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Synthesis of Palmitate Curcumin Ester via Steglich Esterification and Evaluation of Antimicrobial Activity and Toxicity against *Daphnia magna*

Mentari Zikri Anty*[1], Azimatur Rahmi[2], M. Sulthon Nurharmansyah Putra[3], Minandre Wiratama[4], Sumi Hudiyono[5]

[1,2,3,4] Department of Chemistry, Universitas Pertahanan RI, Kawasan IPSC Sentul, Bogor, West Java, Indonesia

[5] Department of Chemistry, Universitas Indonesia, Jl. Lingkar Kampus Raya, Depok, West Java, Indonesia

e-mail: mentarizikri17@gmail.com

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Abstract

Curcumin possesses a wide range of biological activities; however, its practical application is constrained by poor aqueous solubility and low bioavailability. Chemical modification via esterification with fatty acids offers a promising strategy to enhance its physicochemical properties and biological performance. This study aimed to synthesize palmitate curcumin esters through Steglich esterification and to evaluate their antimicrobial activity as well as their toxicity toward *Daphnia magna*. The synthesis was performed by reacting curcumin with palmitic acid in the presence of N, N-dicyclohexylcarbodiimide (DCC) and 4 dimethylaminopyridine (DMAP) as coupling agents. The resulting mono- and dipalmitate curcumin esters were subsequently purified using column chromatography and characterized by Fourier Transform Infrared (FT-IR) and UV–Visible spectroscopy to confirm the successful formation of ester bonds. Antimicrobial activity was assessed against *Staphylococcus aureus* and *Escherichia coli* using the disk diffusion method, while acute toxicity was evaluated using *Daphnia magna* through LC_{50} determination. The findings revealed that both ester derivatives exhibited relatively weak antimicrobial activity, with inhibition zones ranging from 7.00 to 8.33 mm. In contrast, toxicity evaluation demonstrated that monopalmitate–curcumin exhibited higher toxicity (LC_{50} = 153.65 mg/L) compared to dipalmitate–curcumin (LC_{50} = 412.28 mg/L). This difference is likely attributed to the presence of a free phenolic hydroxyl group in the monoester structure, which may enhance its biological reactivity. Overall, the results suggest that esterification modifies curcumin's properties, although further optimization is required to improve its antimicrobial efficacy while maintaining acceptable toxicity levels.

Keywords: Steglich esterification, curcumin, palmitic acid, antimicrobial activity, toxicity, *Daphnia magna*

1. Introduction

Infectious diseases remain a major public health challenge in Indonesia. A number of emerging infections have been reported, showing increases in both case numbers and geographic spread. Emerging Infectious Diseases (EIDs) are defined as diseases that appear for the first time in a population or previously existing diseases that rapidly increase in

incidence or expand to new geographic areas (re-emerging infectious diseases). EIDs can be caused by viral and bacterial infections (Gunawan et al., 2022). In Indonesia, the Ministry of Health has identified several diseases with the potential to cause public health emergencies and attract global concern, including cholera, meningitis, yellow fever, hantavirus infection, Severe Acute Respiratory Syndrome (SARS), Ebola, Japanese encephalitis, acute poliomyelitis, anthrax, Nipah virus infection, and avian influenza (Kementrian Kesehatan RI, 2025). Therefore, strengthening early detection systems and enhancing prevention and control measures are essential to effectively manage infectious diseases. One promising approach under development is the utilization of fatty acids as potential sources for drug development.

Certain saturated and unsaturated fatty acids have been reported to modulate the immune system, enhance immune responses, and exhibit antimicrobial, antiviral, and antiparasitic activities (Afroze et al., 2024). Moreover, the development of such compounds may help address the growing global challenge of antibiotic resistance. Previous studies have shown that several unsaturated fatty acids possess inhibitory activity against both Gram-positive and Gram-negative bacteria (Kumar et al., 2020; Yoon et al., 2018). In addition, toxicity studies have indicated that oleic acid–curcumin ester derivatives exhibit strong toxic effects against *Daphnia magna* (Anty et al., 2024).

Fatty acids are generally classified into short-chain and long-chain types. Palmitic acid (C16), a long-chain saturated fatty acid, has been reported to inhibit bacterial enzymatic activity, suppress the growth of protozoa, viruses, and fungi, and exhibit antibacterial properties (Hendy et al., 2020). It has been isolated from *Sargassum polycystum* extracts and demonstrated antibacterial activity against *Escherichia coli* as well as anti-inflammatory effects (Maghfira, 2024). Consequently, palmitic acid holds potential for the treatment of inflammatory conditions, bacterial infections, and viral diseases. Previous research has also reported that esterification of decanoic and palmitic acids with C5-curcuminoid compounds enhances anticancer activity (Sanabria-Ríos et al., 2015). Curcumin is a natural bioactive compound widely studied for its antimicrobial, antioxidant, and anti-inflammatory properties. However, its application is limited by poor aqueous solubility and low bioavailability (Aggarwal et al., 2017; Hewlings, 2017). Chemical modification through esterification with fatty acids represents an effective strategy to improve its physicochemical properties. The presence of phenolic hydroxyl groups in curcumin enables the formation of ester derivatives with long-chain fatty acids, which can increase lipophilicity and support pharmaceutical formulation

Esterification of curcumin with saturated fatty acids represents an effective strategy to modify its chemical and biological properties, potentially improving solubility in non-polar solvents and enhancing therapeutic applications. This process involves the reaction between curcumin, which contains two phenolic groups, and saturated fatty acids with long hydrocarbon chains, facilitating pharmaceutical formulation (Agrawal & Jaiswal, 2022).

Despite these promising findings, studies on the synthesis of palmitate–curcumin esters via Steglich esterification and their evaluation in terms of antimicrobial activity and toxicity remain limited. In particular, the relationship between structural modification and biological effects has not been systematically investigated. Therefore, this study aims to synthesize

palmitate–curcumin esters using the Steglich esterification method and to evaluate their antimicrobial activity and toxicity as potential novel therapeutic agents.

2. Methods

Materials

The materials used in this study included palmitic acid, N,N-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), methanol, ethanol, distilled water, silica gel, hexane, ethyl acetate, nitrogen gas, cotton, n-hexane, Whatman filter paper, dimethyl sulfoxide (DMSO), 1 M H_2SO_4, Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*), nutrient agar, peptone, and beef extract. The ester products were purified using column chromatography with silica gel 60 (70–230 mesh). Qualitative characterization was performed using Fourier Transform Infrared (FTIR) spectroscopy (Shimadzu) and a UV–Visible spectrophotometer (Shimadzu 2600). Toxicity testing was conducted using *Daphnia magna*.

Esterification

The synthesis of palmitic acid–curcumin esters was carried out following a reported procedure (Anty et al., 2024). A total of 20 mmol of palmitic acid was dissolved in 25 mL of dichloromethane, followed by the addition of 9 mmol of the coupling reagent (DCC). The mixture was stirred for 20 minutes at room temperature (25°C). Subsequently, 10 mmol of curcumin and 13.5 mmol of DMAP were added. The reaction mixture was stirred for 20 hours in the dark. After completion, the mixture was diluted with dichloromethane and filtered. The filtrate was then concentrated under reduced pressure to obtain the ester product.

Thin Layer Chromatography (TLC) Analysis

The precursor compounds (palmitic acid and curcumin) and ester products were dissolved in dichloromethane and spotted onto silica gel plates using a hexane:ethyl acetate (1:1, v/v) solvent system. Spot visualization was carried out using 1 M H_2SO_4.

Column Chromatography Purification

The ester products were purified using silica gel column chromatography following Anty et al. (2024). The mobile phase consisted of n-hexane:ethyl acetate (12:1, v/v). Fractions (10 mL) were collected in vials and analyzed by TLC using n-hexane:ethyl acetate (1:1, v/v).

FTIR Characterization

The products were analyzed using FTIR to identify functional groups. Liquid samples were placed on a KRS-5 plate and scanned as thin films after solvent evaporation (6,11).

Antimicrobial Assay

Nutrient agar (NA) was prepared by dissolving it in 100 mL distilled water, heated to boiling, and homogenized. The medium was dispensed into test tubes (10 mL for testing and 5 mL for culture). Nutrient broth (NB) was prepared from beef extract and peptone. Both NA and NB were sterilized in an autoclave at 121°C for 15 minutes, while Petri dishes were sterilized in an oven at 170°C for 2 hours.

Bacterial cultures (*S. aureus* and *E. coli*) were inoculated into sterile NB and incubated for 24 hours. Bacterial density was adjusted to the McFarland standard (0.08–0.1) using a UV–Vis spectrophotometer.

For testing, 200 µL of bacterial suspension was added to each Petri dish, followed by 20 mL of NA. The mixture was homogenized and allowed to solidify. Sterile discs were placed on the agar surface, and 5 µL of sample was applied to each disc. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured.

Toxicity Test

Adult *Daphnia magna* were cultured in well water that had been settled overnight and fed at least every two days (12). Neonates (<24 hours old) were used for toxicity testing.

The experiment included control and sample groups. The control contained well water, DMSO, and 10 neonates. The sample group contained test solutions diluted to 5 mL with well water and 10 neonates. After 24 hours of exposure, mortality was recorded. LC_{50} values were calculated using probit analysis based on mortality percentages.

3. Result and Discussion

Steglich Esterification of Palmitic Acid with Curcumin

The palmitate curcumin ester products obtained consisted of dipalmitate curcumin as a pale yellow solid (0.3326 g) and monopalmitate curcumin as a bright yellow solid (0.5226 g). Thin-layer chromatography (TLC) analysis using an n-hexane:ethyl acetate (1:1, v/v) eluent showed Rf values of 0.91 for dipalmitate curcumin and 0.76 for monopalmitate curcumin.

FT-IR Characterization of Palmitate Curcumin Esters

The FTIR spectrum of dipalmitate curcumin is presented in Figure 1. A noticeable shift in the carbonyl (C=O) absorption band was observed, from 1689 cm^{-1} (carboxylic acid in palmitic acid) to 1753 cm^{-1}, indicating the formation of an ester group. In the fingerprint region, characteristic peaks were identified, including the ketone carbonyl (C=O) at 1631 cm^{-1}, aromatic C=C stretching at 1579 cm^{-1}, aromatic C–O stretching at 1298 cm^{-1}, and C–O–C stretching at 1030 cm^{-1}, confirming the structural features of the synthesized ester compound.

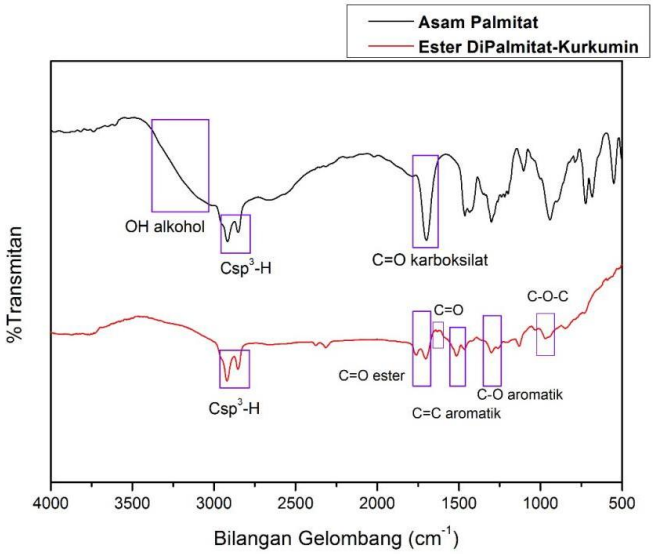

Figure 1. FT-IR Spectrum of Dipalmitate Curcumin Ester

The FTIR spectrum of monopalmitate curcumin is shown in Figure 2. It indicates a shift in the carbonyl (C=O) absorption band from 1689 cm^{-1} (carboxylic acid in palmitic acid) to 1753 cm^{-1}, confirming the formation of an ester group. Additionally, an O–H absorption band is observed at 3258 cm^{-1}. In the fingerprint region, characteristic peaks include the ketone carbonyl (C=O) at 1631 cm^{-1}, aromatic C=C stretching at 1579 cm^{-1}, aromatic C–O stretching at 1298 cm^{-1}, and C–O–C stretching at 1030 cm^{-1}.

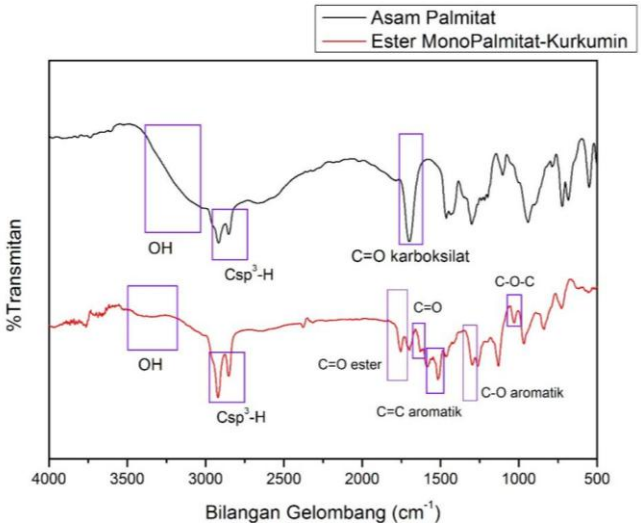

Figure 2. FT-IR Spectrum of Monopalmitate–Curcumin Ester

The FT-IR characterization shown in Figure 2 indicates the presence of functional groups consistent with the target molecule. A key distinction between fatty acid–curcumin diester and monoester products is that the monoester still exhibits a phenolic O–H absorption peak that has not undergone esterification. This confirms the successful formation of the ester products.

UV–Vis Characterization of Palmitate Curcumin Esters

UV–Vis absorption measurements were conducted in the wavelength range of 200–700 nm for both monopalmitate–curcumin and dipalmitate curcumin. The analysis was also performed using sodium hydroxide (NaOH) as a shift reagent to identify unreacted phenolic hydroxyl groups (Pradana, 2014). The UV–Vis spectrum of dipalmitate–curcumin without NaOH shows a main absorption band at 400–410 nm, corresponding to π→π* electronic transitions within the conjugated curcuminoid system, while absorption in the UV region at 200–230 nm is attributed to π→π* transitions of the aromatic rings. These results indicate that the esterification process does not disrupt the primary chromophore of curcumin but instead modifies only the phenolic hydroxyl groups. Upon the addition of NaOH as a shift reagent, no spectral shift was observed for the dipalmitate–curcumin ester. This is due to the absence of free phenolic hydroxyl groups in the structure, as both OH groups have been esterified and are therefore unable to undergo deprotonation by NaOH (M et al., 2018).

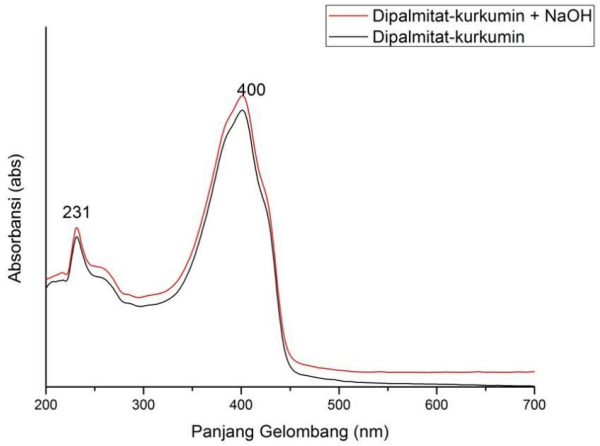

Figure 3. UV–Vis Spectrum of Dipalmitate Curcumin Ester Product

Based on Figure 4, the UV–Vis spectrum of the monopalmitate curcumin ester product shows a bathochromic shift, indicating the presence of a phenolic hydroxyl group (Swantara et al., 2016). This shift occurs due to the deprotonation of the phenolic hydroxyl group, which is sensitive to the basic NaOH reagent, resulting in the formation of a phenolate ion. This process enhances electron delocalization within the conjugated π-system and lowers the energy of the π→π* transition. As a result, the energy gap decreases, making electron excitation easier and causing the absorption wavelength to shift toward longer wavelengths.

In addition, this shift is also influenced by the presence of a hydroxyl auxochrome in the chromophore (L et al., 2019; Suhartati, 2006).

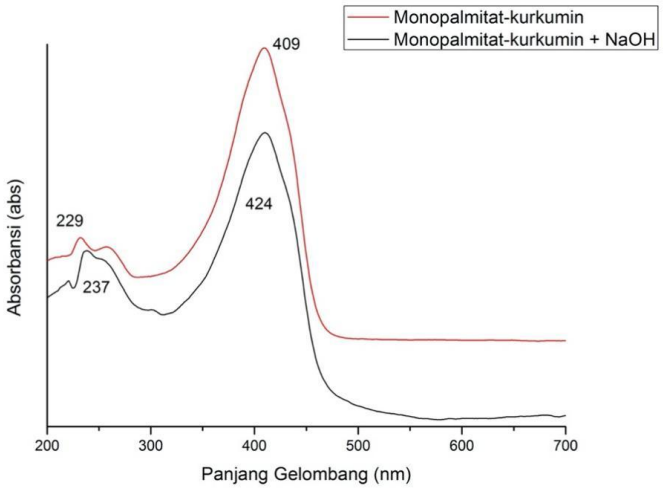

Figure 4. UV–Vis Spectrum of Monopalmitate Curcumin Ester Product

Antimicrobial Activity Test

The antibacterial activity of each precursor and ester product was evaluated against the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *Escherichia coli*. In this study, the samples generally produced larger inhibition zones against *Escherichia coli* compared to *Staphylococcus aureus*. The results of the antimicrobial activity observations are presented in Table 1.

Table 1. Measurement Results of Microbial Inhibition Zone Diameters

Test Sample	Inhibition Zone Diameter (mm)	
	S. aureus	*E. coli*
Monopalmitate–Curcumin	7.00	7.50
Dipalmitate–Curcumin	7.00	8.33
Palmitic Acid	6.50	6.67
DMSO (–)	–	–
Chloramphenicol (+)	13.33	17.00

Based on the results presented in Table 1, all tested samples exhibited weak antimicrobial activity against both bacterial strains, as indicated by the relatively small inhibition zones. The monoester product showed lower effectiveness against the Gram-negative bacterium *Escherichia coli*, which may be attributed to the presence of an outer membrane composed of hydrophilic lipopolysaccharides. This structure acts as a barrier to

macromolecules and hydrophobic compounds, thereby increasing bacterial resistance to such antimicrobial agents (Naulidia et al., 2020).

Antimicrobial activity may occur through several mechanisms, including inhibition of bacterial quorum sensing (QS) systems, interaction with DNA leading to bacteriostatic effects, disruption of RNA and protein synthesis, interaction with cellular proteins or enzymes, and damage to the cell wall and membrane (Zheng et al., 2020). In this context, the hydroxyl (–OH) groups present in the ester products may contribute to bacterial cell lysis and reduced membrane permeability, ultimately impairing cellular function and leading to bacterial cell death, as reflected by the observed inhibition zones (Yaqoob et al., 1994).

The antimicrobial activity of fatty acids is also influenced by factors such as carbon chain length, degree of unsaturation (number of double bonds), and the configuration of these bonds (cis or trans) (Yoon et al., 2018). Short- to medium-chain fatty acids generally exhibit stronger antimicrobial effects, particularly in the form of monoglycerides, whereas long-chain saturated fatty acids tend to be less effective. Additionally, the number and position of double bonds in fatty acids with 12–22 carbon atoms play a significant role in antimicrobial activity. Cis double bonds enhance antimicrobial efficacy compared to straight-chain fatty acids, while trans configurations are generally less active (Yoon et al., 2018). In line with these findings, palmitic acid demonstrated relatively small inhibition zones due to its long saturated carbon chain. Antimicrobial activity is commonly classified based on inhibition zone diameter as strong (>14 mm), moderate (10–13 mm), weak (7–9 mm), and inactive (<6 mm), according to standard classifications used in non-clinical disk diffusion studies (Balouriri et al., 2016; Davis & Stout, 1971).

Toxicity Test on *Daphnia magna*

This study was conducted in two stages, namely a preliminary test and a definitive test. The preliminary test was carried out to determine the optimal conditions under which the test organisms exhibited less than 10% mortality, while the definitive test was performed by refining the sample concentration based on the results of the initial observations (Edwin et al., 2017). The preliminary test was conducted under three conditions: well water only, well water with 2% DMSO, and well water with 5% DMSO. DMSO was used as a solvent for the ester products and varied to evaluate the tolerance of *D. magna* to the solvent. The results of the preliminary test are presented in Table 2. Based on these findings, the optimal condition for subsequent testing was identified as well water with 2% DMSO, as it resulted in a mortality rate of less than 10%.

Table 2. Preliminary Toxicity Test Results of *Daphnia magna*

Condition	Population (individuals)	Mortality (%)	
		24 Hours	48 Hours
Well Water	10	0.00	80.00
Well Water + 2% DMSO	10	3.33	96.67
Well Water + 5% DMSO	10	26.67	100.00

The next stage was the definitive test, the results of which are presented in Table 4. Based on the data, for each ester product as well as the precursor compounds, higher concentrations correspond to increased mortality of *Daphnia magna*. The highest mortality percentage for each ester product was observed at a concentration of 1000 ppm. In the toxicity assessment, LC_{50} values were calculated to determine the concentration of a compound capable of causing 50% mortality in the test organism (*D. magna*). The LC_{50} value was obtained from the regression equation ($y = a + bx$), where $y = 5$ represents 50% mortality as the dependent variable, allowing the determination of x as the independent variable. The obtained x value was then converted into its antilogarithm to yield the LC_{50} value.

Table 3. Definitive Toxicity Test of *Daphnia magna*

Sample	Concentration (mg/L)	Log [C]	Mortality (%)	Probit Value	LC_{50} (mg/L)
Control	–	–	0	–	–
Curcumin	50	1.69	30.0	4.47	84.84
	100	2.00	56.7	5.16	
	250	2.39	76.7	5.72	
	500	2.69	100.0	8.71	
	1000	3.00	100.0	8.71	
Palmitic Acid	50	1.69	20.0	4.16	125.24
	100	2.00	30.0	4.74	
	250	2.39	66.7	5.42	
	500	2.69	83.3	5.96	
	1000	3.00	100.0	8.71	
Dipalmitate–Curcumin Ester	50	1.69	0.0	0.00	412.28
	100	2.00	10.0	3.71	
	250	2.39	33.3	3.16	
	500	2.69	66.7	5.42	

Sample	Concentration (mg/L)	Log [C]	Mortality (%)	Probit Value	LC_{50} (mg/L)
	1000	3.00	96.7	6.82	
Monopalmitate–Curcumin Ester	50	1.69	23.3	3.06	153.65
	100	2.00	46.7	4.91	
	250	2.39	73.3	5.62	
	500	2.69	80.0	5.84	
	1000	3.00	100.0	8.71	

Based on the toxicity results, the observed LC_{50} values can be interpreted according to the toxicity classification proposed by Anty et al. (2024), as presented in Table 4. The LC_{50} value of monopalmitate–curcumin is lower than that of dipalmitate curcumin, indicating higher toxicity. This difference is likely due to the presence of a free phenolic hydroxyl (–OH) group in the monoester, which may disrupt cell membrane permeability. In addition, the compound may act as a stomach poison, interfering with the digestive system of *Daphnia magna* and ultimately causing mortality (Anty et al., 2024). The toxic properties of a compound are strongly influenced by its chemical structure and functional groups. Previous research by Alfi N. (2017) on essential oils reported that their toxicity is associated with the presence of functional groups such as hydroxyl (–OH) and carbonyl (C=O), which can contribute to biological reactivity and toxic effects.

Table 4. Toxicity Levels Based on LC_{50} Values

LC_{50} (mg/L)	Toxicity Level
0-100	Highly Toxic
100-500	Moderately Toxic
500-1000	Low Toxicity
>1000	Non-Toxic

4. Conclusion

Steglich esterification of palmitic acid with curcumin successfully produced dipalmitate–curcumin and monopalmitate–curcumin esters. The formation of these ester products was confirmed through FT-IR and UV–Vis characterization. Antimicrobial testing revealed that dipalmitate–curcumin exhibited greater activity against *Escherichia coli*, while monopalmitate–curcumin showed higher activity against *Staphylococcus aureus*. Toxicity evaluation using *Daphnia magna* indicated that monopalmitate–curcumin is more toxic than dipalmitate–curcumin, which is likely attributed to the presence of a free phenolic hydroxyl group in the monoester structure.

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