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Research Article

Development of Chamomile Lyotropic Liquid Crystal Nanoparticle: Anti-Inflammatory Potential

Pengembangan Nanopartikel Kristal Cair Liotropik Kamomil: Potensi Antiinflamasi

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ABSTRACT

Chamomile is renowned for its anti-inflammatory properties. Lyotropic Liquid Crystal Nanoparticle (LLCN) offers better enhancement of the bioavailability and efficacy of natural products. This study evaluated the LLCN system's effectiveness in improving chamomile extract's anti-inflammatory activity and compliance with nanoparticle standards. Chamomile 70% ethanol extract was prepared, and the total phenolic and flavonoid content was measured. The extract was formulated into LLCN, and the particle size, polydispersity index, zeta potential, stability, and entrapment efficiency were evaluated. Anti-inflammatory activity was tested in vitro using the protein denaturation inhibition method. The extract showed high phenolic and flavonoid content. The characterisation results demonstrated that the LLCN system met the required specifications, exhibiting particle size values of 230.366±3.412 nm, polydispersity index of 0.217±0.034, a zeta potential of -23.763±0.756 mV, and an entrapment efficiency of 84.060%±0.100. Stability testing indicated that storage at 4°C was more effective than room temperature, preserving a smaller particle size and improving overall stability. The LLCN system significantly enhanced the extract's anti-inflammatory activity, as demonstrated by a lower IC_{50} value than the viscous extract. In conclusion, the LLCN system enhances the therapeutic potential of chamomile extract and represents a promising strategy for developing phytochemical-based anti-inflammatory formulations.

Keywords: Anti-inflammatory, Chamomile, LLCN

ABSTRAK

Kamomil memiliki sifat antiinflamasi dari senyawa aktifnya. Sistem Nanopartikel Kristal Cair Liotropik (LLCN) dapat meningkatkan ketersediaan hayati dan efektivitas senyawa tersebut. Penelitian ini mengevaluasi kemampuan sistem LLCN dalam meningkatkan aktivitas antiinflamasi ekstrak kamomil dan kesesuaiannya dengan standar nanopartikel. Ekstrak kamomil diperoleh melalui ultrasonikasi dengan etanol 70%, lalu dilakukan penetapan kandungan fenolik dan flavonoid total. Ekstrak diformulasikan ke dalam sistem Nanopartikel Kristal Cair Liotropik dan dikarakterisasi berdasarkan ukuran partikel, indeks polidispersitas, potensial zeta, stabilitas, dan efisiensi penjeratan. Aktivitas antiinflamasi diuji secara in vitro menggunakan metode

penghambatan denaturasi protein. Hasil menunjukkan ekstrak kamomil mengandung fenolik dan flavonoid kadar tinggi. Karakterisasi memenuhi spesifikasi nanopartikel dengan ukuran 230,366±3,412 nm, indeks polidispersitas 0,217±0,034, potensial zeta -23,763±0,756 mV, dan efisiensi penjeratan 84,060%±0,100. Penyimpanan pada suhu 4°C lebih efektif dibandingkan suhu kamar. Formulasi Nanopartikel Kristal Cair Liotropik secara signifikan meningkatkan aktivitas antiinflamasi ekstrak, ditunjukkan dengan nilai IC_{50} yang lebih rendah. Sistem ini mampu meningkatkan potensi terapeutik ekstrak kamomil dan menawarkan strategi menjanjikan dalam pengembangan formulasi antiinflamasi berbasis fitokimia.

Kata Kunci: *Antiinflamasi, Kamomil, LLCN*

INTRODUCTION

Inflammation is the body's natural reaction to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a complex biological process involving the activation of various types of immune cells and the release of pro-inflammatory cytokines. While inflammation is an important part of the immune system, uncontrolled or chronic inflammation can cause tissue damage and trigger various chronic diseases. Anti-inflammatory agents are required to treat these conditions. These agents include Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), corticosteroids, and cytokine inhibitors (1). In recent years, increasing attention has been directed towards phytochemicals derived from herbal plants as alternative anti-inflammatory agents to synthetic drugs. Notably, compounds such as flavonoids, classified under phenolic compounds, have shown promising anti-inflammatory potential (2).

Herbal plants play an important role in drug development, mainly due to their ability to synthesise secondary metabolites with significant biological activity. Chamomile extract, in particular, is widely recognised for its potent anti-inflammatory properties, primarily due to its rich content of flavonoids and other bioactive compounds. The active constituents are mainly present in the flowers and are commonly formulated as infusions, essential oils, or extracts. Chamomile contains over 120 constituents. Phenolic compounds, including phenolic acids and flavonoids, dominate chamomile extracts' content. Chamomile extract is particularly recognised for its high content of flavonoids and phenolic compounds, which are known to possess potent anti-inflammatory properties. The active constituents, primarily present in the flowers, include apigenin, luteolin, and quercetin, which are believed to exert anti-inflammatory effects by inhibiting the Cyclooxygenase (COX) enzyme, reducing pro-inflammatory cytokine production and preventing oxidative stress, whilst the phenolic acids also contribute to these effects by scavenging free radicals and reducing inflammation markers (3).

Ongoing research continues to understand the mechanisms and potential uses of this plant's anti-inflammatory properties in health and medicine. In vitro studies have demonstrated that both aqueous and alcohol extracts of chamomile exhibit anti-inflammatory activity, with measurable effects observed at concentrations as low as 200 µg/mL (4). Moreover, hydroalcoholic chamomile extract administered at high doses of 800 mg/kg and 1000 mg/kg has been shown to reduce paw and ear swelling and alleviate abdominal pain in animal models. Although chamomile possesses a wide range of bioactive compounds, its anti-inflammatory efficacy at lower doses remains limited, indicating the need for formulation strategies to enhance its activity. Innovative approaches, such as nanoparticle-based delivery systems, may offer solutions for increasing the efficacy and reducing the required dosages of chamomile extracts (5).

This study focuses on the Total Flavonoid Content (TFC) and Total Phenolic Content (TPC) tests due to their pivotal role in chamomile's anti-inflammatory properties. Although chamomile contains other bioactive compounds, flavonoids and phenolic acids are dominant and well-documented for their significant contributions to anti-inflammatory mechanisms. These compounds also synergise with other constituents within the extract, amplifying their overall therapeutic effects. The study aims to establish a direct correlation between these compounds and their anti-inflammatory efficacy by evaluating

flavonoid and phenolic content. It offers a targeted approach to improve bioactivity while reducing the required therapeutic dosage (6).

Lyotropic Liquid Crystal Nanoparticles (LLCNs) are an outstanding platform for drug delivery, serving as nanocarriers for therapeutic agents. LLCNs have various advantages, including efficient and targeted drug delivery and improving the physicochemical stability of active ingredients, thereby enhancing overall drug stability. LLCNs can also improve the bioavailability and therapeutic activity of an active ingredient (7). It is important to note that LLCNs are not an administration route but a nanocarrier system that can be further developed into oral or topical formulations, depending on the intended therapeutic use. Oral formulations offer the advantage of systemic absorption, while topical applications allow for localised delivery with minimised systemic side effects (8). Based on these considerations, this study aims to explore the LLCN system formulated with chamomile flower extract and assess its effectiveness in enhancing anti-inflammatory activity. The experimental design includes TFC and TPC assays, particle size analysis, zeta potential measurement, and entrapment efficiency assessment. Anti-inflammatory activity will be tested in vitro using the protein denaturation inhibition method.

The urgency of this study lies in the increasing demand for natural anti-inflammatory agents as safer alternatives to conventional synthetic drugs, which often have adverse side effects. Despite the well-documented anti-inflammatory properties of chamomile, the requirement for high doses to achieve significant therapeutic effects limits its clinical application (9). By incorporating chamomile extract into an LLCN system, this study addresses the critical need to enhance its active constituents' bioavailability and therapeutic efficacy while minimising the required dose (7).

The novelty of this research stems from its formulation of chamomile extract within an LLCN system, which has not been extensively studied. This innovative approach combines the anti-inflammatory potential of chamomile with the advanced drug delivery capabilities of LLCN, offering a promising strategy for improving therapeutic outcomes and expanding the practical applications of chamomile in medicine (10).

Materials and Methods

Materials

Chamomile (*Matricaria chamomilla* L.) flower powder was obtained from the Functional Service Unit of Traditional Health Services at Dr. Sardjito Tawangmangu General Hospital and was identified at the same location. The materials used in this study included 70% ethanol (C_2H_6O, Cipta Kimia, Indonesia) as the extraction solvent, 50% methanol (CH_3OH, Merck, Germany) for phytochemical screening, concentrated hydrochloric acid (HCl, 37%, Merck, Germany), 2 N hydrochloric acid (HCl, Merck, Germany), acetic acid anhydride ($C_4H_6O_3$, Merck, Germany), chloroform ($CHCl_3$, Merck, Germany), sulphuric acid (H_2SO_4, 95-98%, Merck, Germany), iron(III) chloride ($FeCl_3$, Merck, Germany), magnesium (Mg, Merck, Germany), and distilled water or aquadest (H_2O, Bratachem, Indonesia). Dragendorff's and Mayer's reagents (Merck, Germany) were used for alkaloid screening. Quercetin ($C_{15}H1_0O_7$, Sigma-Aldrich, USA) and gallic acid ($C_7H_6O_5$, Sigma-Aldrich, USA) were used as TFC and TPC determination standards, respectively. Aluminium chloride 2% ($AlCl_3$, Merck, Germany), sodium acetate 0.1 M (CH_3COONa, Merck, Germany), and Folin–Ciocalteu reagent (Merck, Germany) were used in the TFC and TPC analysis. Sodium carbonate 10% (Na_2CO_3, Merck, Germany) was also used in the TPC assay. Glyceryl monooleate ($C_{21}H_{40}O_4$, Gattefossé, France) and Poloxamer 407 (Sigma-Aldrich, USA) were used to prepare LLCN. For particle characterisation, distilled water (H_2O) was used to dilute the sample for Dynamic Light Scattering analysis. Bovine Serum Albumin (BSA, Sigma-Aldrich, USA), Tris Buffer Saline (TBS, Sigma-Aldrich, USA), Lowry C reagent, and Lowry D reagent (Sigma-Aldrich, USA) were used in the anti-inflammatory assay. Diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$, Sigma-Aldrich, USA) was a positive control in the in vitro anti-inflammatory activity test. All materials used were of analytical grade.

Methods

1. Loss on Drying of Powder

The loss on drying was determined using a moisture balance device (Ohaus MB45, USA), as modified from (11). Two grams of powder were weighed and placed on a calibrated plate at 105°C. The sample was levelled and heated until the device indicated completion.

2. Extraction

The extraction was carried out using the ultrasonication method with 70% ethanol as the solvent. A 1:10 weight-to-volume ratio was applied between chamomile powder and solvent. The chamomile powder was weighed, placed in a glass bottle, mixed with the solvent, and homogenised. Extraction was performed using a probe sonicator (SONICS Vibra-Cell, USA) at a fixed frequency of 35 kHz. Parameters included 20 minutes of ultrasonication, ultrasonic power of 300 W, and a constant temperature of 75°C. Following extraction, the filtrate was filtered and concentrated using a rotary vacuum evaporator (IKA RV 10, Germany) to obtain a viscous extract. The extract yield was then calculated (12).

3. Phytochemical Screening

Phytochemical screening was conducted with modifications from Emilia *et al*. (13). 10 mg of extract was dissolved in 1-2 mL of 50% methanol (CH_3OH) with gentle heating, followed by the addition of magnesium (Mg), and 4-5 drops of concentrated hydrochloric acid (HCl, 37%). The presence of flavonoid aglycones is indicated by a colour change to red or orange, known as the Cyanidin Reaction or Shibata Reaction. For alkaloid screening, 10 mg of extract was dissolved in 1.5 mL of 2% hydrochloric acid (HCl), and the solution was divided equally into three test tubes. The first tube served as a control, while the second was added with 2-3 drops of Dragendorff's reagent and the third with 2-3 drops of Mayer's reagent. The presence of alkaloids is indicated by the formation of turbidity or a brownish-orange precipitate in Dragendorff's reagent and a yellowish-white precipitate in Mayer's reagent. Steroid and terpenoid screening involved dissolving 10 mg of extract in 0.5 mL of acetic anhydride ($C_4H_6O_3$), mixing it with 0.5 mL chloroform ($CHCl_3$), and transferring the mixture to a dry tube. Then, 1-2 mL of sulphuric acid (H_2SO_4, 95-98%) was dripped along the tube wall using a pipette to initiate the Liebermann-Burchard reaction. A colour change from red to green or purple between the layers indicates the presence of steroids or triterpenoids. For tannin screening, 10 mg of extract was diluted in 2 mL of distilled water, followed by the addition of 3 drops of iron(III) chloride ($FeCl_3$). A blue-black colour indicates the presence of gallic tannins, while a blackish-green colour indicates catechol tannins. The saponin screening test was carried out by dissolving 10 mg of extract in 10 mL of hot water. After cooling, the solution was shaken vigorously for 10 seconds. The presence of saponins is confirmed if a stable froth forms (1-10 cm high) and lasts at least 10 minutes. The froth remained stable when one drop of 2 N hydrochloric acid (HCl) was added.

4. TFC

TFC was determined using a colourimetric method with quercetin ($C_{15}H_{10}O_7$) as the standard solution. For the analysis, 1 mL of the test or standard solution was mixed with 1 mL of 2% aluminium chloride ($AlCl_3$), 1 mL of 0.1 M sodium acetate (CH_3COONa), and 2 mL of distilled water (H_2O). After incubation at room temperature for 30 minutes, absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer. A calibration curve was constructed using quercetin standard solutions at concentrations of 20, 30, 40, 50, and 60 ppm to quantify the TFC in the sample (14).

5. TPC

TPC in the chamomile flower extract was determined using the Folin-Ciocalteu method. For the analysis, 1 mL of the test or standard solution was mixed with 5 mL of distilled water (H_2O), 1 mL of diluted Folin-Ciocalteu reagent, and 3 mL of 10% sodium carbonate (Na_2CO_3). After incubation for 1 hour, absorbance was measured at the maximum wavelength. A calibration curve was constructed using gallic acid ($C_7H_6O_5$) standard solutions at 30, 50, 70, 90, and 110 ppm concentrations to quantify the TPC in the sample (14).

6. Preparation of LLCN

The LLCN system was formulated using the top-down method. Initially, glyceryl monooleate ($C_{21}H_{40}O_4$) was heated on a magnetic stirrer at 60°C. Glyceryl monooleate, ethanol (C_2H_6O), and distilled water (H_2O) were added to the chamomile extract and stirred with a magnetic stirrer at 150 rpm to obtain the oil phase. Poloxamer 407 was weighed and dissolved in distilled water (H2O) using a magnetic stirrer for the water phase. This aqueous phase was then gradually added to the oil phase and homogenised using a sonicator for 5 minutes. The composition of the LLCN formulation, including

the proportions of chamomile extract, glyceryl monooleate, poloxamer 407, ethanol, and distilled water, is presented in **Table 1** (15).

Table 1. Chamomile Extract LLCN Formula

Materials	Amount (g)
Chamomile Extract	0.02
Glyceryl Monoleate	10
Ethanol	5
Aquadest	1.8
Poloxamer 407	1
Aquadest	82.18
Total	100

7. Characterisation of LLCN

Particle size and polydispersity index characterisation were carried out using a Particle Size Analyzer (Malvern Zetasizer, Malvern Instruments, UK) tool that utilises the Dynamic Light Scattering method. A total of 1 mL of sample was taken and placed into the cuvette. Next, the cuvette is inserted into the holder, and the analysis is carried out using the appropriate instrument to achieve particle size and polydispersity index. The zeta potential characterisation process was carried out using a Zetasizer device (Malvern Zetasizer, Malvern Instruments, UK). For this, the formulation was diluted in water to the required concentration. Then, the diluted sample is placed into a cuvette, and measurements are taken using the Zetasizer device to determine the zeta potential (16). LLCN then underwent the physical stability testing process, which refers to the duration a drug maintains its physical properties over a specific storage period. Samples were stored for 30 days at controlled room temperature (30°C) and cold temperature (4°C), after which particle size and zeta potential were re-measured. The final results were compared to baseline values to assess changes (17). The entrapment efficiency characterisation process was carried out using UV-Vis spectrophotometry (Shimadzu UV-1800, Shimadzu, Japan). The LLCN solution of the chamomile extract was diluted in a ratio of 1:1000, and then the absorbance was measured in the wavelength range of 200 to 500 nm. After that, the suspension was centrifuged (Gemmy PLC, Taiwan) at 20.000 rpm for 20 minutes. A supernatant of 0.1 mL was taken to measure the absorbance at the predetermined maximum wavelength. The percentage of LLCN uptake from the chamomile extract was calculated based on the data obtained through the formula (18):

$$\%EE = \frac{(Qt\text{-}Qs)}{Qt} x100\%$$

Description:
EE = Entrapment Efficiency
Qt = Amount of LLCN of chamomile extract added
Qs = Amount of LLCN of chamomile extract detected in the supernatant

8. In Vitro Anti-inflammatory Activity Assay

The anti-inflammatory activity assay was assessed by measuring the inhibition of BSA denaturation. The absorbance of each sample was determined based on the residual reaction between denatured BSA and anti-inflammatory compounds. Based on established pharmaceutical research standards, a sample was considered to exhibit anti-inflammatory activity if the inhibition percentage exceeded 20%. Diclofenac sodium (positive control) solution was prepared at concentrations of 15, 20, 25, 30, 35, and 40 ppm, the chamomile extract and LLCN extract at concentrations of 10, 20, 30, 40, 50, and 60 ppm. Each test solution was mixed with Lowry C reagent, Lowry D reagent, and 0.2% BSA in TBS, followed by incubation and heating. Absorbance was measured at 657 nm using a UV-Vis spectrophotometer (19).

Data Analysis

Data obtained from the anti-inflammatory activity testing of chamomile flower extract and LLCN were analysed using the one-way ANOVA statistical test with SPSS version 23.0 software. This test evaluated significant differences between samples, including the diclofenac sodium, chamomile viscous extract, and chamomile extract LLCN. The one-way ANOVA test, which compares the means of more than two groups, was conducted at a significance level of 0.05 to determine whether there are statistically significant differences.

RESULTS

1. Loss on Drying of Powder

Determination of drying shrinkage of chamomile powder was carried out using a moisture balance device and replicated 3 times. Results show a loss on drying of 6.333%±0.058, which conforms to the standard of 6.8% (11).

2. Extraction

The yield was calculated, showing that 500 grams of chamomile flower powder extracted by the ultrasonication method produced 179 grams of thick extract with a yield of 35.8%.

3. Phytochemical Screening

Table 2. Phytochemical Screening of Chamomile Extract

Phytochemical Screening		Literature Source (13)	Result	Description
Flavonoid		Presence of red or orange flavonoid aglycones	Red in colour	+
Alkaloid	Mayer	There is a yellowish-white precipitate	No precipitation	-
	Dragendorf	Turbidity or brownish-orange sediment present	No precipitation	-
Steroid and Triterpenoid		Colour change from red to green or purple between the two layers of solution	No purple ring	-
Tannin		Blackish green in colour	Blackish green in colour	+
Saponin		There is a stable froth	There is a stable froth	+

4. TFC

The maximum wavelength for absorbance was observed at 431 nm. TFC analysis was carried out by making a linear regression equation from the standard curve, resulting in the equation y = 0.0146x - 0.0575. Based on this equation, the total flavonoids in the sample were calculated and expressed as milligrams of Quercetin Equivalent per gram of sample (mg QE/g), which means that the amount of flavonoids detected in the sample is equivalent to a certain amount of quercetin, the standard used for calibration (14). The TFC of the ethanol extract of chamomile flowers was 14.220 ± 0.125 mg QE/g.

5. TPC

The maximum wavelength for TPC measurement was obtained at 653 nm. Analysis of TPC was carried out by making a linear regression equation from the standard curve, resulting in the equation y = 0.0082x - 0.0289. The TPC of the chamomile ethanol extract was 63.562±0.230 milligram Gallic Acid Equivalent per gram sample (mg GAE/g) (14).

6. LLCN Preparation Results

LLCN is a nanoparticle type formed from Lyotropic Liquid Crystal, which is a self-assembly structure formed from surfactants or other amphiphilic molecules in a solvent. The LLCN exhibited a uniform, milky white, and opalescent macroscopic appearance. This visual characteristic aligns with descriptions found in the literature for various LLCN systems, indicating a well-structured liquid crystalline arrangement. This structure causes strong Rayleigh scattering of visible light, leading to an opaque external appearance (20).

7. Characterisation of LLCN

Table 3. Particle Size, Polydispersity Index, and Zeta Potential Test Results of LLCN Chamomile Extract Before and After Storage Period

	Controlled Room Temperature (30°C)		Cold Temperature (4°C)		Standard (32)	Description
	Day 1	Day 30	Day 1	Day 30		
Particle Size (nm)	230.366± 3.412[a]	280.766± 12.37[b]	207.433± 1.415[a]	205.233± 1.823[b]	<1000 nm	Conforms to standard
Polydispersity Index	0.217± 0.034[a]	0.440± 0.032[b]	0.124± 0.016[a]	0.345± 0.017[b]	0.01-0.07	Conforms to standard
Zeta Potential (mV)	-23.763± 0.756[a]	-30.600± 1.156[b]	-25.583± 0.543[a]	-29.673± 0.854[b]	±30 mV	Conforms to standard

Different superscript letters ([a],[b]) indicate statistically significant differences between Day 1 and Day 30 ($p<0.05$, independent t-test).

A t-test was done to analyse particle size, polydispersity index, and zeta potential. The t-test results showed a significant difference in particle size, polydispersity index, and zeta potential, with a significance value (sig 2-tailed) <0.05, indicating statistically meaningful differences. At cold temperature (4°C), particle size slightly decreased from 207.433±1.415 nm on day 1 to 205.233±1.823 nm on day 30, which is ideal as a smaller particle size is preferred. The polydispersity index increased from 0.124±0.016 to 0.345±0.017, approaching the expected standard, indicating better stability in particle size distribution. Meanwhile, the zeta potential changed from -25.583±0.543 mV to -29.673±0.854 mV, aligning more closely with the required stability threshold for the dispersion system. Based on these results, storage at 4°C is more suitable than at controlled room temperature to maintain a smaller particle size and enhance the system's overall stability (21).

The entrapment efficiency analysis was carried out by making a linear regression equation based on the standard calibration curve. The linear regression equation obtained is y = 0.005x + 0.0166. This linear regression equation is then used to calculate the entrapment efficiency utilising the amount of LLCN chamomile extract added minus the amount of LLCN chamomile extract detected in the supernatant and multiplied by 100%. Optimal entrapment efficiency is achieved when the absorption efficiency approaches 100%. It indicates that almost the entire active substance is successfully encapsulated into the nanoparticle to be maximally utilised in therapy. In addition, a good entrapment efficiency is generally considered to be above 60%, which indicates that more than half of the initial active substance can be absorbed into the nanoparticle delivery system. The EE of LLCN average result was obtained at 84.060%±0.100, exceeding the standard threshold of >60% (22).

8. In Vitro Anti-Inflammatory Activity Assay

The anti-inflammatory activity of diclofenac sodium (positive control), chamomile viscous extract, and chamomile extract LLCN was evaluated by determining their IC_{50} values, representing the concentration required to inhibit 50% of BSA denaturation. The positive control used diclofenac sodium test results showed an IC_{50} value of 19.984±0.229 ppm, which confirms that diclofenac sodium showed better anti-inflammatory activity than chamomile (IC_{50} value of 52.041±0.066 ppm). The assay for LLCN of chamomile extract test results showed an IC_{50} value of 21.703±0.235 ppm, almost equivalent to diclofenac sodium, which has an IC_{50} value of 19.984±0.229 ppm, indicating that chamomile extract LLCN has higher anti-inflammatory activity than the extract. A lower IC_{50} value indicates stronger anti-inflammatory activity, reflecting a higher potency in inhibiting protein denaturation. This inhibition is directly correlated with anti-inflammatory activity, as the prevention of protein denaturation mimics the protection of cellular proteins during inflammatory conditions. Thus, the samples' ability to inhibit BSA denaturation demonstrates their potential as effective anti-inflammatory agents. A comparison of IC_{50} values for each sample is presented in **Figure 1**.

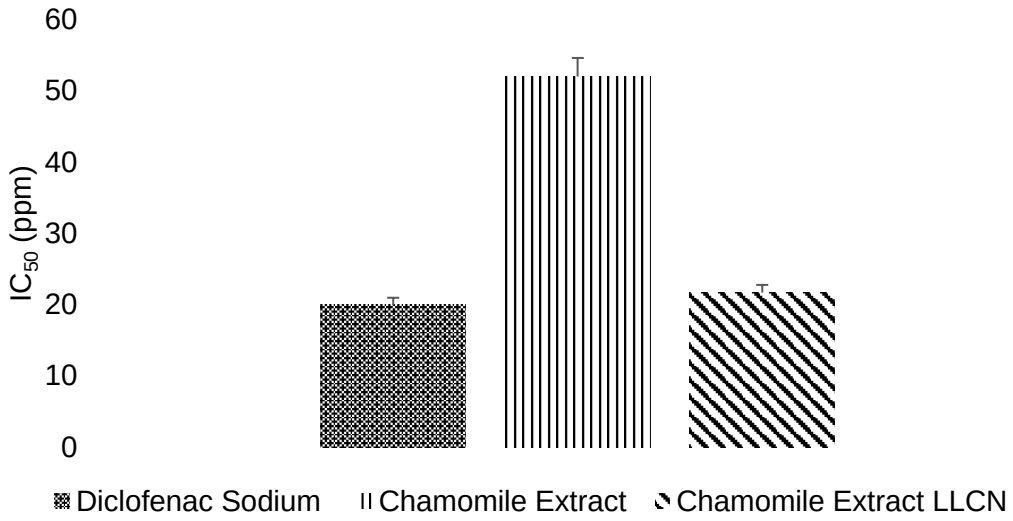

Figure 1. Histogram of IC_{50} values presented in ppm of diclofenac sodium, chamomile extract, and chamomile extract LLCN in the anti-inflammatory activity assay

DISCUSSION

Chamomile powder was identified successfully, confirming that the analysed samples were indeed chamomile flower powders. Loss on drying results show that the average drying shrinkage of chamomile flower powder after three repetitions is 6.333%, with a standard deviation of 0.058. The extraction of chamomile flowers was obtained with a yield of 35.800%, and a study recorded a yield range between 20-40% depending on the extraction parameters used. In this context, the yield of 35.800% indicates that the ultrasonication method effectively extracts bioactive compounds from chamomile flowers. This result is within the optimal range, indicating that the applied extraction process is good and efficient since the higher the extract yield, the more active substance compounds are extracted (23).

As shown in **Table 2**, phytochemical screening results confirmed the presence of flavonoids, tannins, and saponins. These compounds are essential in providing pharmacological effects, particularly anti-inflammatory activity. Flavonoids, which are polyphenolic compounds, exhibit strong antioxidant activity. This antioxidant property contributes significantly to their anti-inflammatory effects by scavenging reactive oxygen species and reducing oxidative stress, which are key factors in the inflammatory process. The ability of flavonoids to modulate inflammatory signalling pathways further underscores their dual role as antioxidants and anti-inflammatory agents, making them vital contributors to the therapeutic effects of chamomile extract. Among the major flavonoids in chamomile, apigenin has been shown to have significant anti-inflammatory properties. Apigenin is known to inhibit Cyclooxygenase-2 (COX-2) expression and histamine production from mast cells, as well as reduce

the production of Interleukin-6 (IL-6) and Interleukin-8 (IL-8), all of which play a role in the inflammatory response (4). Tannins, another class of polyphenolic compounds identified in the ethanol extract, are recognised for their protein-precipitating properties and astringent effect. Tannins have been reported to have anti-inflammatory activity by inhibiting enzymes involved in the inflammatory process and reducing cell membrane permeability, which helps reduce swelling and inflammation. Similarly, saponins were also found in chamomile flower extract. These compounds are known for their broad activities, including as anti-inflammatory agents. Saponins can interact with cell membranes, interfere with inflammatory signalling, and modulate the body's immune response. These effects help reduce inflammation and accelerate the healing process (24).

In testing flavonoid compounds, the quercetin standard was used as a comparison. The TFC value indicated that in each gram of chamomile flower extract sample, flavonoids were equivalent to 14.220±0.125 milligrams of quercetin. Study-reported findings showed variations in TFC, with results of 5.465±1.060 mg QE/g in water extracts and 12.64±2.3 mg QE/g in ethanol extracts (25). In this context, the results obtained fell within the range of commonly observed total flavonoid levels. Variations in total flavonoid levels are primarily influenced by several factors, including differences in extraction methods and types of solvents (26).

The TPC was also evaluated, and results indicated that each gram of chamomile flower extract sample contained phenolic compounds equivalent to 63.562±0.230 mg GAE/g. A study found that the TPC of chamomile flower extracts ranged from 37.8 to 57.2 mg GAE/g (27). In this context, the results obtained were within the reported total phenol levels range. Variations in total phenol levels are often attributed to differences in extraction methods, types of solvents, plant growth and storage conditions, and genetic variations among chamomile varieties (26).

Flavonoids and phenolic compounds are well-documented for their anti-inflammatory activity due to their ability to modulate several inflammatory pathways. Flavonoids inhibit key enzymes such as COX and Lipoxygenase (LOX), reducing the synthesis of pro-inflammatory mediators like prostaglandins and leukotrienes. Phenolic compounds contribute to anti-inflammatory effects by scavenging Reactive Oxygen Species (ROS) and inhibiting Nuclear Factor-Kappa B (NF-κB) activation, which is a central pathway in inflammation (3). As demonstrated in this study, the levels of these compounds in chamomile extract correlate with its ability to mitigate inflammatory responses. Higher concentrations of flavonoids and phenolics in the extract enhance its efficacy, as these compounds act synergistically to suppress oxidative stress and modulate inflammatory signalling. These findings underscore the importance of these bioactive compounds in developing chamomile-based anti-inflammatory therapies (28).

In drug development, designing advanced carrier systems is essential for enhancing drug delivery, stability, and bioavailability. Vesicular systems composed of lipid-based structures and stabilising agents have been widely explored for their ability to improve drug penetration, particularly in transdermal and topical applications (29). These systems share similarities with LLCN, which possesses a crystalline, regular, periodic internal structure, often forming a nanometre lattice, giving it unique physical and chemical properties such as thermal stability and better drug storage ability (30). The LLCN formulation consisted of two distinct phases designed to optimise the stability and efficacy of the nanoparticle system. The decision to use a single formula in this study, rather than introducing variations in extract doses or polymer types, was driven by the need for consistency and control over the formulation's characteristics. Maintaining a standardised formula allowed for a more precise evaluation of the effects of each component, such as glyceryl monooleate ($C_{21}H_{40}O_4$) and Poloxamer 407, on the stability and bioavailability of the LLCN. The study focuses on understanding the interactions between the surfactants, co-surfactants, and solvents by using a single formula, without the added variability that might come from altering the extract dose or polymer. Therefore, by keeping the formula consistent, the study aimed to ensure that any observed effects could be attributed to the formulation's core components and their interaction rather than external variations. This approach ensured a more precise and more reliable assessment of the LLCN system's properties and its potential application for therapeutic use (31).

Characterisation of nanotechnology involves the analysis of molecules or particles smaller than 1000 nanometres in size (32). The test results before and after the storage period show that the particle size of all formulas is in the nanoparticle range, which is less than 1000 nm. However, during the storage period, there was a change in particle size in LLCN due to agglomeration between particles. The results

of polydispersity index testing before and after the storage period showed a narrow particle size distribution with polydispersity index values in the range of 0.124-0.440, which could be due to the use of surfactants that effectively improve the homogeneity of the extract compounds. The polydispersity index shows the size distribution of nanoparticles in a preparation. In this study, the polydispersity index was in the range of 0.01 to 0.07 for monodisperse particles. The monodisperse nanoparticles can improve the stability of nanoparticle systems because they have a homogeneous particle size, shape, and weight. The higher the polydispersity index, the more particles are aggregated, or in other words, the more unstable the preparation is (33).

The zeta potential test results show that the zeta potential value of LLCN chamomile extract has not exceeded the threshold value of ±30 mV. Nanoparticles with zeta potential values below -30 mV or above +30 mV show high stability. Dispersion systems with low zeta potential values are more prone to aggregate formation due to the presence of Van der Waals forces in inter-particle interactions (34). This low zeta potential value indicates that the chamomile extract LLCN will remain physically stable (35). Stability in the LLCN system is a critical factor for its pharmaceutical application, with storage temperature playing a significant role in maintaining its physicochemical properties.

Statistical analysis using a t-test revealed that storage at 4°C significantly influenced particle size, polydispersity index, and zeta potential compared to room temperature. Smaller particle sizes, as observed at 4°C, are associated with improved bioavailability and cellular uptake, essential for enhancing therapeutic efficacy (36). Though slightly increased, the polydispersity index remained within the acceptable range (<0.05), indicating moderate uniformity and system stability, consistent with findings from nanoparticle-based delivery systems. Additionally, the zeta potential shifted towards more negative values, aligning with stability requirements, as values exceeding ±25 mV are known to prevent particle aggregation through strong electrostatic repulsion. These findings underscore the importance of controlled storage conditions, particularly cold temperatures, in preserving the stability and functionality of nanoparticle systems. The LLCN system's ability to maintain optimal characteristics at 4°C highlights its potential for developing stable and effective chamomile-based anti-inflammatory formulations, supported by recent advancements in nanocarrier research (37).

Optimal entrapment is achieved when the entrapment efficiency approaches 100%, which indicates that almost the entire active substance is successfully encapsulated within the nanoparticles and available for therapeutic use. In addition, a good entrapment efficiency is generally considered to be above 60%, which indicates that more than half of the initial active substance can be absorbed into the nanoparticle delivery system (22). The high entrapment efficiency average of 84.060%±0.100 is essential because it can increase the therapeutic potential of the chamomile flower extract. With good entrapment efficiency, the amount of active compound required to achieve a significant anti-inflammatory effect can be optimised. It means that lower doses can be used to achieve the same effect, which in turn can reduce potential side effects and improve the safety profile of the nanoparticle formulation (38).

The IC_{50} value represents the concentration of extract required to inhibit 50% of protein denaturation. As shown in **Figure 1**, the LLCN formulation of chamomile extract demonstrated improved anti-inflammatory activity compared to the chamomile viscous extract, as indicated by its lower IC_{50} value. This enhancement may be attributed to the nanostructured system of LLCN, which increases the surface area, improves solubility, and facilitates better interaction with biological targets. Additionally, LLCN enhances the stability and bioavailability of active compounds, potentially prolonging their therapeutic effects. Compared to conventional chamomile extract, the LLCN formulation exhibits notable improvements in efficacy. However, while LLCN enhances the performance of chamomile extract in this assay, further comparative studies with alternative nanocarrier systems and different formulation strategies are needed to understand its advantages fully. The anti-inflammatory activity of diclofenac sodium (positive control), chamomile viscous extract, and chamomile extract LLCN was assessed based on their IC_{50} values, representing their ability to inhibit BSA denaturation. Diclofenac sodium exhibited an IC_{50} value of 19.984±0.229 ppm, while chamomile extract showed an IC_{50} value of 52.041±0.066 ppm. The LLCN formulation of chamomile extract yielded an IC_{50} value of 21.703±0.235 ppm, approaching the result observed with diclofenac sodium. A lower IC_{50} value signifies a greater capacity to inhibit protein denaturation, an important indicator of anti-inflammatory potential. These findings suggest that the LLCN system enhances the bioactivity of chamomile extract, making it a promising candidate for further development in inflammatory therapy (7,39).

Data obtained from the anti-inflammatory activity testing of chamomile flower extract and LLCN were statistically analysed using normality, homogeneity, and one-way ANOVA tests with SPSS software. The statistical analysis applied to the data included tests for normality using the Shapiro-Wilk method and homogeneity tests, which confirmed that the data were normally distributed and homogeneous (sig >0.05). However, the significant differences (sig <0.05) observed in the one-way ANOVA test underscore the variability in IC_{50} values among the three groups. Additional statistical analyses are required to fully validate these findings, especially considering the need for more comprehensive variable data that directly compare LLCN technology with other formulation strategies. The homogeneity test also shows sig >0.05, meaning the sample is homogeneous. However, the one-way ANOVA test showed sig <0.05, indicating a significant difference in IC_{50} values between diclofenac sodium, chamomile viscous extract, and LLCN of chamomile extract, having abnormal data distribution due to a significant difference in IC_{50} values. Based on the description above, there is a significant difference between the three variables, and it can be concluded that the LLCN of chamomile extract has significantly higher anti-inflammatory activity when compared to chamomile thick extract. Still, it is also not equivalent to the positive control, namely diclofenac sodium.

The formation of the LLCN system significantly enhances the anti-inflammatory activity of chamomile extract, as evidenced by the lower IC_{50} value compared to the viscous extract. This improvement is attributed to the unique characteristics of the LLCN system, including its optimal particle size, polydispersity index, zeta potential, and entrapment efficiency. The smaller particle size of LLCN facilitates better cellular uptake and bioavailability. At the same time, the stable zeta potential ensures colloidal stability, preventing aggregation and maintaining the integrity of the encapsulated active compounds. Additionally, the high entrapment efficiency of the LLCN system ensures that a more significant proportion of the bioactive compounds in chamomile extract are effectively delivered to the target site. These properties collectively contribute to the enhanced anti-inflammatory activity observed in vitro, demonstrating the potential of LLCN technology to improve the therapeutic efficacy of phytochemical-based formulations. Further studies are needed to explore the full scope of LLCN's advantages, including its potential for controlled and sustained release and its applicability in other delivery routes.

CONCLUSION

This study confirmed that the LLCN system effectively enhances the anti-inflammatory activity of chamomile extract, as demonstrated by a lower IC_{50} value (21.703±0.235 ppm) compared to the extract alone (52.041±0.066 ppm). The improved activity is attributed to the LLCN system's characteristics, including optimal particle size, polydispersity index, zeta potential, and high entrapment efficiency (84.060%±0.100), all of which collectively to improved bioavailability and therapeutic potential. Additionally, the LLCN formulation met nanoparticle standards in terms of stability and physicochemical properties, with superior performance when stored at 4°C. These findings indicate that LLCN technology offers a promising approach for improving the anti-inflammatory efficacy of chamomile extract while maintaining compliance with nanoparticle formulation criteria. These results provide a strong rationale for future in vivo studies focusing on anti-inflammatory efficacy, pharmacokinetic profile, and broader therapeutic applicability of chamomile extract LLCN in various inflammation-associated disease models.

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