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Original Article

FORMULATION OF MEMORY SUPPORT TARGETED NANOSTRUCTURED LIPID CARRIERS (NLCS) LOADED WITH KELULUT HONEY EXTRACT PRODUCED WEST KALIMANTAN

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ABSTRACT

Objective: The purposes of this study were to find active compounds of the secondary metabolites, screen and determine the comparison of solid lipid, liquid lipid, and surfactant to produce the optimal NLCs formulation, analyze characteristic optimal NLCs loaded honey extract, and analyze the effectiveness of the optimal NLCs formulation as memory support *in vitro*. The Design-Expert software used for optimal NLCs kelulut honey extract formulation.

Methods: The research method begins with the extraction process using ethanol solvent's maceration method. The formulation of NLCs begins with screening honey extracts for various solid lipids, liquid lipids, and surfactants. Next, the formulation design uses a D-optimal mixture design to obtain 14 run variations of glyceryl monostearate: tocopherol oil: tween 20 and evaluation using transmittance response, pH, and entrapment efficiency. The data on the response is entered into the software to obtain the optimal NLCs honey extract formula. The optimal NLCs honey extract were evaluated for transmittance, pH, entrapment efficiency, particle size, transmission electron microscope (TEM), Fourier transform Infrared Spectroscopy (FTIR), and *in vitro* activity as memory support.

Results: Based on the evaluation, the water content of honey extract was 5.74 %±0.144; metals present in honey are Pb (0.003 mg/kg) and Cd (0.175 mg/kg). Phenolic and Total Flavonoids are 25.91 mg GAE/g±0.15 extract and 182.36 mg GAE/g extract±0.28. The optimal NLCs obtained combined glyceryl monostearate: tocopherol oil: tween 20 with a 0.5: 5.54: 23.96 composition ratio. The optimal NLCs kelulut honey extract formulation for transmittance value was 94.58%±1.54, pH was 6.59±0.15, and Entrapment efficiency was 99.89 %±0.09. Determination for particle size was 327 nm±0.57, and TEM and FTIR provided details on their structure. Evaluation for memory support *in vitro*, IC_{50} NLCs optimal formula 61.99±0.34; honey without extract 72.59±0.79; honey extract 38.55±0.24; and NLCs of base optimal formula without extract 829.81±0.93. The real-time stability shows optimal NLCs honey extract stable in real-time stability and freeze-thaw.

Conclusion: NLCs from honey extract can be formulated from optimal NLCs using Design-Expert software. NLCs from honey extract has physical characteristics according to requirements and is stable. *In vitro* antioxidant studies revealed that the optimal formulation NLCs loaded honey extract had higher activity memory support with IC_{50} 61.99±0.34.

Keywords: NLCs, Lipid, Honey extract, Memory support, D-Optimal mixture design

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INTRODUCTION

Online game addiction is called internet addictive disorder (IGD) [1], and it is considered an international disease that causes response inhibition, memory deficit, and brain damage [2, 3]. Antioxidant compounds can prevent and overcome memory deficits due to free radicals, control memory disorders, decrease lipid hydroperoxides, increase antioxidant enzyme activity in the brain, and improve cognition [4]. Natural products such as honey continue to provide benefits.

The nectar of flowers by honeybees (Apis mellifera; Family: Apidae) [5]. Can made natural product called honey. Since 5500 y ago, honey has been used by humans [6]. Honey is claimed to have many health benefits, including antioxidant, anti-inflammatory, antibacterial, antidiabetic, respiratory, gastrointestinal, cardiovascular, and nervous system protection effects [6-12]. Honey contains vitamins, minerals, amino acids, organic acids, and secondary metabolites: flavonoids, phenolic acids, proteins, amino acids, ascorbic acid, hydroxymethylfurfuraldehyde (HMF), and enzymes [13]. The composition of honey is influenced by seasonal variations and the geographic location from where the nectar is collected. The composition of honey consists of about 82% water, carbohydrates, proteins, phytochemicals, antioxidants, and minerals that determine its potential and medical properties. The sugar content in honey consists of fructose (38.2%), glucose (31.2%), disaccharides and several other tri-saccharides and higher saccharides (9%) and sucrose (0.7–1%) [5, 14, 15]. The phenolic content of certain types of honey is not always known, and this causes difficulty in determining whether there is an effect associated with honey consumption or its content, so further experiments on honey are needed in different areas [16]. Several types of honey are endemically developed in West Kalimantan. Drugs acting on the brain require a targeted delivery system capable of crossing the physical barrier to the central nervous system (CNS), namely the blood-brain barrier (BBB) [17]. Nanotechnology has been widely developed, and one of them is its application in carrier systems to provide protection and as a transfer of hydrophobic and hydrophilic bioactive compounds so that their stability and bioavailability can be increased [18]. Nanostructured Lipid Carriers (NLC) use a lipid-based carrier system with solid and liquid lipid matrices stabilized with surfactants. NLC was developed to facilitate the dispersion of hydrophobic bioactive compounds in hydrophilic systems [19, 20]. The NLC system has high encapsulation capability, controlled release, is thermodynamically stable, and can increase the bioavailability of bioactive compounds [19, 21]. The NLC system is widely applied in the pharmaceutical field because of its ability to deliver drugs to the target. It can also control drug release, with the nanoparticle size causing bioactive components to more accurately reach target cells or receptors in the body [22]. NLC has several advantages, namely high drug loading capacity due to its imperfect crystal structure and its ability to facilitate drug penetration through the stratum corneum due to its small size, its ability to hydrate the skin, increased drug solubility in lipids, high encapsulation ability, controlled release, thermodynamically stable and able to increase the bioavailability of bioactive compounds [19, 21, 23, 24]. From research, NLCs formulation potential approach for sustained release of drug which may reduce systemic side effects, increase skin retention time

and duration of action, increase skin retention time, decreases systemic absorption of the corticosteroid, thereby avoiding side effects [25]. Caffeine formulated in NLC preparations can increase hair growth higher than preparations on the market [26].

The purpose of this study is to find drugs that are targeted to the brain and derived from local honey from West Kalimantan. Honey targeted to improve memory must contain active compounds of secondary metabolites, design and formulation, evaluation of NLCs madu extract, and *in vitro* memory test using antioxidants. The novelty of this study is the honey produced by local beekeepers in West Kalimantan, which has never been studied for its active compound content, design formulation NLCs, stability test, and *in vitro* antioxidant activity.

MATERIALS AND METHODS

Materials and excipient

The materials used in this study is a digital analysis balance (Ohaus PA214, USA), glassware (pyrex), a vortex mixer (Thermolyne), UV-Vis Spectrophotometer (Shimadzu tipe 2450), a one cm-sized quartz cuvette, homogenizer (IKA RW20), hotplate with magnetic stirer (DLab), glassware (pyrex), stopwatch, vortex mixer (Thermolyne), ultrasonicator (J. P. Selecta), Particle Size Analyzer (Beckman coulter), FTIR (Thermo Scientific), TEM (a magnetic stirrer (Stuart CB162), pH meter (HANNA), filter paper, a micro pipette Socorex®(0,5–10; 5–50; 50-200, 200–1000 µl), an eppendorf tube, a rotary evaporator (Heldolph tipe Hei-VAP), an oven (Memmert), a waterbath (Memmert tipe WNB14), an aluminum foil, a thermometer. The excipients used in this study are Kelulut honey was selected for the study from Paloh (Sambas district), Kalimantan Barat, Indonesia. Ethanol 70% (Esa Multi), Ethanol 96% (Merck), N-heksan (Esa Multi), ethyl acetate (Merck), chloroform (Merck), methanol (Merck), aquadest (Dwicentra), quercetin (Sigma aldrich), Gallic acid (Sigma aldrich), DPPH (Smartlab), reagen Folin Ciocalteu, Na_2CO_3 (Merck), $AlCl_3$(Merck), CH_3COOH (Merck), HCl (Merck), serbuk Mg, $FeCl_3$, glyseril monostearat (Evonik), cetyl alcohol, cetyl palmitate, VCO, tocopherol oil, castor oil, soya oil, olive oil, Tween 20, Tween 80, PEG 400, Cremophore EL, propylenglikol, nipagin.

Honey organoleptic test

Honey is tested for taste, aroma, texture

Honey moisture test

The moisture content test on the powder was carried out using the moisture balance method. This method is carried out by weighing 1 gram of the preparation and then inserting it into a measuring device. Observe until the % water content is indicated on the tool. A good water content is less than 10% [27].

Preparation of extract honey

Honey was extracted by maceration using 70% ethanol; it was for 24 h while being repeatedly shaken and macerated. The filtrate was then evaporated further on the hot plate. The remaining solvent was removed by placing the remaining residue in the dryer for±24 h [28].

Quantitative test for determination of total phenolic content (TPC)

Amount of 1 g of honey sample was extracted with 5 ml of 40% methanol/acid water (v/v, pH = 2, HCl). Then, the sample was stirred for 15 min with a magnetic stirrer. From the extract, 0.2 ml was mixed with 2 ml of Folin-Ciocalteu reagent 1:10 and 1.8 ml of Na_2CO_3 7.5% (w/v). Samples were kept in the dark for 20 min, and absorbance was measured at 750 nm using a UV spectrometer [29]. Gallic acid as a standard solution ranging from 0–400 mg/l were used to obtain a calibration curve [30].

Quantitative determination of total flavonoids (TFC)

A total of 5 ml of honey solution (0.1 g/ml) was mixed with 5 ml of 2% aluminum chloride (AlCl3). Flavonoid-aluminum complexes were formed after 10 min of incubation at 25 °C. Complex formation was measured at 430 nm using a UV-Vis spectrophotometer. Quercetin (0–100 mg/l) was used as a standard chemical to prepare the calibration curve [31, 32].

Solid lipid and liquid lipid screening

Lipid screening was performed on glyceryl monostearate, cetyl palmitate, cetyl alcohol, a combination of cetyl palmitate and cetyl alcohol (1:1), and cetyl palmitate and cetyl alcohol (1:4), tween 80, tween 20, PEG 400, dan cremphore oil. One gram of each lipid was melted to a temperature of 5 °C above the melting point, 25 mg of condensed extract was added, and the mixture was stirred. The selected lipids are lipids that can dissolve the highest extract without any precipitate left behind, and when the lipid solidifies, it can become a single phase. The selected lipids are used in the next stage [33].

Orientation of solid lipid, liquid lipid, and surfactant composition formulas with D-optimal design

Optimization of the NLCSs formula was carried out using the D-Optimal Design method. Furthermore, 14 runs were obtained on the mixed composition for the optimized three components: GMS, Tocopherol oil, and Tween 20. The lipid phase was prepared by mixing selected solid and liquid lipids and melting them until they reached a temperature of 5 °C above their melting point. Meanwhile, the water phase is made by mixing distilled water and surfactant [34, 35]. The lipid phase (GMS and tocopherol oil) is melted at 65 C or 5 °C above the melting point. The water phase (Tween 20, propylene glycol, nipagin, distilled water) was mixed homogeneously. Mix the liquid and water phases on a magnetic stirrer for and homogenizer, then sonicate. Leave at room temperature for 1 h before measurement.

Evaluation of 14 runs of solid lipid, liquid lipid and surfactant composition with D-optimal design

Transmittance evaluation

The prospective preconcentrate formula is 100.0 µl plus distilled water to a final volume of 5 ml. Homogenization of the mixture was carried out with the help of a vortex for 30 seconds. The absorption of the solution that was obtained was measured at a wavelength of 650 nm to know the value of clarity [36]. The more evident it is, or the absorbance is closer to the absorbance of distilled water, it indicates that the emulsion droplets have reached nanometer size.

pH evaluation

NLCs pH measurements are carried out by dipping the electrode on the pH meter into the NLCs honey extract, and the pH results will appear on the screen after a few moments. Before use, the pH meter is calibrated with a buffer solution of pH 7 and 4. The electrode used is rinsed with distilled water before and after measurement. NLCSs of 100 µl were added up to 5 ml with distilled water then homogenized by inverting it. Readings on the pH meter are taken after 5 min to ensure the numbers are stable and do not the number move anymore [37].

Determination of NLCs honey extract entrapment efficiency

NLCs were weighed as much as 1 g dissolved in ethanol. Then centrifuged at 6000 rpm for 15 min to separate the lipid and water phases. The supernatant was dissolved in ethanol and then read using a UV-VIS spectrophotometer at 425 nm and 757 nm (wavelengths for quercetin and gallic acid).

Particle size analysis

Measurements using the Particle Size Analyzer (PSA) tool determine the size and distribution of nanoparticles. A total of 1 ml of NLCSs was mixed with aquadest, add 5 ml, then homogenized by tossing for 1 min. After that, 3 ml was taken and put into a cuvette for analysis. The particle size data is the average particle size, particle size distribution, and deviation standard [17, 35].

Determination transmission electron microscope

The morphology of the NLC was observed by TEM using one drop of sample spread on a 200-mesh copper grid coated with a carbon membrane; TEM photos were taken using several magnifications to obtain a precise image [38].

Determination fourier transform infrared spectroscopy (FT-IR)

Fourier transform infrared spectroscopy (FT-IR) The infrared absorption characteristics of NMP bulk drug, physical mixtures, and

NMP-NLC lyophilized powder were compared by FT-IR (IRTracer-100; Shimadzu, Kyoto, Japan). The crystal form of the drug was detected by the changes in the characteristic absorption peaks or the hydrogen bond formation between the components. The KBr tablet method prepared the sample, and an FT-IR analyzer recorded the FT-IR spectra.25 Briefly, samples were squeezed to a fine powder, added with anhydrous KBr at a mass ratio of 1:5 (sample: KBr), and compressed to a thin pellet. The pressure was 5 tons for 3 min in a hydraulic press, and the scanning range was 500–4,000 cm-1 at a resolution of 1.0 cm^{-1} [39].

Stability evaluation

Stability testing of NLCs optimal formula was held using the real-time and freeze-thaw method. In the real-time method, the NLCs samples were stored at 27±2 °C for one month (30 d) [40]. In the freeze-thaw method, the NLC samples were stored at 4±2 °C for 24 h and moved at 40±2 °C for 24 h (counted as one cycle), then repeated up to six cycles (12 d) [41]. The organoleptic, transmittance, pH values and EE were evaluated every cycle.

DPPH method of antioxidant *in vitro* test

Determination of Maximum Wavelength of 0.1 mmol. DPPH Solution tested the free radical scavenging activity of DPPH mangosteen peel by determining the maximum wavelength (λmax) of 0.1 mmol DPPH in methanol using UV-Vis spectrophotometry. The absorbance of the solution was read at a wavelength of 400-800 nm [42].

RESULTS AND DISCUSSION

Organoleptic

This honey was taken in July 2022 from the Kelulut area. The honey selected for the study was freshly from Paloh (Sambas district) with a golden brown color, thick consistency, and characteristic honey aroma.

Water content

The water content test was intended to determine the percentage of water content in the honey used as the sample. The water content in honey dramatically affects the quality of the preparation. The higher the water content in honey, the more excellent the opportunity for the growth of microorganisms in it [43]. The test results showed that the water content is 5.74 %±0.144. The water content in honey is responsible for its stability against fermentation and granulation. The general requirement for honey is to have a water content below 18.6% [44]. The Codex Alimentarius was revised, and honey quality parameters were established [45]. Honey with good quality should have a moisture content of not more than 20 g 100 g-1. Honey with a high water content has a more significant potential for fermentation, so the fermentation must be considered, and appropriate storage is needed [46]. Water content is the second largest constituent of honey. It is influenced by several factors, including interest, geographic origin of nectar, soil characteristics, climatic conditions, conditions at harvest, degree of ripening, manipulation by beekeepers during harvest, extraction, processing methods, and storage conditions [47]. Indonesia has a wide variety of honey because of its diverse climate and flora, and the resulting honey depends directly on the geographical conditions of its origin and has specific physicochemical properties.

Test for the metal content in honey

Based on the test results, metals present in honey are Pb (0.003 mg/kg) and Cd (0.175 mg/kg). The measurements of heavy metal contamination were tested on arsenic, lead, and cadmium. Based on the test results, the metal contamination levels generally do not exceed the limits set in the test parameters. Heavy metal contamination testing is critical to standardize medicinal plants because it can be dangerous and toxic to health [48]. Heavy metals will accumulate in plants, and many studies have proven that heavy metals are at risk of toxic effects [49].

Determination of flavonoid and fenolic content

Gallic acid and quercetin have their main properties as antioxidants. Gallic acid and quercetin equivalents are general references for measuring the amount of phenolic and flavonoid compounds contained in a sample or material [50]. Levels of total phenolic compounds in honey extract using gallic acid. Phenolic compounds are reacted under alkaline conditions so that proton dissociation occurs to form phenolic ions. Based on the regression equation for gallic acid absorption, it is y = 0.0126x+0.0014 with an r of 0.9984. Quercetin is a flavonoid from the flavonol group, with a keto group on the C-4 atom and a hydroxyl group on the neighboring C-3 and C-5 atoms [51]. The quercetin regression equation is Y=0.0358 x-0.0259. The standard curve equation for gallic acid and quercetin obtained a linear relationship between absorbance and concentration with a correlation coefficient of>0.9974. The results of measuring honey extract's total phenolic and flavonoid content were 25.91 mg GAE/g±0.15 extract and 182.36 mg GAE/g extract±0.28, respectively. In research on several honeys worldwide, the phenolic content was found to be 13.88 mg GAE/100 g (in acacia honey) and 142.61 mg GAE/100 g (in acacia honey, Serbia). Total flavonoid content ranged between 0.44 mg QE/100 g in acacia honey and 3.97 mg QE/100 g in mint honey. This data shows that the data obtained varies significantly from one type of honey to another and from one country to another [52].

Honey solubility test data in liquid, solid and surfactant oils

Table 1: Solubility test data for honey in liquid, solid oil and surfactants

No.	Sample	Solubility	Colour	Clarity	Transmittance*
Solid lipid					
1	GMS	dissolved	Dark yellow	Turbid	6.18±0.023
2	CA: CP 4:1	not dissolved	Upper layer: cloudy white, bottom layer: dark yellow	Turbid	4.16±0.021
3	Cetyl palmitate	not dissolved	Upper layer: white like crystal, bottom layer: dark yellow	Unclear	40.52±0.00
4	Cetyl alcohol	not dissolved	Upper layer: yellow, bottom layer: dark yellow	Turbid	1.15±0.015
5	CA: CP 1:1	not dissolved	Upper layer: clear, bottom layer: dark yellow	Clear	17.25±0.008
Liquid lipid					
6	VCO	not dissolved	Upper layer: clear, bottom layer: dark yellow	Clear	86.48±0.004
7	Tocopherol oil	dissolved	Clear, yellow	Clear	81.88±0.05
8	Castor oil	not dissolved	Upper layer: clear yellow, bottom layer: dark yellow	Clear	84.24±0.02
9	Soya Oil	not dissolved	Upper layer: clear yellow, bottom layer: dark yellow	Clear	88.02±0.006
10	Olive Oil	not dissolved	Upper layer: clear yellow, bottom layer: dark yellow	Clear	89.18±0.004
Surfactant					
11	Tween 20	dissolved	Turbid, yellow	Turbid	89.02±0.003
12	Tween 80	not dissolved	Upper layer: clear yellow, bottom layer: dark yellow	Clear	91.71±0.009
13	PEG 400	not dissolved	Yellow	Clear	96.96±0.001
14	CEL	not dissolved	upper layer: clear yellow, bottom layer: dark yellow	Clear	91.73±0.012

*(All values are mean±SD; n=3)

The results of solubility testing show that the oils that can dissolve honey extract are GMS, tocopherol oil, Tween 20, and PEG 400. The presence of oil or liquid lipids in the NLC system gives the NLC system advantages in terms of drug entrapment because, in general, the drug ingredients are more soluble. In oil rather than liquid lipids [19], the presence of oil can reduce the regularity of the lipid matrix crystal lattice due to differences in the carbon chain lengths of lipids and oils [53]. In other studies, it was found that the higher the liquid lipid concentration in the NLC system, the higher the explanation capacity and the smaller the size of the particles formed [54].

Tocopherol oil is a chain-breaking antioxidant that prevents the spread of free radical reactions. It is the main and reference antioxidant that interferes with auto-oxidation, reacting with lipid radicals as an electron donor and converting free radicals into more stable species. Tocopherol into NLC will have improved stability and sustained release. GMS is a non-ionic group that contains high molecular alcohol components and is an emulsifier whose increased concentration can increase consistency and viscosity [55]. The selected surfactant is Tween 20. Increasing Tween 20 as a surfactant will also increase the solubility of the drug because apart from being an emulsifying agent, Tween 20 can also be a solubilizing agent. Tween 20 was chosen as a surfactant component because the high HLB of tween 20 makes it more hydrophilic. The hydrophilic groups in tween 20 produce higher solubility of simvastatin [56].

NLC formulations consist of solid lipids, liquid lipids, and surfactants. All excipients selected for NLC preparations must be under the GRAS (Generally Regarded as Safe) category, namely in the safe category [48]. The selection of lipids, both liquid lipids and solid lipids, varies significantly from one active substance to another. This is because the selection of the type of lipid is usually based on the solubility of the active substance or drug in the lipid material [57]. Solid lipids, as the main ingredient in the formation of NLC are responsible for ensuring the formation of solid particles at room temperature, play a role in loading capacity and play a role in maintaining the stability of the active substance [57, 58]. Liquid lipids change the regularity of the solid lipid matrix crystal lattice to irregular, increasing the charge capacity or space for active compounds [59]. Surfactants as emulsifying agents used in NLC preparations significantly impact the preparation's dispersion and stability [57]. Surfactants reduce the interfacial tension between the lipid mixture and the dispersing agent (water) to help prevent the particles from forming aggregates to maintain size [58]. Non-ionic surfactants are surfactants whose alkyl groups are uncharged. At the same time, the skin has an ionic charge, so using ionic surfactants will irritate the skin because the ions repel each other. So, non-ionic surfactants are safer, compatible with the skin, and have slightly irritating properties when used topically [60]. Tween 20 is an amphiphilic molecule, a hydrophilic non-ionic surfactant that can produce stable, solubilizing, oil-in-water emulsions. When added to an oil/water mixture, the hydrophilic head accommodates into the water phase and the hydrophobic tail into the oil phase. Furthermore, there is a reduction in interfacial tension, and the possibility of separation in the oil/water phase becomes small [61]. The NLCs formulation also uses 10% propylene glycol as a cosurfactant in this study. In other studies using propyleneglycol, a high percentage of transmittance was obtained [17].

Characterization NLCs 14 run

Transmittance response

Based on the ANOVA test, normal plot of residual curve analysis, and lack of fit analysis, the quadratic model in the equation was a valid and appropriate model for transmitting. This was also strengthened by the lack of fit analysis results, which showed that p-value 0, 52>0.05 at the 95% significance level. These showed no significant difference between the experimental and predicted data from the proposed model. The quadratic p-value model 0.03<0.05 showed a significant difference in clarity from using different compositions of solid lipid, liquid lipid, and surfactants.

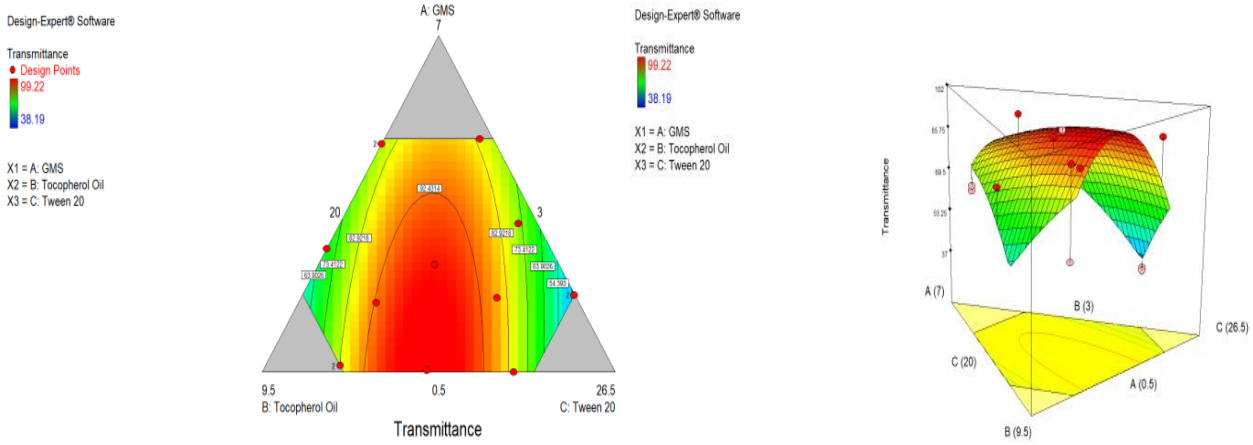

Fig. 1: Contour plot of transmittance response: X1 = A: GMS, X2 = B: Tocopherol oil, X3 = C: Tween 20 (Design Expert® Ver. 7.0.0.)

The D-Optimal design equation obtained can be seen in equation 1.

$$Y = -39{,}26\,(A) - 137.57\,(B) - 8.72\,(C) + 2.17\,(A)(B) + 2.98\,(A)(C) + 7.86\,(B)(C) \ldots . (1)$$

Information:

Y = Transmittance

A = Gliserol mono stearat

B = Tocopherol oil

C = Tween 20

Based on the Figure, the blue color contour plot showed the smallest transmittance value, followed by green and red. The fig. showed that Tween 20 has a role in increasing the transmittance value in the honey extract of NLCs formulation. Combining Tocopherol oil and Tween 20 increases the highest transmittance response; the interaction between GMS and Tween 20 is next. The interaction between GMS and Tocopherol oil has the slightest effect on the transmittance response but is more significant than using the NLC component alone.

Transmittance test results can be seen in table 1. Based on observations, most runs showed a clear display, except at runs 4 and 10 using GMS: Tocopherol oil: Tween 20 with a ratio of (1,983: 3,017: 25). The results of the transmittance test in this study showed that tween 20 in the excess composition caused. Transmittance describe clarity NLCs; one of the NLCs characteristics that needs to be measured affects the globul size. Based on the contour plot of transmittance data and the equation. The transmittance value determined by each optimized component and is also influenced by

the interaction between the components. Interactions between GMS and Tocopherol oil, GMS and Tween 20, and Tocopherol oil and Tween 20 had positive values. Components without combination had negative values. This negative interaction showed that the interaction reduced the transmittance value. A combination of Tocopherol oil and Tween 80 gave the most significant effect, indicated by the magnitude of the coefficients in the equation. Tween 20 influenced the transmittance value greater than GMS and tocopherol oil. The interaction of the two components formed NLC with a clear and stable display, which could increase the NLC transmitting value. The more precise the emulsion, the smaller the particle size, and the more turbid the emulsion, the larger the particle size. Surfactants in the NLC formula must be able to reduce the interface tension between the oil and the dispersing medium. A large amount of surfactant can reduce the interface tension and the droplet's size. The size of the droplet can be predicted from the emulsion's clarity level (transmittance value), the more precise the formed emulsion, the smaller the achieved droplet size [62]. In another research, propylene glycol as a co-surfactant exhibited the smallest globular size 3.10-6.04 µm and hence this composition was used further to implement the factorial design as design of experiments [63].

Based on observations, most runs showed a straightforward appearance, except for runs 4, and 10 using GMS: Tocopherol oil: Tween 20 with a ratio (1,983:3,017:25). Transmittance describes the clarity, which is one of the characteristics of NLCs that needs to be measured because it affects particle size. Suppose the system has a minimal globule size through which light passes. In that case, the light emission will be continued so that the color of the solution appears transparent, and the resulting transmittance is even more excellent. Large amounts of surfactant can reduce interfacial tension and reduce droplet size. Based on the contour plot of the transmittance data and their equations, it is known that the transmittance value is determined by each optimized component and influenced by the interaction between components. The interaction between GMS and Tocopherol oil, GMS and Tween 20, and Tocopherol oil and Tween 20 has a positive value. Components without a combination have a negative value. This negative interaction shows that the interaction reduces the transmittance value. The combination of Tocopherol oil and Tween 80 provides the most significant influence, as indicated by the large coefficient in the equation. Tween 20 influences transmittance values more than GMS oil and tocopherol. The interaction of these two components forms an NLC with a clear and stable appearance, which ultimately can increase the transmission value of the NLC.

pH response

Based on the ANOVA test, normal plot residual curve analysis, and lack of fit analysis, the quadratic model in the equation is a valid and appropriate model for pH. The lack of fit analysis results showed a p-value of 0.22>0.05 at the 95% significance level. This shows no significant difference between the experimental and predicted data from the proposed model. The quadratic p-value model of 0.02<0.05 indicates a significant difference in pH from using different compositions of solid lipids, liquid lipids, and surfactants.

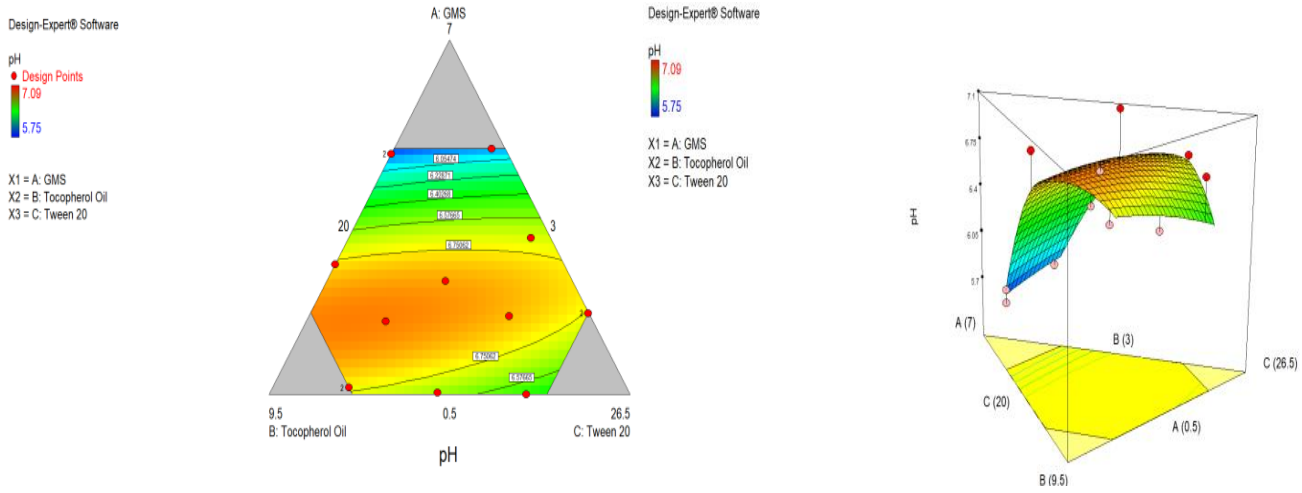

Fig. 2: Contour plot respon pH: X1 = A: GMS, X2 = B: Tocopherol oil, X3 = C: Tween 20 (Design Expert® Ver. 7.0.0.)

The D-Optimal design equation obtained can be seen in Equation 2.

$$Y = -3.07\,(A) + 0.094\,(B) + 0.177\,(C) + 0{,}101\,(A)(B) + 0.102\,(A)(C) + 0.011\,(B)(C) \ldots . (2)$$

Information:

Y = pH

A = Gliserol mono stearat

B = Tocopherol oil

C = Tween 20

Based on the image, the blue Contour Plot shows the smallest pH value, followed by green, yellow, and red. The red color shows the most excellent pH. The increase influences this red area in Tween 20. This shows that Tween 20 increases the pH value in the NLCs formulated honey extract. Combining GMS and Tween 20 increases the pH response the highest. Next in sequence, those that increase the pH response are tocopherol oil, a combination of Tocopherol oil with Tween 20 and GMS. The single GMS component had the most minor influence on the pH response.

Entrapment efficiency (EE) response

Based on the ANOVA test, normal residual curve analysis, and lack of fit analysis, the quadratic model in the equation is a valid and suitable model for Entrapment Efficiency (EE). The results of the ANOVA test show that the p-value is at a significance level of 95%, meaning that the quadratic model is the suitable model to explain the effect of components and their interactions on Entrapment Efficiency (EE). This is also reinforced by the lack of fit analysis results, which shows a p-value of 0.48>0.05 at the 95% significance level. This shows no significant difference between the experimental and predicted data from the proposed model. The quadratic p-value model 0.01<0.05 indicates a significant difference in Entrapment Efficiency (EE) from using different compositions of solid lipids, liquid lipids, and surfactants.

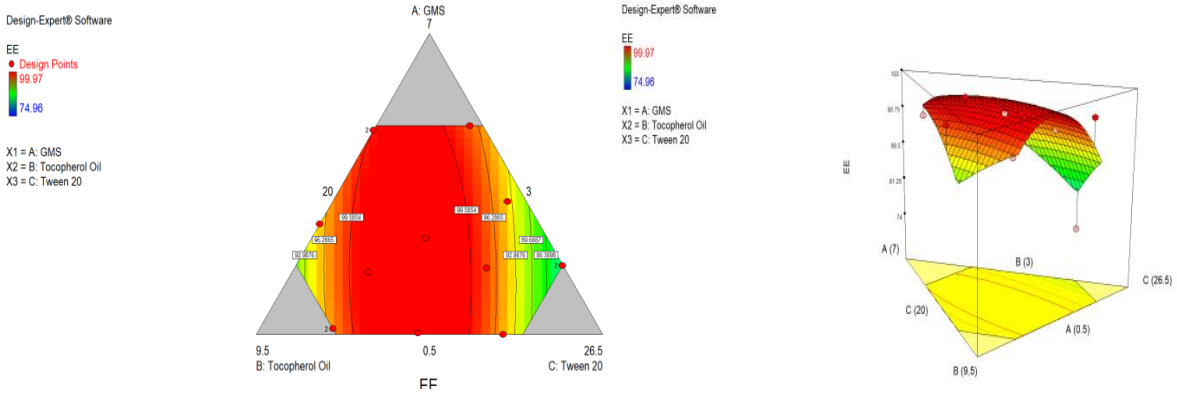

Fig. 3: Contour plot respon entrapment efficiency (EE): X1 = A: GMS, X2 = B: Tocopherol oil, X3 = C: Tween 20 (Design Expert® Ver. 7.0.0.)

The D-Optimal design equation obtained can be seen in Equation 3.

$$Y = -7.61\ (A) - 41.69\ (B) - 0.53\ (C) + 0.87\ (A)(B) + 0.83\ (A)(C) + 2.52\ (B)(C) \quad \ldots\ldots (3)$$

Information:

Y = Entrapment Efficiency (EE)

A = Gliserol mono stearat

B = Tocopherol oil

C = Tween 20

Based on the Figure, the green color contour plot showed the smallest EE value, followed by yellow, orange, and red. The red color indicated the greatest EE. This red area was affected by an increased combination of Tocopherol oil with Tween 20. This showed that combination increases the EE value in the honey extract of NLCs formulation. Combining Tocopherol oil with Tween 20 increases the highest EE response. The following sequence of things that increase the EE response is the interaction of GMS with Tocopherol oil, the interaction of GMS with Tween 20, and then the use of Tween 20, GMS, and Tocopherol oil alone. GMS is a glyceryl monostearate lipid that can form stable emulsions because it has two hydroxyl groups, which are polar and nonpolar, and its solubility in lipids is very high compared to other lipid polymers [64].

The single Tocopherol oil component had the most minor influence on the EE response. Entrapment Efficiency (EE) and Drug Loading (DL) Capacity The amount of free drug in the sample is measured to determine the entrapment efficiency and drug loading capacity of the sample using the ultrafiltration method. Entrapment efficiency studies are necessary to determine the high performance of the formulation. In this NLC formulation, entrapment efficiency in the run was 4.96-99.92% in a system with 9.84±0.041% drug loading capacity in lipid concentration. High EE is required in any drug nano-carrier system, including NLC. In addition, the amount of drug trapped in the nano-carrier also determines the performance of the drug delivery system because it affects the rate of drug release from the system [65]. The addition of drugs in the NLC system minimizes interface retention between the lipid matrix and the liquid phase, which reduces the free energy at the boundary of the lipid phase and the combined drug [66]. A high loading capacity is intended because it will ultimately increase the efficiency of drug adsorption [48]. Drug loading is defined as the process of incorporating the drug into the carrier system, while entrapment efficiency describes the effectiveness of the drug loaded into the carrier. The solubility of the drug in the lipids ensures maximum loading in the NLC system [67]. Therefore, the high honey-loading capacity in the examined NLC system is due to the excellent solubility of honey in the NLC lipid phase. The formation of disordered lipid crystals in NLCs has increased the loading capacity. Incomplete crystal formation in lipid nanoparticles can be achieved by mixing solid lipids with liquid lipids, leading to higher NLC drug loading [68].

NLCs honey extract optimal formula

The optimal formulation results obtained from the D-optimal design are formulas with a ratio of GMS: Tocopherol oil: Tween 20 (0.50:5.54:23.96) with a desirability value of 0.964. Desirability values close to one indicate that the response variable selected for formula optimization can reach the optimal point according to the desired target. The results of determining the composition of GMS, Tocopherol, and Tween 20 oil obtained showed that the composition of Tween 20 as a surfactant was only able to form a homogeneous mixture if the composition ratio was greater than that of GMS, Tocopherol oil. The higher the amount of surfactant in the ratio, the better the interaction balance is achieved, but there are limitations.

Characterization NLCs honey extract optimal formula

Verification of optimal formula

Verification is carried out by comparing the optimal NLCs experimental results with software predictions. Based on the probability value for each response, a p-value>0.05 was obtained, and it was concluded that there was no significant difference between the predicted results of the D-Optimal design in the Design-Expert software and the results of experimental observations.

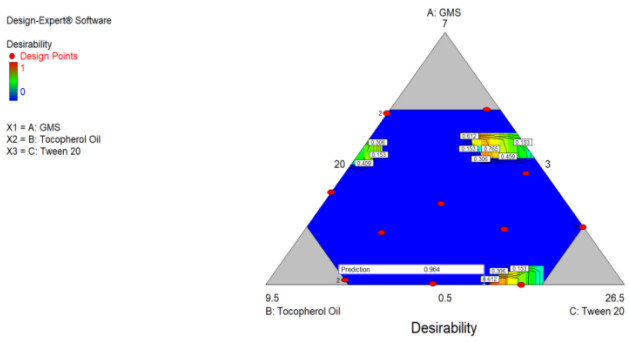

Fig. 4: Desirability of NLCs honey extract (Design expert® Ver. 7.0.0.)

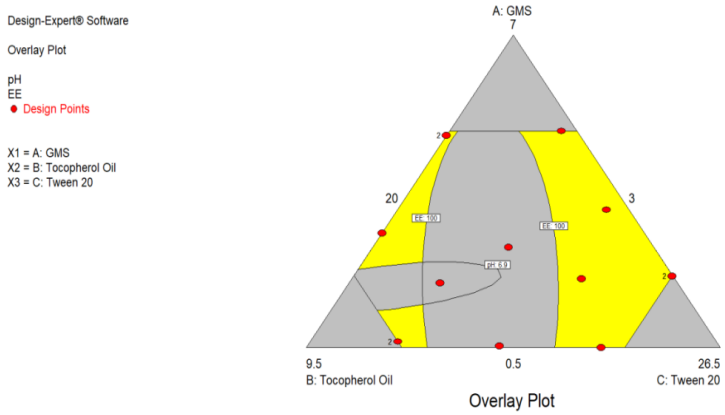

Fig. 5: Superimposed of NLCs honey extract optimal formula, (Design Expert® Ver. 7.0.0.)

Based on the optimal formula produced by the D-Optimal design in Design-Expert software version 7.0.0, this formula is predicted to produce a transmittance of 94.35, a pH of 6.56, and an EE of 99.73. Based on fig. 5, the contour plot is generated from the transmittance, pH, and EE responses. The superimposed results provide a yellow area that has an optimal response. This area provides an optimal prediction formula with a desirability of 0.964.

Table 3: Evaluation NLCs honey extract optimal formula

Evaluation	Prediction from software design expert	Average from research	SD
pH	6.56	6.59	0.15
Transmittance	94.35	94.58	1.54
Entrapment Efficiency	99.73	99.89	0.09

(Prediction from software Design Expert, n=1, from software, values from reasearch are mean±SD; n=3)

The NLCs observation results were compared with the results of the predictive response produced by the optimal formula for the simplex lattice design. Verification is then done using the One Sample T-Test in the OpenStat software. Data analysis with SPSS using one sample T-Test. In the transmittance test parameters, p-value = 0.852>0.05 was obtained, so there was no difference between the predictions of the Design-Expert software using the simplex lattice design method and the experimental results using the optimal NLCs formula. In the pH test, p-value = 0.713>0.05 was obtained, showing no difference between software predictions and experimental results. Furthermore, the EE test obtained a p-value = 0.103>0.05, meaning there was no significant difference between software predictions and experimental results.

Characterization NLCs honey extract optimal formula with transmission electron microscopy (TEM)

TEM evaluation aims to look at microstructural analysis, defect identification, interface analysis, crystal structure, atomic arrangement in crystals, and nanometer-scale elemental analysis.

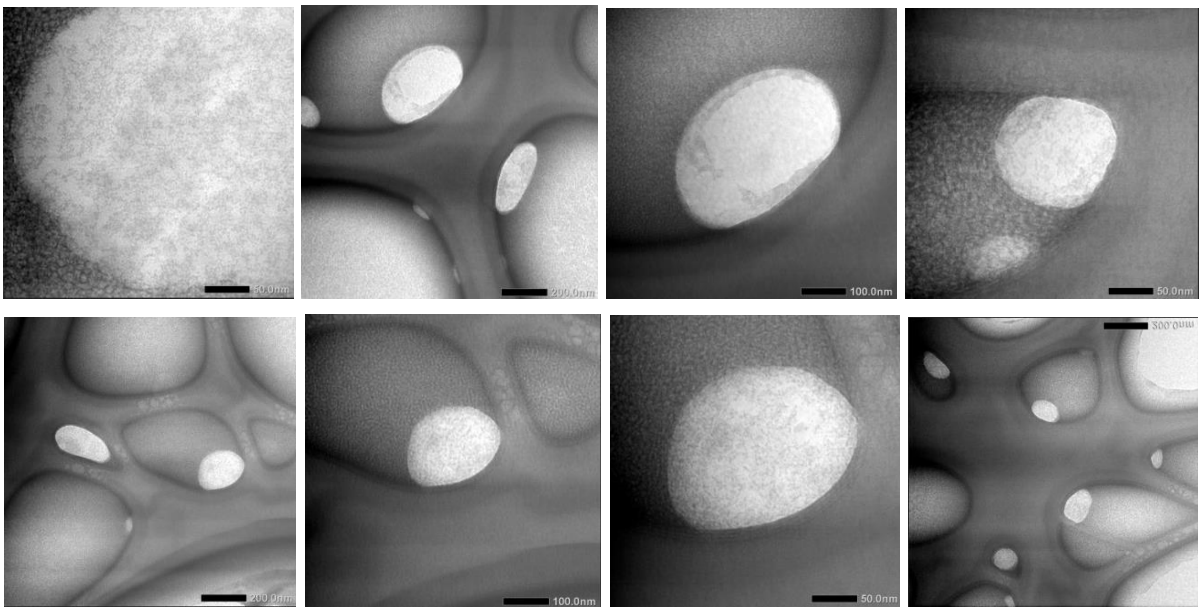

Fig. 6: TEM NLCs honey extract optimal formula. The magnifications are 40.000 x

Morphological tests are needed in NLC characterization, which aims to determine the shape and internal structure of NLC, which already contains active drug ingredients. Lipid crystals generally have a non-spherical platelet shape. The non-spherical shape of lipid nanoparticles has advantages, namely affecting NLC stability, entrapment efficiency (EE), drug loading, drug location in the NLC, and drug release rate, having a large surface area, short diffusion path, and low lipid layer compared to the non-spherical shape of lipid nanoparticles. Round requires more surfactant to stabilize the NLC [19]. This follows the NLCs of optimal formula honey with a high surfactant composition, namely 25.96. This morphology test can also determine the particle size of an NLC. The particle size determined by TEM was shown to be smaller than that determined by dynamic laser light scattering techniques. Based on observations, it was obtained that the NLCs of the optimal formula honey had separate particles, no lumps, oval shape, and smooth and uniform size, ranging from 100 nm to 200 nm. The optimal formula for honey NLCs was obtained using the high-pressure homogenization method.

Characterization NLCs honey extract optimal formula with fourier transform infrared spectroscopy (FTIR)

The purpose of this test is to analyze the functional groups contained in the sample. The analysis uses the UATR (Universal attenuated total reflectance) or Universal Transmittance method. As shown in fig. 8, it was observed that there were several peaks characteristic of drug 3342.02 (-OH stretch, strong bond broadening); 2925.42 (CH stretch, strong bond); 2094.22 (C=C=N, weak bond); 1992.2 (aromatic C-H, weak bond); 1639.5 (C=C alkene, medium bond); 1459.14 (CH2, weak bond); 1080.05 (C-O, medium bond); 927.66 (C-O, strong bond) was found to be similar to the spectrum of honey as shown in the following image. This revealed no physicochemical interactions between the drug and the excipients in the NLC formulation.

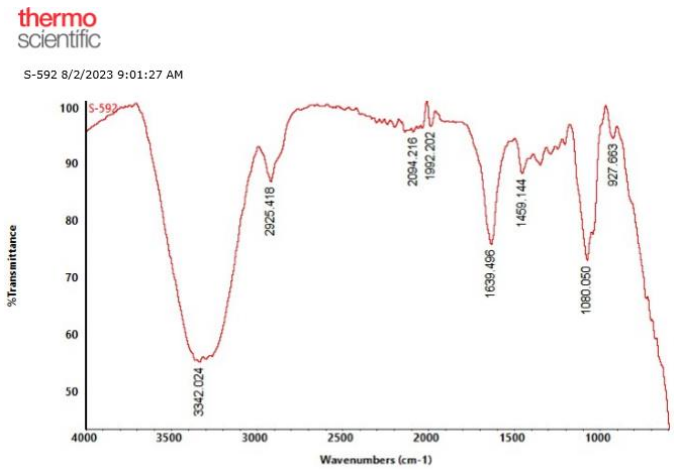

Fig. 7: FTIR profile NLCs honey extract optimal formula

In a study conducted by Erejuwa *et al.*, (2012) [69], it was found that honey contains chemical compounds in the form of polyphenols and flavonoids, which act as antioxidants. This follows the results of the phytochemical and FTIR tests conducted on Trigona spp. The test contained phenolics and flavonoids. Phenolic compounds are compounds that have antioxidant effects. Meanwhile, flavonoids are chemical compounds that come from a combination of several phenols. Honey is a natural product of Apis mellifera bees from nectar or honeydew. It has a distinctive sweet taste due to the high concentration of sugars, namely glucose and fructose. Fructose is an essential sugar in honey, about 36% range. Glucose with an average value of 25%. Significant changes were also found in the 900-750 cm^{-1} area, confirming the variable saccharide configuration [39].

Characterization NLCs honey extract optimal formula with particle size analyzer (PSA)

The test was carried out to determine the size of the nanoparticles contained in the sample. Based on the test results, it is known that the average size of NLCs is 327 nm±0.572. This proves that the NLCs prepared are capable of producing nanostructures. The droplet size decreases as the surfactant concentration increases [70]. Surfactants can cause a decrease in the interfacial film and stabilize it, resulting in a tiny droplet diameter.

In contrast, adding a co-surfactant can cause a more expansive interfacial film [71, 72]. The relative proportions of surfactant and co-surfactant cause variations in droplet size [73]. The oil composition also affects the particle size of NLCs [74]. Oil can increase the ability of NLCs to carry drugs but causes the nanoemulsion to become more prominent, so the ratio of oil used is always smaller than surfactants [75]. The droplet size of NLCs can regulate the effective drug release [73, 76, 77]. In another study, the average droplet size was obtained. The PI (polydispersity index) value indicates the homogeneity of the nanoemulsion particles. The PI value obtained from testing with aquadest media was 0.316. A polydispersity index (PDI) of less than 0.5 indicates a uniform globule size distribution [78, 79], so it can be concluded that the particle size distribution of NLCs is uniform, and the nanoemulsion preparation method has good reliability [59]. Samples with PDI values close to 0 indicate monodispersion samples, while PDI values<1 indicate polydispersion samples [80]. In other studies, storage can increase the particle size of the NLCs system when stored for 30 d, but the increase in size is still on the nanoscale and is still in a stable condition; this can be caused by the aggregation of particles during storage. Lv *et al.* (2016) reported that NLC Brucea javanica oil experienced a slight increase in particle size from 181.5 to 195 nm after being stored for 30 d, partly due to particle aggregation during storage. In addition, the size of nanoparticles has a large specific surface area, which can affect the increase in the bioavailability of bioactive compounds in digestion [81]. The NLC system, which has a size in the range of 100-600 nm, has optimal absorption potential in the human gastrointestinal tract [82]. As depicted in fig. 7, the optimal formula honey NLCs on observation gives a regular and relatively uniform shape showing good particle dispersion.

Determination stability test

Stability Tests of NLC Formulations Stability tests include accelerated and long-term stability tests. Stability tests are carried out to detect possible changes during storage [83]. The physical instability of an emulsion-based carrier system can be detected by observing the occurrence of creaming, separation of the oil phase, or sedimentation of components with a high density. This can be seen when the NLC has been subjected to centrifugation, heating, or cooling [84]. The optimal formula in this study found that the surfactant ratio more dominantly affects the system's stability. This

is because surfactants play an essential role in stabilizing oil-based systems. Surfactants can reduce surface tension by quickly preventing particle aggregation and recrystallization, stabilizing the interfacial tension [85]. Other researchers [86, 87] also reported that surfactant concentration and composition can affect the stability of the NLC system. According to Ziani, *et al.*, (2012) the ratio of surfactant oil and the type of oil can affect the stability of the colloidal dispersion system [87]. Macroscopic carrier systems can be formed at lipid: surfactant ratios above 1:2 and are affected by the manufacturing method used. Lipid-based carrier system formulations can be easily formed using higher surfactant concentrations [88, 89]. Lipids, as the main ingredient of lipid nanoparticles, can affect the capacity and release of bioactive compounds that will be carried in the NLC system later. Higher concentrations of lipids can affect the encapsulation efficiency of the bioactive compounds that are carried. The higher the concentration of lipids, the higher the load of bioactive compounds carried and the effect on the desired NLC entrapment efficiency. In addition, using higher lipid concentrations can reduce the water composition in the system, which helps the system to be more stable for a more extended period and efficient during the system manufacturing process [19]. The cycling test aims to determine the stability of NLCs preparations against varying temperature stress. The cycling test results conducted for six cycles found that the NLCs formula remained stable, as indicated by the absence of discoloration, precipitation, and crystallization. This shows that the NLCs formula has good physical stability when stored at 4 ºC and 40 ºC for 24 h, respectively, in the transmittance and adsorption efficiency tests, but changes in the pH parameter. Data analysis with SPSS using the ANOVA test showed that the transmittance test parameters obtained p-value = 0.774>0.05, the pH test obtained p-value = 0.00<0.05, and the EE test obtained p-value = 0.686>0.05 EE. These results indicate that honey NLCs are stable during storage with cycling tests on transmittance and EE evaluation but unstable at pH. Data analysis with SPSS using the Paired Sample Test to see a comparison between the evaluation of optimal formula honey NLCs and basic NLCs showed that the transmittance test parameters obtained p-value = 0.053>0.05, the pH test obtained p-value = 0.494>0.05 and test EE obtained p-value = 0.005<0.05 EE, meaning that there is no difference in transmittance and Ph, but different in EE. This aligns with previous studies where there were significant differences in particle size during storage with freeze-thaw cycles. There is an increase in particle size, which indicates the coalescence of small particles or coalescence [89]. The physical instability of an emulsion-based carrier system can be detected by observing the occurrence of creaming, separation of the oil phase, or sedimentation of components with a high density. This can be seen when NLCs has been subjected to centrifugation, heating, or cooling [37]. The results of the real-time stability test for 60 d showed that the NLCs formula remained stable, as indicated by the absence of color changes, precipitation, and crystallization. This shows that the optimal formula for honey NLCs has good physical stability when stored at 25 C for 60 d in transmittance, pH, and adsorption efficiency tests. In the stability test of the optimal formula NLC base, it was found that the base was unstable in response to pH and EE. Data analysis with SPSS using the ANOVA test showed that the transmittance test parameter obtained p-value = 0.107>0.05, the pH test obtained p-value = 0.886>0.05, and the EE test obtained p-value = 0.871>0.05 EE. These results indicate that honey NLCs were stable after storage for 60 d in all evaluations. Data analysis on the NLC basis shows that the transmittance test parameters obtained p-value = 0.537>0.05, the pH test obtained p-value = 0.001<0.05, and the EE test obtained p-value = 0.007<0.05 EE. These results indicate that the base NLCs were stable after storage for 60 d in the transmittance test but were unstable in the pH and EE tests. Data analysis with SPSS using the Paired Sample Test to see the comparison between the evaluation of optimal formula honey NLCs and base NLCs shows that the transmittance test parameters obtained p-value = 0.202>0.05, the pH test obtained p-value = 0.00<0.05 and the EE test obtained p-value = 0.042<0.05 EE, meaning there is no difference in transmittance, but there is a difference in pH and EE. The results of data analysis in real-time stability tests show that honey NLCs are stable in storage compared to base NLCs, meaning that the presence of honey in NLCs can increase the stability of the preparation.

Antioxidant *in vitro*

The results of UV-Vis spectra on the test show that the wavelength value is at 515.25 nm and is included in the range of visible light wavelengths. The maximum wavelength of 0.1 mmol of DPPH is 515 nm [90]. The time used for the interaction between the test sample and DPPH in this study was 30 min. This can also be seen visually with a change in the purple color, fading and slightly yellowish after an incubation period of 30 min. This color change occurred due to a compound in the sample that donates a hydrogen atom to the DPPH radical to reduce it to a more stable form, namely 1, 1-diphenyl-2-picrylhydrazine. Several studies have shown that the reaction time is 30 min and has been investigated in research [91].

Table 4: IC_{50} value

Sample	IC_{50} (ppm)
Honey	72.59±0.79
Ethanol Extract	38.55±0.24
NLCs of honey extract optimal formula	61.99±0.34
NLCs of base optimal formula without extract	829.81±0.93

(All values are mean±SD; n=3)

Based on the test, it is known that the honey and the ethanol extract have antioxidant activity. The ethanol extract was included in the robust category, while the NLCs of honey extract optimal formula and honey without preparation were in the strong category. NLCs of base optimal formula without extract have no antioxidant activity. A compound is categorized as a powerful antioxidant if the IC_{50} value is less than 50 g/ml and a strong category if the IC50 value is between 50-100 g/ml. The smaller the IC_{50} value, the stronger the antioxidant activity [92]. The DPPH radical scavenging activity of phenolic and flavonoid compounds. Flavonoid compounds act as antioxidants because they have a hydroxyl group that can release protons as hydrogen ions. The hydrogen ion has only one proton and no electrons, so the radical electron contained in the nitrogen atom in the DPPH compound binds to hydrogen ions and generates reduced DPPH [93]. Radicals in DPPH can be reduced when reacting with hydrogen donors contained in phenolic compounds [94].

The 70% ethanol extract has a small IC_{50} value because it is a polar solvent that can extract phenolic compounds. Phenolic compounds are antioxidant compounds that can donate hydrogen atoms to DPPH free radicals. The extraction of active compounds was with 70% ethanol as solvent. The liquid solvent in the extraction process is the optimal solvent for the active or efficacious content compounds so that these compounds can be separated from the material and other constituent compounds, and the extract contains only most of the desired content compounds. The main factors for consideration in selecting filter fluids are selectivity, ease of work and processes, economy, environmental friendliness, and safety [48].

CONCLUSION

The obtained NLCs of the optimal honey extract were a combination of GMS: Tocopherol oil: Tween 20 with a composition ratio of 0.5: 5.54: 23.96. Based on the evaluation, the water content of honey extract was 5.74 %±0.144; metals present in honey are Pb (0.003 mg/kg) and Cd (0.175 mg/kg). Phenolic and Total Flavonoids are 25.91 mg GAE/g±0, 15 extract and 182.36 mg GAE/g extract±0.28. The transmittance value was 94.58%±1.54, pH was 6.59±0.15, and Entrapment efficiency was 99.89±0.09. The average droplet size was 327 nm±0.57, and TEM and FTIR provided details on their structure. Optimal NLCs loaded honey extract had higher activity memory support with IC_{50} 61.99±0.34. The real-time stability shows optimal NLCs honey extract stable in real-time stability and freeze-thaw cycle.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare thet they have no conflict of interest.

REFERENCES

1. Kuss DJ, Louws J, Wiers RW. Online gaming addiction? motives predict addictive play behavior in massively multiplayer online role-playing games. Cyberpsychol Behav Soc Netw. 2012;15(9):480-5. doi: 10.1089/cyber.2012.0034, PMID 22974351.
2. Han DH, Bolo N, Daniels MA, Arenella L, Lyoo IK, Renshaw PF. Brain activity and desire for internet video game play. Compr Psychiatry. 2011;52(1):88-95. doi: 10.1016/j.comppsych.2010.04.004, PMID 21220070.
3. WHO. Addictive behaviours: gaming disorder; 2018. https://www.who.int/news-room/Q-Adetail/addictive-behaviours-gaming-disorder.
4. Chou MY, Chen YJ, Lin LH, Nakao Y, Lim AL, Wang MF. Protective effects of hydrolyzed chicken extract (Probeptigen®/Cmi-168) on memory retention and brain oxidative stress in senescence-accelerated mice. Nutrients. 2019;11(8):1-21. doi: 10.3390/nu11081870, PMID 31408929.
5. Tashkandi H. Honey in wound healing: an updated review. Open Life Sci. 2021;16(1):1091-100. doi: 10.1515/biol-2021-0084, PMID 34708153.
6. Samarghandian ST, Farkhondeh T, Samini F. Honey and health: a review of recent clinical research. Pharmacognosy Res. 2017;9(2):121-7. doi: 10.4103/0974-8490.204647, PMID 28539734.
7. Abedi F, Ghasemi S, Farkhondeh T, Azimi Nezhad M, Shakibaei M, Samarghandian S. Possible potential effects of honey and its main components against COVID-19 infection. Dose Response. 2021;19(1):1559325820982423. doi: 10.1177/1559325820982423, PMID 33867892.
8. Khleifat K, Qaralleh H, Allimoun M, Al Khlifeh ES, Tawarah N. Antibacterial and antioxidant activities of local honey from Jordan. Trop J Nat. 2021;5(3):470-7. doi: 10.26538/tjnpr/v5i3.10.
9. Margaoan R, Topal E, Balkanska R, Yucel B, Oravecz T, Cornea Cipcigan M. Monofloral honeys as a potential source of natural antioxidants, minerals and medicine. Antioxidants (Basel). 2021;10(7):1-48. doi: 10.3390/antiox10071023, PMID 34202118.
10. Martinez Armenta C, Camacho Rea MC, Martinez Nava GA, Espinosa Velazquez R, Pineda C, Gomez Quiroz LE. Therapeutic potential of bioactive compounds in honey for treating osteoarthritis. Front Pharmacol. 2021;12:642836. doi: 10.3389/fphar.2021.642836, PMID 33967778.
11. Nohair SFA. Antidiabetic efficacy of a honey-royal jelly mixture: a biochemical study in rats. Int J Health Sci (Qassim). 2021;15(4):4-9. PMID 34285683.
12. Wadi MA. *In vitro* antibacterial activity of different honey samples against clinical isolates. BioMed Res Int. 2022;2022:1560050. doi: 10.1155/2022/1560050, PMID 35097108.
13. Dafalla NE, El-Sarrag M, Osman KA, Ali SA. Determination of flavonoids in sudanese honey samples and plant sources collected from different places in sudan. Int J Agric Innov Res. 2014;2(4):545-53.
14. Bonerba E, Panseri S, Arioli F, Nobile M, Terio V, Di Cesare F. Determination of antibiotic residues in honey in relation to different potential sources and relevance for food inspection. Food Chem. 2021;334:127575. doi: 10.1016/j.foodchem.2020.127575, PMID 32707361.
15. Escuredo O, Seijo MC. Authenticity of honey: characterization, bioactivities and sensorial properties. Foods. 2022;11(9):1-4. doi: 10.3390/foods11091301, PMID 35564024.
16. Olas B. Honey and its phenolic compounds as an effective natural medicine for cardiovascular diseases in humans? Nutrients. 2020;12(2):1-14. doi: 10.3390/nu12020283, PMID 31973186.
17. Pratiwi L, Sari R, Apridamayanti P. Design and characterization of nanospray with self-nanoemulsifying drug delivery system using sinergistic combination of Melastoma malabathricum l. fraction and gentamicin. Int J App Pharm. 2021;13(2):254-63. doi: 10.22159/ijap.2021v13i2.40094.
18. Mcclements DJ, Xiao H. Is nano safe in foods? establishing the factors impacting the gastrointestinal fate and toxicity of organic and inorganic food-grade nanoparticles. NPJ Sci Food. 2017;1(1):6. doi: 10.1038/s41538-017-0005-1, PMID 31304248.
19. Tamjidi F, Shahedi M, Varshosaz J, Nasirpour A. Nanostructured lipid carriers (NLC): a potential delivery system for bioactive food molecules. Innov Food Sci Emerg Technol. 2013;19:29-43. doi: 10.1016/j.ifset.2013.03.002.
20. Riskiana N, Sundaryono A, Nurhamidah. Ethnopharmaceutical literature study, acute toxicity test of hydnopytum sp. on the histology of the mouse liver. Bencoolen J Pharm. 2021;1(1):1-10. doi: 10.33369/bjp.v1i1.15585.
21. Hung LC, Basri M, Tejo BA, Ismail R, Nang HLL, Abu Hassan HA. An improved method for the preparations of nanostructured lipid carriers containing heat-sensitive bioactives. Colloids Surf B Biointerfaces. 2011;87(1):180-6. doi: 10.1016/j.colsurfb.2011.05.019, PMID 21652183.
22. Mohanraj VJ, Chen Y. Nanoparticles-a review. Trop J Pharm Res. 2007;5(1):561-73. doi: 10.4314/tjpr.v5i1.14634.
23. Ghasemiyeh P, Mohammadi Samani S. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages. Res Pharm Sci. 2018;13(4):288-303. doi: 10.4103/1735-5362.235156, PMID 30065762.
24. Patel DK, Tripathy S, Nair SK, Kesharwani R. Nanostructured lipid carrier (NLC) a modern approach for topical delivery: a review. World J Pharm Pharm Sci. 2013;9(3). doi: 10.22270/jddt.v9i3-s.2750.
25. Mohan DC, Suresh A, Mukundan S, Gupta S, Viswanad V. Development and *in vitro* evaluation of nanolipid carriers of clobetasol propionate and pramoxine hydrochloride for topical delivery. Int J App Pharm. 2018;10(3):28-36. doi: 10.22159/ijap.2018v10i3.24171.
26. Makky AM, El-Leithy ES, Hussein DG, Khattab A. Skin targeting of an optimized caffeine nanostructured lipid carrier with improved efficiency against chemotherapy-induced alopecia. Int J App Pharm. 2022;14(4):235-50. doi: 10.22159/ijap.2022v14i4.44681.
27. Komala O, Noorlaela E, Dhiasmi A. Antibacterial test and anti-acne mask formulation containing cinnamon (cinnamomum burmanni nees and t. nees). Ekologia. 2018;18(1):31-9.
28. Akhir RAM, Bakar MFA, Sanusi SB. Antioxidant and antimicrobial activity of stingless bee bread and propolis extracts. AIP Conf Proc. 2017:1-7. doi: 10.1063/1.5005423.
29. Biesaga M, Pyrzyńska K. Stability of bioactive polyphenols from honey during different extraction methods. Food Chem. 2013;136(1):46-54. doi: 10.1016/j.foodchem.2012.07.095, PMID 23017391.
30. Pauliuc D, Dranca F, Oroian M. Antioxidant activity, total phenolic content, individual phenolics and physicochemical parameters suitability for Romanian honey authentication. Foods. 2020;9(3):2-22. doi: 10.3390/foods9030306, PMID 32182719.
31. Pontis JA, Costa LAM, Da Silva SJR, Flach A. Color, phenolic and flavonoid content, and antioxidant activity of honey from roraima, Brazil. Food Sci Technol. 2014;34(1):69-73. doi: 10.1590/S0101-20612014005000015.
32. Isla MI, Craig A, Ordonez R, Zampini C, Sayago J, Bedascarrasbure E. Physicochemical and bioactive properties of honeys from Northwestern Argentina. LWT-Food Science and Technology. 2011;44(9):1922-30. doi: 10.1016/j.lwt.2011.04.003.
33. Fernandes AV, Pydi CR, Verma R, Jose J, Kumar L. Design, preparation and *in vitro* characterizations of fluconazole loaded nanostructured lipid carriers. Braz J Pharm Sci. 2020;56:1-14. doi: 10.1590/s2175-97902019000318069.
34. Jafar G, Abdassah M, Rusdiana T, Khairunisa R. Development and characterization of precirol ato 88 base in nanostructured

lipid carriers (Nlc) formulation with the probe sonication method. Int J App Pharm. 2021;13(3):43-6. doi: 10.22159/ijap.2021.v13s3.8.

35. Rosli NA, Hasham R, Aziz AA, Aziz R. Formulation and characterization of nanostructured lipid carrier encapsulated Zingiber zerumbet oil using ultrasonication technique. J Adv Res Appl Mech. 2015;11(1):16-23.
36. Patel J, Kevin G, Patel A, Raval M, Sheth N. Design and development of a self-nanoemulsifying drug delivery system for telmisartan for oral drug delivery. Int J Pharm Investig. 2011;1(2):112-8. doi: 10.4103/2230-973X.82431, PMID 23071930.
37. Pratiwi L, Fudholi A, Martien R, Pramono S. Physical and chemical stability test of snedds (Self-nanoemulsifying drug delivery system) and nanoemulsion ethyl acetate fraction of garcinia mangostana L. Trad Med J. 2018;23(2):84-90. doi: 10.22146/mot.28533.
38. Liu Y, Wang L, Zhao Y, He M, Zhang X, Niu M. Nanostructured lipid carriers versus microemulsions for delivery of the poorly water-soluble drug luteolin. Int J Pharm. 2014;476(1-2):169-77. doi: 10.1016/j.ijpharm.2014.09.052, PMID 25280882.
39. Kędzierska Matysek MK, Matwijczuk A, Florek M, Barłowska J, Wolanciuk A, Matwijczuk A. Application of ftir spectroscopy for analysis of the quality of honey. Bio Web Conference: Contemporary Research Trend In Agricultural Engineering. 2018;10:1-15. doi: 10.1051/bioconf/20181002008.
40. Dantas MGB, Reis SAG, Damasceno CMS, Rolim LA, Rolim Neto PJ, Carvalho FO. Development and evaluation of stability of a gel formulation containing the monoterpene borneol. Scientific World Journal. 2016;2016:7394685. doi: 10.1155/2016/7394685, PMID 27247965.
41. Kumar A, Dua JS. Formulation and evaluation of itraconazole niosomal gel. Asian J Pharm Res Dev. 2018;6(5):76-80. doi: 10.22270/ajprd.v6i5.425.
42. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (dpph) for estimating antioxidant activity. Songklanakarin J Sci Technol. 2004;26:211-9. doi: 10.15416/ijpst.v4i2.10456.
43. Alqarni AS, Owayss AA, Mahmoud AA. Physicochemical characteristics, total phenols and pigments of national and international honeys in Saudi Arabia. Arab J Chem. 2016;9(1):114-20. doi: 10.1016/j.arabjc.2012.11.013.
44. International Honey Commission. Harmonised methods of the international honey commission. Liebefeld, Switzerland: Swiss Bee Research Centre; 2009. p. 63.
45. Nordin A, Sainik NQAV, Chowdhury SR, Saim AB, Idrus RBH. Physicochemical properties of stingless bee honey from around the globe: a comprehensive review. J Food Compos Anal. 2018;73:91-102. doi: 10.1016/j.jfca.2018.06.002.
46. Bobis O, Moise AR, Ballesteros I, Reyes ES, Duran SS, Sanchez Sanchez J. Eucalyptus honey: quality parameters, chemical composition and health-promoting properties. Food Chem. 2020;325:126870. doi: 10.1016/j.foodchem.2020.126870, PMID 32387927.
47. Ministry of Health of the Republic of Indonesia. General standard parameters of medicinal plant extracts. Jakarta: Ministry of Health of the Republic of Indonesia. Thing; 2000. p. 13-31.
48. Patel DK, Patel K, Dhanabal S. Phytochemical standardization of aloe vera extract by HPTLC techniques. J Acute Dis. 2012;1(1):47-50. doi: 10.1016/S2221-6189(13)60011-6.
49. Dean J. Extraction techniques in analytical science. London: john Wiley and Sons LTD; 2009. p. 43-6.
50. Lee KW, Kim YJ, Lee HJ, Lee CY. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J Agric Food Chem. 2003;51(25):7292-5. doi: 10.1021/jf0344385, PMID 14640573.
51. Azizah DN, Kumolowat E, Faramayuda F. Determination of flavonoid levels AlCl3 method in methanol extract of cocoa fruit peel (Theobroma cacao L.). Kartika Pharmaceutical Scientific Journal. 2014;2(2):45-9. doi: 10.26874/KJIF.V2I2.14.
52. Albu A, Radu Rusu R, Simeanu D, Radu Rusu C, Pop IM. Phenolic and total flavonoid contents and physicochemical traits of Romanian monofloral honeys. Agriculture. 2022;12(9):1-15. doi: 10.3390/agriculture12091378.
53. Souto EB, Muller RH. Lipid nanoparticles (Solid lipid nanoparticles and nanostructured lipid carriers) for cosmetic, dermal, and transdermal applications. Drug Deliv Syst Nanoparticulate. 2007:213-29.
54. Hu FQ, Jiang SP, Du YZ, Yuan H, Ye YQ, Zeng S. Preparation and characterization of stearic acid nanostructured lipid carriers by solvent diffusion method in an aqueous system. Colloids Surf B Biointerfaces. 2005;45(3-4):167-73. doi: 10.1016/j.colsurfb.2005.08.005, PMID 16198092.
55. Chen L, Ao F, Ge X, Shen W. Food-grade pickering emulsions: preparation, stabilization and applications. Molecules. 2020;25(14):1-24. doi: 10.3390/molecules25143202, PMID 32674301.
56. Thomas N, Holm R, Garmer M, Karlsson JJ, Mullertz A, Rades T. Supersaturated self-nanoemulsifying drug delivery systems (Super-SNEDDS) enhance the bioavailability of the poorly water-soluble drug simvastatin in dogs. AAPS J. 2013;15(1):219-27. doi: 10.1208/s12248-012-9433-7, PMID 23180162.
57. Zhang C, Luo S, Zhang Z, Niu Y, Zhang W. Evaluation of glabridin loaded nanostructure lipid carriers. J Taiwan Inst Chem Eng. 2017;71:338-43. doi: 10.1016/j.jtice.2016.11.010.
58. Müller RH, Petersen RD, Hommoss A, Pardeike J. Nanostructured lipid carriers (NLC) in cosmetic dermal products. Adv Drug Deliv Rev. 2007;59(6):522-30. doi: 10.1016/j.addr.2007.04.012, PMID 17602783.
59. Ebtavanny TG, Soeratri W, Rosita N. Effect of lipid composition on nanostructured lipid carrier (nlc) on ubiquinone effectiveness as an anti-aging cosmetics. Int J Drug Deliv Technol. 2018;8(3):144-50. doi: 10.25258/ijddt.8.3.5.
60. Lukic M, Pantelic I, Savic S. An overview of novel surfactants for formulation of cosmetics with certain emphasis on acidic active substances. Tenside Surfactants Deterg. 2016;53(1):7-19. doi: 10.3139/113.110405.
61. Rowe RC. Handbook of pharmaceutical excipients, 6th Ed. Apha, editor. (Php) Pharmaceutical Press. Vol. 546. London; 2009. p. 592.
62. Pratiwi L. Novel antimicrobial activities of self-nanoemulsifying drug delivery system ethyl acetate fraction from Garcinia mangostana L. peels against staphylococcus epidermidis: design, optimization, and *in vitro* studies. J Appl Pharm Sci. 2021;11(3):162-71.
63. Hardikar SR, Akbar Ansari DM, Patil NA. Formulation of nanostructured lipid carriers of haloperidol prepared by using clarified butter. Int J App Pharm. 2023;15(2):213-8. doi: 10.22159/ijap.2023v15i2.46759.
64. Kumar R, Yasir M, Saraf SA, Gaur PK, Kumar Y, Singh AP. Glyceryl monostearate based nanoparticles of mefenamic acid: fabrication and *in vitro* characterization. Drug Invent Today. 2013;5(3):246-50. doi: 10.1016/j.dit.2013.06.011.
65. Tiwari G, Tiwari R, Sriwastawa B, Bhati L, Pandey S, Pandey P. Drug delivery systems: an updated review. Int J Pharm Investig. 2012;2(1):2-11. doi: 10.4103/2230-973X.96920, PMID 23071954.
66. How CW, Rasedee A, Abbasalipourkabir R. Characterization and cytotoxicity of nanostructured lipid carriers formulated with olive oil, hydrogenated palm oil, and polysorbate 80. IEEE Trans Nanobioscience. 2013;12(2):72-8. doi: 10.1109/TNB.2012.2232937, PMID 23268387.
67. Rahman HS, Rasedee A, How CW, Abdul AB, Zeenathul NA, Othman HH. Zerumbone-loaded nanostructured lipid carriers: preparation, characterization, and antileukemic effect. Int J Nanomedicine. 2013;8(1):2769-81. doi: 10.2147/IJN.S45313, PMID 23946649.
68. Muller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (nlc) in cosmetic and dermatological preparations. Adv Drug Deliv Rev. 2002;54(1)Suppl 1:S131-55. doi: 10.1016/s0169-409x(02)00118-7, PMID 12460720.
69. Erejuwa OO, Sulaiman SA, Wahab MS. Oligosaccharides might contribute to the antidiabetic effect of honey: a review of the literature. Molecules. 2011;17(1):248-66. doi: 10.3390/molecules17010248, PMID 22205091.
70. Kassem AA, Mohsen AM, Ahmed RS, Essam TM. Self-nanoemulsifying drug delivery system (SNEDDS) with

enhanced solubilization of nystatin for treatment of oral candidiasis: design, optimization, *in vitro* and *in vivo* evaluation. J Mol Liq. 2016;218:219-32. doi: 10.1016/j.molliq.2016.02.081.

71. Fahmy UA, Ahmed OA, Hosny KM. Development and evaluation of avanafil self-nanoemulsifying drug delivery system with rapid onset of action and enhanced bioavailability. AAPS PharmSciTech. 2015;16(1):53-8. doi: 10.1208/s12249-014-0199-3, PMID 25168449.
72. Hosny KM, Banjar ZM. The formulation of a nasal nanoemulsion zaleplon in situ gel for the treatment of insomnia. Expert Opin Drug Deliv. 2013;10(8):1033-41. doi: 10.1517/17425247.2013.812069, PMID 23795561.
73. Singh SK, Verma PR, Razdan B. Glibenclamide-loaded self-nanoemulsifying drug delivery system: development and characterization. Drug Dev Ind Pharm. 2010;36(8):933-45. doi: 10.3109/03639040903585143, PMID 20184416.
74. Fernandez P, Andre V, Rieger J, Kuhnle A. Nano-emulsion formation by emulsion phase inversion. Colloids and Surfaces A Physicochemical and Engineering Aspects. 2004;251(1-3):53-8. doi: 10.1016/j.colsurfa.2004.09.029.
75. Larsen AT, Akesson P, Jureus A, Saaby L, Abu Rmaileh R, Abrahamsson B. Bioavailability of cinnarizine in dogs: effect of SNEDDS loading level and correlation with cinnarizine solubilization during *in vitro* lipolysis. Pharm Res. 2013;30(12):3101-13. doi: 10.1007/s11095-013-1145-x, PMID 23949249.
76. Parmar N, Singla N, Amin S, Kohli K. Study of cosurfactant effect on nanoemulsifying area and development of lercanidipine loaded (SNEDDS) self nanoemulsifying drug delivery system. Colloids Surf B Biointerfaces. 2011;86(2):327-38. doi: 10.1016/j.colsurfb.2011.04.016, PMID 21550214.
77. Badran MM, Taha EI, Tayel MM, Al-Suwayeh SA. Ultra-fine self nanoemulsifying drug delivery system for transdermal delivery of meloxicam: dependency on the type of surfactants. J Mol Liq. 2014;190:16-22. doi: 10.1016/j.molliq.2013.10.015.
78. Balakumar K, Raghavan CV, Selvan NT, Prasad RH, Abdu S. Self nanoemulsifying drug delivery system (SNEDDS) of rosuvastatin calcium: design, formulation, bioavailability and pharmacokinetic evaluation. Colloids Surf B Biointerfaces. 2013;112:337-43. doi: 10.1016/j.colsurfb.2013.08.025, PMID 24012665.
79. Shakeel F, Haq N, Alanazi FK, Alsarra IA. Polymeric solid self-nanoemulsifying drug delivery system of glibenclamide using coffee husk as a low-cost biosorbent. Powder Technol. 2014;256:352-60. doi: 10.1016/j.powtec.2014.02.028.
80. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018;10(2). doi: 10.3390/pharmaceutics10020057, PMID 29783687.
81. Lv W, Zhao S, Yu H, Li N, Garamus VM, Chen Y. Brucea javanica oil-loaded nanostructure lipid carriers (BJO NLCs): preparation, characterization and *in vitro* evaluation. Colloids Surf A Physicochem Eng Aspects. 2016;504:312-9. doi: 10.1016/j.colsurfa.2016.05.068.
82. Weiss J, Decker EA, Mcclements DJ, Kristbergsson K, Helgason T, Awad T. Solid lipid nanoparticles as delivery systems for bioactive food components. Food Biophys. 2008;3(2):146-54. doi: 10.1007/s11483-008-9065-8.
83. Okonogi S, Riangjanapatee P. Physicochemical characterization of lycopene-loaded nanostructured lipid carrier formulations for topical administration. Int J Pharm. 2015;478(2):726-35. doi: 10.1016/j.ijpharm.2014.12.002, PMID 25479097.
84. Mcclements DJ, Rao J. Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Crit Rev Food Sci Nutr. 2011;51(4):285-330. doi: 10.1080/10408398.2011.559558, PMID 21432697.
85. Witayaudom P, Klinkesorn U. Effect of surfactant concentration and solidification temperature on the characteristics and stability of nanostructured lipid carrier (NLC) prepared from rambutan (Nephelium lappaceum L.) kernel fat. J Colloid Interface Sci. 2017;505:1082-92. doi: 10.1016/j.jcis.2017.07.008. PMID 28697547.
86. Karn Orachai K, Smith SM, Phunpee S, Treethong A, Puttipipatkhachorn S, Pratontep S. The effect of surfactant composition on the chemical and structural properties of nanostructured lipid carriers. J Microencapsul. 2014;31(6):609-18. doi: 10.3109/02652048.2014.911374, PMID 24861323.
87. Ziani K, Fang Y, McClements DJ. Fabrication and stability of colloidal delivery systems for flavor oils: effect of composition and storage conditions. Food Res Int. 2012;46(1):209-16. doi: 10.1016/j.foodres.2011.12.017.
88. Salminen H, Aulbach S, Leuenberger BH, Tedeschi C, Weiss J. Influence of surfactant composition on physical and oxidative stability of quillaja saponin-stabilized lipid particles with encapsulated ω-3 fish oil. Colloids Surf B Biointerfaces. 2014;122:46-55. doi: 10.1016/j.colsurfb.2014.06.045, PMID 25016544.
89. Rahmasari D, Rosita N, Soeratri W. Physicochemical characteristics, stability, and irritability of nanostructured lipid carrier system stabilized with different surfactant ratios. JFIKI 2022;9(1):8-16. doi: 10.20473/jfiki.v9i12022.8-16.
90. Rohman A. Analytical pharmaceutical chemistry. Yogyakarta: Student Library; 2007.
91. Martinez Armenta C, Camacho Rea MC, Martinez Nava GA, Espinosa Velazquez R, Pineda C, Gomez Quiroz LE. Therapeutic potential of bioactive compounds in honey for treating osteoarthritis. Front Pharmacol. 2021;12:642836. doi: 10.3389/fphar.2021.642836, PMID 33967778.
92. Kim SJ, Hong EH, Lee BR, Park MH, Kim JW, Pyun AR. A-mangostin reduced er stress-mediated tumor growth through autophagy activation. Immune Netw. 2012;12(6):253-60. doi: 10.4110/in.2012.12.6.253, PMID 23396851.
93. Gurav S, Deshkar SN, Gulkari V, Duragkar VN, Patil A. Free radical scavenging activity of polygala chinensis linn. Pharmacol Online. 2007;2:245-53.
94. Arazo M, Bello A, Rastrelli L, Montelier M, Delgado L, Panfet C. Antioxidant properties of pulp and peel of yellow mangosteen fruits. Emirates J Food Agric. 2011;23(6):517-24.