treating diseases caused by oxidative stress, such as cardiovascular diseases and various types of cancer. Nanoemulsion can increase the bioavailibilty of lipophilic drugs, it also increases absorption by changing intestinal permeability. Hinger *et al.* compared the nanoemulsions formulation with a liposom m THPC (foslip) and found that nanoemulsions have superior biocompatibility compared to the liposomal [27].

Table 1: Composition of nanoemulsion bases at S-CoS ratio of 4:1

Formula	A	B	C	D	E	F
VCO+(S+CoS) (%)	20	30	40	50	60	70
	1:9	1:9	1:9	1:9	1:9	1:9
	2:8	2:8	2:8	2:8	2:8	2:8
	3:7	3:7	3:7	3:7	3:7	3:7
	4:6	4:6	4:6	4:6	4:6	4:6
Ratio of VCO: (S+CoS)	5:5	5:5	5:5	5:5	5:5	5:5
	6:4	6:4	6:4	6:4	6:4	6:4
	7:3	7:3	7:3	7:3	7:3	7:3
	8:2	8:2	8:2	8:2	8:2	8:2
	9:1	9:1	9:1	9:1	9:1	9:1
Water (%)	80	70	60	50	40	30
Total	100	100	100	100	100	100

S-CoS, surfactants-cosurfactants; VCO, virgin coconut oil

Table 2: Droplet size, polydispersity index, and zeta potential of transparent nanoemulsion bases

No	Nanoemulsion bases	Particle size (nm)	Polydipersity index	Zeta potensial (mV)
1	B1	163.9	0.594	-14.7
2	C1	500.5	0.462	-8.36
3	D1	18.45	0.397	-13.9
4	E1	15.73	0.275	-10.1
5	F1	14.10	0.151	-7.43
6	F2	19.04	0.096	-7.47

Table 3: Physicochemical properties of tomato lycopene nanoemulsions (TLN)

Physicochemical properties	TLN1	TLN2	TLN3	TLN4	TLN5	TLN6
pH	5,60	5.49	6.40	5.79	5.76	6.09
Relative Density (g/ml)	1.059	1.049	1.050	1.057	1.067	1.067
Viscosity (cP)	174	272	960	1220	1177	1113
Physical Stability	Stable	Stable	Stable	Stable	Stable	Stable
Particle Size (nm)	14.09	82.52	15.49	14.32	13.91	13.37
Polydispersity Index	0.4247	0.2826	0.2440	0.1630	0.1869	0.0813
Zeta Potential (mV)	-12.4	-8.03	-5.66	-8.86	-14.90	-11.70
Transmittance (%)	98.18	96.18	97.92	98.00	98.70	99.14

CONCLUSION

Lycopene as needle crystals was successfully purified from the chloroform fraction of tomato paste. LC-MS/MS confirmed the identity of lycopene, with a retention time of 17.32 min and a molecular formula m/z 535.4136 (calculated for $C_{40}H_{56}$), negative mode), with the UV-visible spectrum at maximum wavelengths of 457, 484, and 517 nm. The lycopene content in the bioactive fraction (chloroform) of tomato fruit was 8.6668%. The fraction showed weak cytotoxic activity against T47d, DU145, and HeLa cell lines. Formulation optimization resulted in six TLN formulations containing 0.1% lycopene in a base comprising a 1:9 ratio of VCO: (Tween80-PEG400). The formulated lycopene nanoemulsions exhibited a droplet size of 13.37–82.52 nm, zeta potential of (-14.90)–(-5.66) mV, and PDI of 0.0813–0.4247.

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AUTHORS CONTRIBUTIONS

Conceptualization: Farida Rahim, Henny Lucida, Friardi. Data curation: Farida Rahim, Henny Lucida, Friardi, Valdy Filando Sardi. Formal analisis: Farida Rahim, Henny Lucida, Friardi, Andani Eka Putra. Writing-original draft: Farida Rahim, Henny Lucida, Friardi. Writing-review and editing: Friardi.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Arballo J, Amengual J, Erdman JW. Lycopene: a critical review of digestion, absorption, metabolism, and excretion. Antioxidants (Basel). 2021;10(3):342. doi: 10.3390/antiox10030342, PMID 33668703.
2. Chauhan K, Sharma S, Agarwal N, Chauhan B. Lycopene of tomato fame: its role in health and disease. Int J Pharm Sci Rev Res. 2011;10(1):99-115.
3. Trejo Solis C, Pedraza Chaverri J, Torres Ramos M, Jimenez Farfan D, Cruz Salgado A, Serrano Garcia N. Multiple molecular and cellular mechanisms of action of lycopene in cancer inhibition. Evid Based Complement Alternat Med. 2013;2013:705121. doi: 10.1155/2013/705121, PMID 23970935.
4. Kong KW, Khoo HE, Prasad KN, Ismail A, Tan CP, Rajab NF. Revealing the power of the natural red pigment lycopene. Molecules. 2010;15(2):959-87. doi: 10.3390/molecules15020959, PMID 20335956.
5. Wang XD. Lycopene metabolism and its biological significance. Am J Clin Nutr. 2012;96(5):1214S-22S. doi: 10.3945/ajcn.111.032359, PMID 23053559.
6. Li D, Li L, Xiao N, Li M, Xie X. Physical properties of oil-in-water nanoemulsions stabilized by OSA-modified starch for the encapsulation of lycopene. Colloids and Surfaces a: Physicochemical and Engineering Aspects. 2018;552(483):59-66. doi: 10.1016/j.colsurfa.2018.04.055.
7. Ismed F, Putra DP, Arifa N. Phytochemical profiling and antibacterial activities of extracts from five species of sumatran lichen genus stereocaulon. Jordan J Pharm Sci. 2021;14(2):189-202.
8. Sardi VF, Astika A, Jalius IM, Ismed F. Quantification of mangiferin from the bioactive fraction of mango leaves (Mangifera indica L.) and evaluation of wound-healing potential. Jordan J Pharm Sci. 2023;16(3):595-606. doi: 10.35516/jjps.v16i3.652.
9. Myong Kyun R, Min Hee J, Jin Nam M, Woi Sook M, Sun Mee P, Jae Suk C. Un metodo simple para aislar licopeno, a partir de lycopersicon esculentum. Bot Sci. 2013;91(2):187-92. doi: 10.17129/botsci.413.
10. Boileau AC, Merchen NR, Wasson K, Atkinson CA, Erdman JW. Cis-lycopene is more bioavailable than trans-lycopene *in vitro* and *in vivo* in lymph-cannulated ferrets. J Nutr. 1999;129(6):1176-81. doi: 10.1093/jn/129.6.1176, PMID 10356083.
11. Handayani UF, Mahata ME, Rizal Y, Suliansyah I, Wizna W. Potensi dan peningkatan kualitas nutrisi buah tomat (Lycopersicon esculentum) afkir sebagai Pakan ternak unggas. JUPET. 2018;15(1):22. doi: 10.24014/jupet.v15i1.4279.
12. Perdomo F, Cabrera Franquiz F, Cabrera J, Serra Majem L. Influence of cooking procedure on the bioavailability of lycopene in tomatoes. Nutr Hosp. 2012;27(5):1542-6. doi: 10.3305/nh.2012.27.5.5908, PMID 23478703.
13. Chemat Djenni Z, Ferhat MA, Tomao V, Chemat F. Carotenoid extraction from tomato using a green solvent resulting from orange processing waste. J Essent Oil Bear Plants. 2010;13(2):139-47. doi: 10.1080/0972060X.2010.10643803.
14. Davis AR, Fish WW, Perkins Veazie P. A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products. Postharvest Biol Technol. 2003;28(3):425-30. doi: 10.1016/S0925-5214(02)00203-X.
15. Cefali LC, Cazedey EC, Souza Moreira TM, Correa MA, Salgado HR, Isaac VL. Antioxidant activity and validation of quantification method for lycopene extracted from tomato. J AOAC Int. 2015;98(5):1340-5. doi: 10.5740/jaoacint.14-151, PMID 26525253.
16. Ferenczi Fodor K, Renger B, Vegh Z. The frustrated reviewer-recurrent failures in manuscripts describing validation of quantitative TLC/HPTLC procedures for analysis of pharmaceuticals. J Planar Chromatogr Mod TLC. 2010;23(3):173-9. doi: 10.1556/JPC.23.2010.3.1.
17. Rahim F, Putra PP, Ismed F, Putra AE, Lucida H, Molecular Dynamics. Docking and prediction of absorption, distribution, metabolism and excretion of lycopene as protein inhibitor of Bcl2 and DNMT1. Trop J Nat Prod Res. 2023;7(7):3439-44.
18. Kerna AN. A global health preventive medicine overture: lycopene as an anticancer and carcinopreventive agent in the deterrence of cervical cancer liking lycopene. SM Prev Med Public Heal. 2018;2(1):1-5.
19. Teodoro AJ, Oliveira FL, Martins NB, Maia G de A, Martucci RB, Borojevic R. Effect of lycopene on cell viability and cell cycle progression in human cancer cell lines. Cancer Cell Int. 2012;12(1):36. doi: 10.1186/1475-2867-12-36, PMID 22866768.
20. Lindshield BL, Canene Adams K, Erdman JW. Lycopenoids: are lycopene metabolites bioactive? Arch Biochem Biophys. 2007;458(2):136-40. doi: 10.1016/j.abb.2006.09.012, PMID 17067545.
21. Weerapreeyakul N, Nonpunya A, Barusrux S, Thitimetharoch T, Sripanidkulchai B. Evaluation of the anticancer potential of six herbs against a hepatoma cell line. Chin Med. 2012;7(1):15. doi: 10.1186/1749-8546-7-15, PMID 22682026.
22. Kumar A, Kushwaha V, Sharma PK. Pharmaceutical microemulsion: formulation, characterization and drug deliveries across skin. Int J Drug Dev Res. 2014;6(1):1-21.
23. Nirmalayanti NL. Skrining berbagai jenis Surfaktan dan Kosurfaktan. Ilmu Multidispilin. 2021;1(3):158-66.
24. Lu GW, Gao P. Emulsions and microemulsions for topical and transdermal drug delivery. Handbook of non-invasive drug delivery systems; 2010. p. 59-94.
25. Lukic M, Pantelic I, Savic SD. Towards optimal ph of the skin and topical formulations: from the current state of the art to tailored products. Cosmetics. 2021;8(3). doi: 10.3390/cosmetics8030069.
26. Bali V, Ali M, Ali J. Study of surfactant combinations and development of a novel nanoemulsion for minimizing variations in bioavailability of ezetimibe. Colloids Surf B Biointerfaces. 2010;76(2):410-20. doi: 10.1016/j.colsurfb.2009.11.021, PMID 20042320.
27. Hinger D, Grafe S, Navarro F, Spingler B, Pandiarajan D, Walt H. Lipid nanoemulsions and liposomes improve photodynamic treatment efficacy and tolerance in CAL-33 tumor-bearing nude mice. J Nanobiotechnology. 2016;14(1):71. doi: 10.1186/s12951-016-0223-8, PMID 27716314.