

CURCUMINOID NANOEMULSION FROM *Curcuma xanthorrhiza* EXTRACT AND ITS ACTIVITY AS ANTIOXIDANT, ANTIBACTERIAL AND ANTIFUNGAL

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ABSTRACT

Curcuminoids are the main compounds in the rhizome extract of *C. xanthorrhiza* which are beneficial for health. The aim of the research was to study the formulation of curcuminoid nanoemulsions and their biological effects as antioxidant, antibacterial, and antifungal agents. The formulation of the curcuminoid nanoemulsion was carried out using the spontaneous emulsion method, by mixing the curcuminoid extract with oil and tween 80 and adding phosphate buffer pH 7.0. Curcuminoid nanoemulsion products were characterized and tested for their potential biological activity. Antioxidant test using reagent 2,2-diphenyl-1-picrylhydrazyl. Antibacterial test using diffusion method against *Escherichia coli* and *Staphylococcus epidermidis* bacteria. Antifungal test using *Candida albicans*. The curcuminoid nanoemulsion product showed a particle size of 19.5 to 32.1 nm, a polydispersity index < 0.3, and a zeta potential of -4.5 to -9.2 mV. Curcuminoid nanoemulsions have high antioxidant and antifungal activity, while antibacterial activity showed moderate activity. The conclusion is that curcuminoid nanoemulsions from *C. xanthorrhiza* extract can be used effectively as antioxidants and antimicrobial for commercial applications.

Keywords: Antimicrobial, Antifungal, Antioxidant, Curcuminoid, Nanoemulsion

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INTRODUCTION

Curcuma xanthorrhiza Roxb. is a herbal plant that is traditionally used in the treatment of various diseases, including liver disorders, constipation, diarrhea, dysentery, gastric disorders, hemorrhoids, and skin disorders.¹ In Indonesia, this plant is endemic with the local name “temulawak”. The main components in the rhizome of *C. xanthorrhiza* are curcuminoids and essential oils. The curcuminoid components in the extract of *C. xanthorrhiza* mainly consist of curcumin and demethoxycurcumin.^{2,3} Some of the pharmacological effects of curcuminoid compounds include anticancer⁴, antioxidant⁵, anti-inflammatory⁶, anti-osteoarthritis⁷, anti-Alzheimer's⁸, immune-stimulating activity⁹, anti-cholesterol, and triglycerides.¹⁰ However, up to the present time, there are some questions to be resolved in the use of curcuminoids in medicine. It is because of its poor pharmacokinetic profile and its less beneficial therapeutic potential.¹¹ Curcuminoids are known to show properties that are not easily absorbed by the small intestine and are unstable in the metabolic system.¹² Several strategies have been reported such as inhibition of curcuminoid metabolism by coating the ingredients, as well as the formulations with carrier matrices in solid or liquid form. This formulation has been developed to increase absorption and rapid elimination from the body.¹³ The research conducted by Hanna and Saad¹⁴ showed that nano curcumin with the sol-oil method increased the curcumin absorption and bioavailability, and significantly increased the occurrence of Hep-2 cell apoptosis as a marker of DNA damage. Curcumin nanoparticles using PVP-PCL carrier compounds can inhibit tumor growth better than radiation therapy in xenograft model A549.¹⁵ Nanocurcuminoids made using chitosan and alginic acid matrix showed higher antioxidant activity compared to native curcuminoids.¹⁶ Several methods of synthesis of nanocurcuminoids that have been developed include ionic gelation method, emulsification, coacervation or precipitation, and spray drying method.¹⁷ This study aims

to synthesize curcuminoid nanoemulsions by spontaneous emulsion method, by mixing curcuminoid extract into a mixture of oil and surfactant (tween 80). Variations of oil used in this study were virgin coconut oil (VCO) and olive oil. The curcuminoid nanoemulsion product obtained was then characterized and tested for its biological activity.

EXPERIMENTAL

Chemical and Apparatus

The chemicals used include curcuminoid fraction from ethanol extract *C. xanthorrhiza* with demethoxycurcumin 20.97%³, ethanol, aqua dest (Distilled Water), tween 80 (Merck), phosphate buffer pH 7.0 (Merck), ascorbic acid (Aldrich), olive oil, virgin coconut oil (VCO), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Aldrich), chloramphenicol, nystatin, Mueller-Hinton agar (MHA, OXOID), nutrient broth (NB), nutrient agar (NA), paper disk, and aluminum foil. The tools used include digital analytical balance, Buchi Rotavapor R114, magnetic stirrer IKA C-MAG HS7, centrifuge, particle size analysis (PSA) Horiba SZ-100, pH meter, Oswald viscometer, UV-Vis spectrophotometer, and TEM (Transmission Electron Microscopy) FEI Tecnai G2 20 S-Twin.

Curcuminoid Nanoemulsion Preparation

The curcuminoid nanoemulsion formulation was optimized for various oil and surfactant compositions. The oil used is olive oil and VCO. The surfactant used was Tween 80. In this experiment, variations in the ratio of oil and tween were 1:4; 2:4; 3:4; 1:5; and 1:6. A total of 0.01 g of curcuminoid extract was added to the mixture of oil and tween 80 according to the ratio, then homogenized by stirring and heating using a magnetic stirrer at 70°C for 20 minutes. Furthermore, to form an emulsion, phosphate buffer was added while stirring continuously for one hour at a temperature of 70°C. The final volume of each emulsion is 50 mL. The curcuminoid nanoemulsions from each experiment were further characterized, which included analysis of particle size, polydispersity index, zeta potential, pH, viscosity, morphology, and stability. The viscosity of each curcuminoid nanoemulsion was measured using an Oswald viscometer. The curcuminoid nanoemulsion product was tested for physical stability and the stability of the effect of storage at room temperature. A physical stability test of curcuminoid nanoemulsion was carried out by observing the occurrence of sample damage due to centrifugation at 12,000 rpm for 15 minutes. The stability of the curcuminoid nanoemulsion product in storage was carried out by inserting each sample of the curcuminoid nanoemulsion into a test tube and covered with aluminum foil, then left at room temperature for five weeks. Furthermore, the samples were observed for damage every week. The parameters for the occurrence of curcuminoid nanoemulsion damage include the formation of turbidity, cream, and cracks.¹⁸ The formulation of curcuminoid nanoemulsion was also carried out at various concentrations of the curcuminoid extract used. Variations in the concentration of the curcuminoid extract used were 0.01 to 0.05 %. The curcuminoid nanoemulsions formed were also characterized including particle size, polydispersity index, zeta potential, pH, viscosity, and transmittance percentage (%T) as measured by UV-Vis spectrophotometer, and morphological observations using TEM (each experiment was repeated 3 times).

Antioxidant Activity

The antioxidant effect of curcuminoid nanoemulsion using DPPH reagent (2,2-diphenyl-1-picrylhydrazyl) was expressed as IC₅₀ as has been done in a previous study.³ Solutions were made at various concentrations of each curcuminoid nanoemulsion product, reacted with DPPH solution in ethanol, homogenized, and incubated at room temperature for 30 minutes. The absorbance of each sample was measured using a spectrophotometer at 516 nm.

Antibacterial Activity

The antibacterial activity test used two bacterial isolates, namely *Escherichia coli* ATCC 11229 and *Staphylococcus epidermidis* FNCC 0048, with the agar diffusion method as in the previous study.¹⁶ Chloramphenicol (200 mg/mL) was used as a positive control, while distilled water was used as a negative control. The inhibition zone formed around the paper disk was measured at incubation times of 6, 12, 18, and 24 hours. Eight samples (negative control, positive control, and curcuminoid nanoemulsion formulas K-1 to K-5) were placed in different Petri dishes, and each sample was replicated three times.

Antifungal Activity

The antifungal activity test was carried out on *Candida albicans* by diffusion method.¹⁹ Liquid dextrose was poured into a sterile petri dish and allowed to solidify. After solidification, as much as 1mL of the mushroom suspension was spread on the surface of the media so that it was evenly distributed. Paper disks were immersed in each sample of curcuminoid nanoemulsion at various concentrations, positive control (nystatin), and negative control (phosphate buffer pH 7.0), allowed to stand for 15 minutes. Each paper disk was then placed on the surface of the media and incubated at 37°C. The inhibition zone formed around the paper disc was measured at 6 and 12 h incubation.

RESULTS AND DISCUSSION

The optimization data of curcuminoid nanoemulsion formulations on various compositions of oil and surfactants used are shown in Table-1. The oils used in this study were olive oil (BZ1- BZ5) and VCO (AV1-AV5). The variations in the ratio of oil: Tween 80 of 1:4; 2:4; 3:4; 1:5; and 1:6. A total of 0.01 g of curcuminoids were added to each formulation and the final volume of each formula was 50 mL with the addition of phosphate buffer. The characterization of each formula shows that the particle size ranged from 9.4 to 32.0 nm, has a polydispersity index (PI) below 0.2, and the zeta potential ranges from -1.8 to -11.1 mV (Table-1). The curcuminoid nanoemulsion product has a pH of about 6.7 with a slightly thicker viscosity than distilled water. Table-1 shows that curcuminoid nanoemulsions are made with the addition of oil and Tween 80 in various compositions. It shows relatively small, uniform, and relatively stable particle diameter sizes. Curcuminoid nanoemulsion products are brownish-yellow, clear, transparent, and water-soluble, which is known as oil-in-water (O/W) dispersion. Centrifugation at 15,000 rpm for 15 minutes the curcuminoid nanoemulsion product is still relatively stable. The curcuminoid nanoemulsion product using olive oil started to become cloudy in the third week, while the one using VCO remained clear in the fifth week. Curcuminoid nanoemulsion using VCO is more stable than using olive oil. This could be because VCO contains more saturated fatty acids, while olive oil contains more unsaturated fatty acids.²⁰ Fatty acids that have a lot of double bonds will usually be easily damaged compared to saturated fatty acids that don't have double bonds. The resulting damage causes turbidity and sedimentation of the nanoemulsion. The composition of the ratio of oil and Tween 80 affects the diameter size of the curcuminoid nanoemulsion. The VCO-Tween 80 ratio at 1:4 indicates a particle size of 32 nm. The size of the nanoemulsion usually ranges from 20-to 200 nm.²¹ From the optimization data, then the ratio of VCO and Tween 80 with a ratio of 1:4 was used for the formulation of curcuminoid nanoemulsion products at various concentrations of curcuminoids.

Table-1: Curcuminoid Nanoemulsion at Various Compositions of Oil and Surfactants

No	Code	Characterization			
		Particle Size (Z-average nm)	Polydispersity index	Zeta Potential (mV)	Viscosity (cP)
1	Control E	10.2	0.133	-2.5	1.638
2	Control F	10.2	0.106	-1.8	1.526
3	BZ1	10.4	0.173	-8.4	1.521
4	BZ2	12.1	0.174	-4.6	1.526
5	BZ3	10.8	0.120	-6.0	1.656
6	BZ4	9.4	0.125	-11.1	2.027
7	BZ5	9.2	0.101	-9.4	2.542
8	AV1	31.0	0.162	-5.5	1.897
9	AV2	11.4	0.136	-4.3	1.785
10	AV3	14.3	0.136	-7.3	1.651
11	AV4	9.4	0.116	-3.1	1.917
12	AV5	9.4	0.127	-3.7	2.169

Table-2 shows the characteristic data of curcuminoid nanoemulsion in variations of curcuminoid extract from 0.01 to 0.05 g. The curcuminoid nanoemulsion products obtained at various concentrations of curcuminoid extracts had particle sizes of 19.5 to 32.1 nm, polydispersity index <0.3, and showed zeta

potential at -4.5 to -9.2 mV. The curcuminoid nanoemulsions have a clear yellow color with a transmission percentage of about 97%. The morphological shape of the curcuminoid nanoemulsions is in the form of a sphere, with circular walls is Tween 80 which is a surfactant that protects oil and curcuminoids (Figure-1). The characteristics of curcuminoid nanoemulsions are influenced by many factors, including preparation method, type of oil, type of surfactant, the ratio of oil and surfactant composition, and concentration of curcuminoid extract. The curcumin nanoemulsion formulated with oil droplets of medium-chain triglycerides, whey-70 protein, and Tween-80 had a particle size of 141.6 ± 15.4 nm and zeta potential of 6.9 ± 0.2 Mv.²² Curcumin nanoparticles were prepared by dissolving curcumin with DMSO (dimethyl sulfoxide) and mixed with olive oil, without the use of surfactants having a particle size distribution with an average diameter of 28 nm.²³ Antioxidant activity test using DPPH method of curcuminoid nanoemulsion compared to curcuminoid extract has a lower IC₅₀ (Fig.-2). From these data, it can be seen that the curcuminoid nanoemulsion showed increased activity compared to the curcuminoid extract. However, when compared with ascorbic acid, the activity of curcuminoid nanoemulsion and curcuminoid extract was lower. Vitamin C or ascorbic acid is a natural antioxidant that shows very active antioxidant activity. Previous studies have shown that curcuminoid extracts have high antioxidant activity.³ The antioxidant activity test of curcumin in the form of nanoemulsion was more active than curcumin in its original form.²⁴ The research conducted by Shah²⁵ showed higher radical scavenging activity when curcumin encapsulated using chitosan. The results of the curcuminoid nanoemulsion from this study only used VCO oil and Tween 80, phosphate buffer as the aqueous phase and did not use other cofactors. Thus, nanoemulsion curcuminoids are safe to use as natural drugs or antioxidants.

Table-2: Curcuminoid Nanoemulsion at Various Concentration Curcuminoid

Formul a	Curcuminoid (g)	Characterization					
		pH	Viscosity (cP)	%T	Particle Size (Z-average nm)	Polydispersity Index	Zeta Potential (mV)
K-1	0.01	6.9	1.897	98.8	28.3	0.248	-6.6
K-2	0.02	6.9	1.896	97.7	19.5	0.151	-9.2
K-3	0.03	6.8	1.981	97.5	21.3	0.123	-5.8
K-4	0.04	6.8	2.020	97.6	25.7	0.15	-4.5
K-5	0.05	6.8	1.992	97.3	32.1	0.267	-7.7

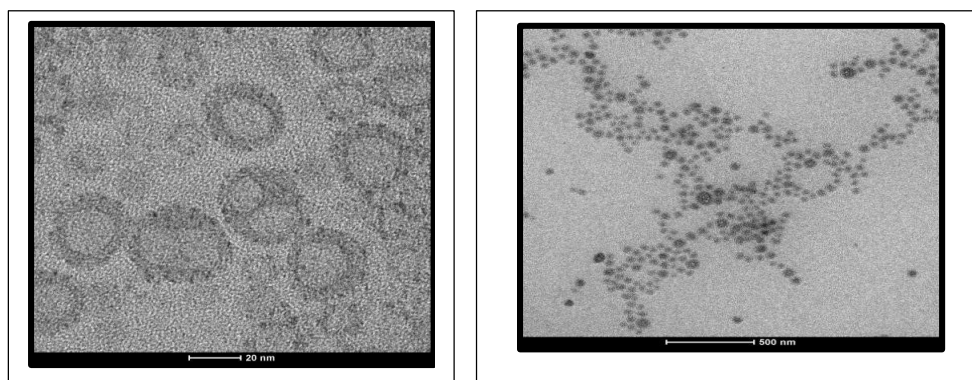


Fig.-1: The Morphological Shape of The Curcuminoid Nanoemulsion by TEM At 20 & 500 Nm (K-5)

The antibacterial activity of the curcuminoid nanoemulsion is shown in Fig.-3. The bacteria used were *S. epidermidis* and *E. Colli*. Observation of the diameter inhibition zone was carried out at incubation times of 6, 12, 18, and 24 hours. The optimal growth phase of *S. epidermidis* and *E. Colli* bacteria is 6-24 hours. This measurement used positive control chloramphenicol, while the negative control used aqua dest. The results showed that the curcuminoid nanoemulsion (K1-K5) showed moderate activity, and was relatively stable at incubation at 6 to 24 hours. Previous studies showed that the antibacterial activity of curcuminoid extracts against *E. Colli* and *S. epidermidis* bacteria showed moderate activity.³ The curcumin nanoemulsion with chitosan and linseed oil showed higher antibacterial activity than the fish oil

nanoemulsion with chitosan.²⁶ Likewise, the results of Cho²⁷ showed that the nanoemulsion of *C. xanthorrhiza* oil was a strong antimicrobial against *Streptococcus mutants*. The mechanism of antimicrobial activity of several natural compounds can occur through various methods, namely inhibiting nucleic acid synthesis, forming complex bonds with cell walls, inhibiting cell wall proteins, inhibiting cell wall permeability, and inhibiting microbial metabolism.²⁸

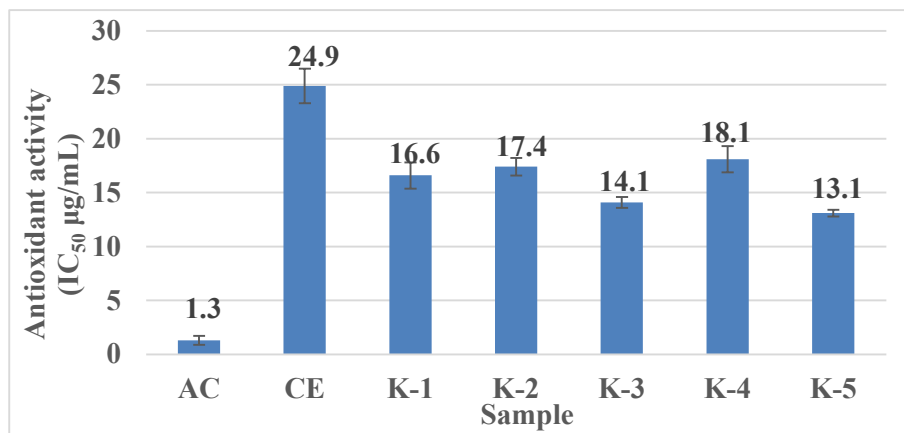


Fig.-2: Antioxidant Activity of Curcuminoid Nanoemulsion (K-1 To K-5) Compared to Ascorbic Acid (AC, and Curcuminoid Extract (CE)

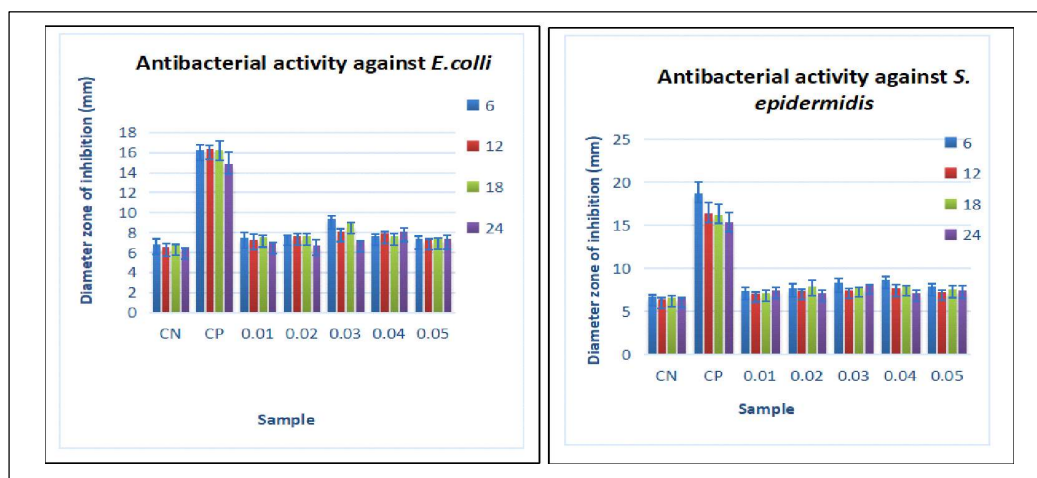


Fig.-3: Antibacterial Activity of Curcuminoid Nanoemulsion (K-1 To K-5), CN (Negative Control, Aquadest), and Positive Control (CP, Chloramphenicol) against *E. Colli* and *S. Epidermidis* at 6, 12, 18, and 24 Hours

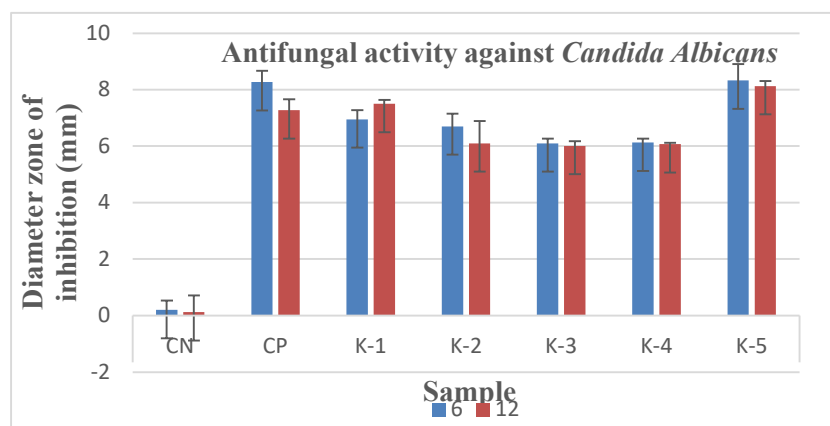


Fig.-4: Antifungal Activity of Curcuminoid Nanoemulsion (K-1 To K-5), CN (Negative Control, Aquadest), and Positive Control (CP, nystatin 200 Mg/ml) against *Candida Albicans* at 6 And 12 Hours

The antifungal activity against *Candida albicans* from curcuminoid nanoemulsion is shown in Fig.-4. As negative control was aqua dest, positive control was nystatin 200 g/mL. From these data, all curcuminoid nanoemulsion products have high antifungal activity. The results of this study are consistent with previous studies showing that curcumin nanoemulsions or in combination with piperine and honey were highly effective against *C. glabrata* (ATCC 2001) and *C. albicans* (ATCC10231).²⁹

CONCLUSION

The curcuminoid nanoemulsion formulation can be made by spontaneous emulsion by mixing oil, tween 80, and phosphate buffer. The curcuminoid nanoemulsion product obtained at various concentrations of curcuminoid extract from 0.01 to 0.05% had a particle size of 19.5 to 32.1 nm, polydispersity index < 0.3, and zeta potential -4.5 to -9.2 mV. The curcuminoid nanoemulsion product showed antioxidant, antibacterial, and antifungal activity. Thus, curcuminoid nanoemulsions can be developed as drugs, as well as additives in food and cosmetics.

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