The amount of CA found in this study is higher than those in requirement of *Flos Lonicerae* meterial in both Chinese and Vietnamese Pharmacopoeia [6],[7]. The findings indicated that the yield of these active components of *Flos Lonicerae* varied with habitats.

5. Conclusion

Two main compounds were isolated and identified as CA and DCQA. A reliable and simple method was developed successfully for the quantitative analysis of two these major components using HPLC-DAD. The method was validated in terms of specificity, accuracy, precision, and system suitability of the main compounds. The results of this study might be beneficial for the standardization and developing the quality control profile of *Flos Lonicerae* by the phytopharmaceutical industries.

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QUANTITATIVE ANALYSIS OF TOTAL POLYPHENOLS IN THE LEAVES OF *TERMINALIA CATAPPA* L. BY UV-VIS ABSORPTION SPECTROSCOPY

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Summary

Quantitative Analysis of total Polyphenols in the Leaves of *Terminalia catappa* L. by UV-Vis Absorption Spectroscopy

An UV-Vis absorption spectroscopy method had been developed and validated to quantify total polyphenols in the leaves of *Terminalia catappa* L. (TC). The TC leaf powder was extracted by ultrasonic method with 50% ethanol, followed by the reaction with Folin-Ciocalteu phenol reagent and photometric measurement at 730 nm. The developed method exhibited good linearity (R^2 = 0.9957) within a range of 1-8 µg/ml and LOD and LOQ were found to be 0.033 and 0.100 µg/ml, respectively. The developed method was also found accurate, and precise according to the regulatory guidelines. The developed method was applied to measure the total polyphenol content in samples collected from various provinces in Vietnam.

Keywords: *Terminalia catappa, Total polyphenols, UV-Vis.*

1. Introduction

Terminalia catappa L. (TC) is a popular plant in Vietnam and several Southeast Asian countries [1]. In traditional medicine, some parts of this plant were used to treat in treatment of different diseases, such as diarrhea (bark) [2] and fever (leaf) [3]. In Taiwan, TC was used as a traditional herb to treat hepatitis [4]. Previous investigations on TC also reported the antioxidant [5], anti-inflammatory [6], and antibacterial effects [7] of the leaves. These results were potential to develop several new antioxidant, anti-inflammatory and antibacterial products from TC. The chemical composition of TC has been reported, including tannins, flavonoids, alkaloids, polyphenols, saponins…[8],[9], with tannins, flavonoids, and phenolic acids known as the main compounds in the leaves. According to O. Q. Allyn et al., 95% ethanol extract from the leaves contained total polyphenol content of 208.722 mg GAE/g extract [7]. In addition, H. V. Annegowda et al reported that the ethanol extract from the dried leaves of TC contained total polyphenol content at 238.26 ± 1.12 mg GAE/g extract [10]. In Vietnam, there was not any monograph on TC in Vietnamese Pharmacopoeia V. To our knowledge, no previous research about the quantitative analysis of the total polyphenols in the leaves of TC has been published. For these reasons, quantitative analysis of total polyphenols in the leaves of TC by UV-Vis absorption spectroscopy was conducted to standardize the materials from this herbal materials.

2. Materials and methods

2.1. Materials

The TC leaves samples include young leaves collected in Gia Vien district, Ninh Binh Province (B3); Dai Tu district, Thai Nguyen Province (B6); Quang Xuong district, Thanh Hoa Province (B7) in February 2021. Mature leaves samples were collected in Lam Thao District, Phu Tho Province (B1); Ngan Son District, Bac Kan Province (B2); My Duc District, Hanoi City (B5); Huu Lung District, Lang Son Province (B9) and Nguyen Binh district, Cao Bang province (B10) in June and July 2021. Old leaves samples were collected in Na Hang district, Tuyen Quang Province (B4), and Tam Dao district, Vinh Phuc Province (B8) in October 2021. In which, sample B5 was used for method development and validation. All samples were dried at 60^0C, cut and ground to a small size, sieved through the No. 710 sieve, stored in a sealed plastic bag, and put in a dry place.

The standard gallic acid was supplied by Chengdu Biopurify Phytochemicals Ltd., with a purity of 99.8%. The solvents (96% ethanol, water). The reagent sodium carbonate was at a purity level for analysis. Folin-Ciocalteu reagent was purchased from Sigma-Aldrich. The instruments that were used in the research included the scale balance Precisa XT 620M; analytical balance Mettler Toledo XPE105 (Mettler Toledo, Switzerland), moisture analyzer Precisa XM60 (Precisa, Switzerland), ultrasonic bath WUC-D22H (Daihan Scientific, Korea), UV-Vis spectrophotometer U5100 (Hitachi, Japan), heating oven Memmert (Memmert, Germany).

2.2. Methods

2.2.1. Analytical condition optimization:

Examine absorption wavelengths: prepare blank sample, testing sample, and standard sample (gallic acid). Measure the absorption spectroscopy on UV-Vis spectrophotometry in the range from 300-800 nm to determine λ max for further analysis.

Examine coloring reaction duration: conduct the coloring reaction with a testing sample and measure the absorption of the coloring solution at different time (10, 20, 30, 40, 60, 90, 120, and 180 minutes after starting time) to choose the most reasonable duration.

Examine the solvent: extract the sample with water, 30% ethanol, 50% ethanol, 70% ethanol, and 96% ethanol.

Examine the extraction duration: test with ultrasonication in 15, 30, 45, and 60 minutes, respectively.

Examine the extraction times: extract the sample 1, 2, or 3 times with the solvent and duration that were inspected before.

The reaction was carried out at room temperature according to the reference of earlier publications [11],[12] and Vietnamese Pharmacopoeia V's guidelines [13]. Refer to earlier publications to select 1 ml of Folin-Ciocalteu (diluted 5 times by water) [11] and 4 ml of 7% sodium carbonate reagent [11],[12] for coloration reaction.

2.2.2. Sample preparation and color reaction:

After optimizing the analytical conditions, the procedure for sample preparation and color reaction is performed as follows.

Prepare the standard solution: the stock solution was prepared by dissolving completely an accurate amount of gallic acid(around 5,00 mg) into 50% ethanol in a brown volumetric flask of 50 ml to obtain the solution at a concentration of 0.1 mg/ml. From the stock solution, prepare a range of increasing concentration standard solutions at 1, 2, 4, 5, 6, and 8 µg/ml by diluting precisely 100, 200, 400, 500, 600, and 800 µl stock solutions in 2 ml water in 10 ml volumetric flask. Add 1 ml Folin-

Ciocalteu reagent (diluted 5 times by water), 4 ml 7% sodium carbonate and water. The standard solutions were mixed thoroughly and incubated for 40 minutes at room temperature before measured absorption at 730 nm.

Prepare the blank solution: same procedure as the standard solution preparation, however using 50% ethanol instead of the stock solution.

Prepare the sample solution: 0.5000 grams of the leaf powder was mixed with 40 ml 50% ethanol in a 250 ml Erlenmeyer flask and sonicated for 30 minutes. The mixture was filtered through a filter paper, transferred all the residues into a flask, and extracted with 30 ml 50% ethanol. The solution was combined in a 100 ml volumetric flask. Add 50% ethanol and mixed thoroughly to obtain solution A. From solution A, diluted 100 µl of solution A with 2 ml water in a 10 ml volumetric flask, added 1 ml reagent (diluted 5 times by water), 4 ml 7% sodium carbonate, and water. Mixed carefully, incubated at room temperature for 40 minutes, and measured absorption at 730 nm.

2.2.3. Method validation:

The method was evaluated according to the guideline of AOAC [14] and ICH [15] including the suitability of the system, linearity range, precision (repeatability and intermediate precision), accuracy, limits of detection (LOD) and quantification (LOQ).

2.2.4. Data analysis:

Experimental results were calculated using Microsoft Excel software using T-test with a 95% confidence interval ($\alpha = 0.05$).

The total polyphenol content was calculated according to the below formation:

$$\textit{The total polyphenol content}\ (\text{mg GAE/g}) = C \times D \times \frac{V}{m \times \frac{100-H}{100}}$$

(mg GAE/g)

With C: the total polyphenol concentration in the sample solution calculated from the calibration curve (µg/ml); D: the dilution of sample solution (D=100); V is the volume of sample solution (ml); m: the weight of the sample (mg); H: humidity (%).

3. Results

3.1. Analytical condition optimization

Examine the condition of spectrophotometry

Examine the wavelength for spectrophotometry: Fig. 1 showed λ max placed in the range from 725 nm to 735 nm. The wavelength 730 nm was chosen.

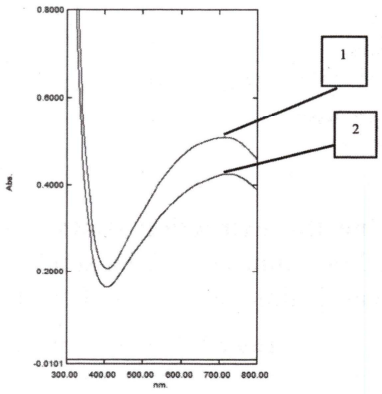

Fig. 1. The UV-Vis spectra of the standard solution (1) and sample solution from the leaves of TC (2)

Examine the coloring reaction time: the result in Fig. 2 showed that the absorption increased and reached the maximum in the range of 40-90 minutes and decreased gradually until the end of the reaction (180 minutes). For time-saving purposes, 40 minutes was chosen for the duration of the reaction.

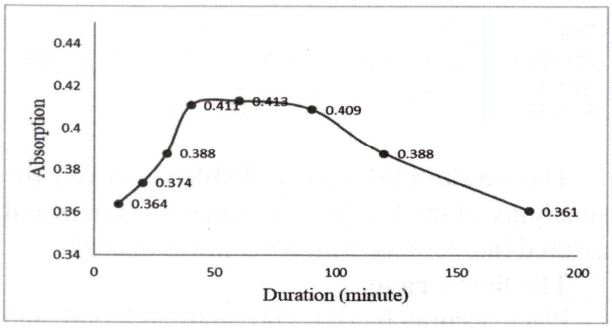

Fig. 2. The chart describes the dependence of absorption on the reaction time

Sample preparation:

Examine the extraction solvent: 50% ethanol showed the highest polyphenolic content so 50% ethanol was the most suitable solvent. The results were presented in **Table 1**.

Table 1. The results of examining the extraction solvent of preparing sample method

The solvent															
Solvent	Water			30% Ethanol			50% Ethanol			70% Ethanol			96% Ethanol		
Total polyphenol content (mg GAE/g)	60.40	61.75	59.53	60.24	61.21	62.23	63.58	65.84	63.83	58.53	60.65	59.99	52.89	54.83	53.95
Average total polyphenol content (mg GAE/g)	60.56			61.23			64.42			59.72			53.89		
RSD (%)	1.85			1.63			1.92			1.82			1.81		

Examine the extraction duration: the samples were extracted in 30, 45, and 60 minutes showing the similarity in total polyphenol content. For time-saving purposes, 30 minutes was chosen for the extraction duration. The results were presented in **Table 2**.

Table 2. The results of examining the extraction duration of preparing sample method

Extraction duration												
Duration (minutes)	15			30			45			60		
Total polyphenol content (mg GAE/g)	57.45	59.62	59.26	63.64	65.96	64.30	63.65	64.78	65.81	63.60	64.57	65.73
Average total polyphenol content (mg GAE/g)	58.78			64.64			64.75			64.63		
RSD (%)	1.98			1.85			1.67			1.65		

Examine the extraction times: the samples extracted 2 or 3 times showed a similar result and higher than 1 time extracted. For this reason, extracting 2 times for 30 minutes each time was the most suitable method for sample extraction. The results were presented in **Table 3**.

Table 3. The results of examining the extraction times of preparing sample method

Times of extraction									
Times of extraction	1			2			3		
Total polyphenol content (mg GAE/g)	55.27	57.42	56.59	64.07	63.42	65.89	64.81	65.91	63.58
Average total polyphenol content (mg GAE/g)	56.43			64.46			64.76		
RSD (%)	1.92			1.99			1.80		

3.2. Method validation

The suitability of the system: Carry out photometric measurements 6 times the solution of 2 µg/ml gallic acid. The results were presented in **Table 4**.

Table 4. The results of examining the suitability

Time	1	2	3	4	5	6
Absorption	0.274	0.267	0.265	0.277	0.275	0.269
Average	0.271					
RSD (%)	1.78					

The results observed in **Table 4** proved the suitability of the UV-Vis instrumental system and assured the stability with RSD lower than 2%

The linear range

Place accurately 100, 200, 400, 500, 600, and 800 µl of stock solution in a 10 ml volumetric flask, add 2 ml water, and mix thoroughly. Add 500 µl Folin-Ciocalteau reagent, mix carefully, and let it stand for 5 minutes. Add 4 ml 7% sodium carbonate and water to the volumetric level, mix, and incubate at room temperature for 40 minutes before measuring UV absorption. Using the least squares method to determine the linear regression equation and correlation coefficient between the solution concentration and the absorption.

Each concentration was conducted to measure UV absorption 3 times, and the average value was calculated. The results were presented in **Table 5** and **Fig. 3**.

Table 5. The results of examining the linear range

The gallic acid concentration in the solution (µg/ml)	1.01	2.01	4.03	5.03	6.04	8.05
Absorption	0.157	0.271	0.521	0.650	0.788	1.107
Linear regression equation	y = 0.1334x + 0.0004					
Correlation coefficient	R^2 = 0.9957					

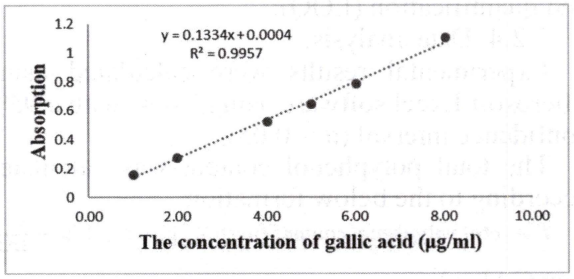

Fig. 3. The calibration line describes the dependence of absorption on gallic acidconcentration at 730 nm

In the linear range, the linear regression equation was y= 0.1334x + 0.0004 with the correlation coefficient was R^2 = 0.9957. Therefore, it can be concluded that this developed method conforms the linearity.

Precision: the results were presented in the **Table 6**.

Table 6. The precision results

Ngày 1			Ngày 2		
Sample weight (mg)	absorption	Total phenol content (mg GAE/g)	Sample weight (mg)	absorption	Total phenol content (mg GAE/g)
500.2	0.403	65.73	500.3	0.391	64.53
501.2	0.396	64.46	501.4	0.397	65.38
501.7	0.401	65.21	501.2	0.393	64.75
501.1	0.409	66.59	501.4	0.409	67.36
500.8	0.411	66.96	500.6	0.402	66.31
499.5	0.391	63.86	499.7	0.389	64.28

Ngày 1			Ngày 2		
Sample weight (mg)	absorption	Total phenol content (mg GAE/g)	Sample weight (mg)	absorption	Total phenol centent (mg GAE/g)
Average		65.47	Average		65.43
RSD (%)		1.83	RSD (%)		1.82
The average of total polyphenol content in total 12 times = **65.45 mg GAE/g** RSD = **1.74%**					

RSD values of each day and in total 2 days were both lower than 2%. T-test calculation with reliability of 95% (α = 0.05) showed that there is no difference in the results between 2 days. It is reliable that the method achieved high repeatability, stability, and precision.

Accuracy

To evaluate the accuracy of the method, add a precise amount of gallic acid into the sample which contained total polyphenol content equal to 50% of the original sample to make a list of samples with 80%, 100%, and 120% polyphenolic concentration (respectively mix exactly about 9.0 mg; 15.0 mg; 20.0 mg gallic acid with 0.2500 g sample powder in the 250 ml Erlenmeyer flask, then add 40 ml 50% ethanol and sonicated for 30 minutes. The mixture was filtered through a filter paper, transferred all the residues into a flask, and extracted with 30 ml 50% ethanol. The solution was combined in a 100 ml volumetric flask. Add 50% ethanol and mixed thoroughly to obtain solution B. From solution B, diluted 100 µl of solution B with 2 ml water in a 10 ml volumetric flask, added 1 ml Folin-Ciocalteu reagent (diluted 5 times by water), 4 ml 7% sodium carbonate, and water. Mixed carefully and incubated at room temperature for 40 minutes.). Each concentration was processed and measured UV absorption according to the above method and repeated 3 times.

The results of accuracy were performed in **Table 7**.

Table 7. The accuracy testing results

% polyphenolic	Added gallic acid (mg)	Sample weight (mg)	Total existent polyphenol content (mg GAE)	Absorption	%	% Average	%RSD
79.71	8.97	0.2512	15.09	0.316	95.45	93.64	1.74
79.33	8.83	0.2505	15.05	0.311	93.19		
80.16	9.05	0.2497	15.00	0.312	92.29		
99.84	15.07	0.2516	15.12	0.391	93.96	92.28	1.77
100.33	15.17	0.2508	15.07	0.388	92.17		
100.53	15.28	0.2516	15.12	0.387	90.71		
117.56	20.46	0.2520	15.14	0.456	92.90	93.61	1.95
116.58	20.14	0.2517	15.12	0.450	92.24		
117.39	20.28	0.2504	15.05	0.460	95.68		

The accuracy results showed that the recovery rate of gallic acid ranges from 92-105% with %RSD lower than 2%. The lowest was 90.71% and the highest was 95.68%. From these results, it is observed that the accuracy of the method was acceptable.

Limits of detection (LOD) and quantification (LOQ)

To determine LOD and LOQ, measure the absorbance of the blank 20 times. Calculate LOD and LOQ according to the formula:

$$LOD = \frac{3{,}3\ SD}{a} \text{ và } LOQ = \frac{10\ SD}{a}$$

With: SD is the standard deviation of the absorbance of the blank; a is the slope in the linear regression equation representing the dependence between absorbance and gallic acid concentration (according to the results of building the linear regression equation, the value of a is 0.1334).

The results of determining the LOD and LOQ values are presented in **Table 8.**

Table 8. The results of determining the limits of detection (LOD) and quantification (LOQ) of the analytical method

Times	1	2	3	4	5	6	7	8	9	10
Abs	0.002	0.002	0.001	0.001	0.001	0.004	0.004	0.003	0.004	0.001
Times	11	12	13	14	15	16	17	18	19	20
Abs	0.003	0.001	0.002	0.004	0.001	0.004	0.001	0.004	0.001	0.001
SD(20)	0.001333									
LOD	LOD = 0.033 (µg/ml)									
LOQ	LOQ = 0.100 (µg/ml)									

3.3. Determine the total polyphenol content in several TC samples

With the optimized processing method and spectrophotometry condition, several TC samples collected from different provinces in Viet Nam were analyzed and evaluated for the total polyphenol content. Each sample is quantitatively analyzed 3 times to get the average value of the total phenol content. The results were shown in **Table 9**.

Table 9. The results of several TC samples

Sample	Sampling location	Sample collection time	Humidity (%)	Sample weight (mg)	Abs	Total phenol content (mg GAE/g)	Average total polyphenol content (mg GAE/g)	RSD (%)
B1	Lam Thao, Phu Tho	7/2021	8.25	501.0	0.372	61.64	**62.74**	1.81
				501.5	0.386	63.90		
				500.5	0.378	62.70		
B2	Ngan Son, Bac Kan	7/2021	8.39	500.1	0.365	60.67	**59.94**	1.69
				500.0	0.363	60.35		
				500.6	0.354	58.78		
B3	Gia Vien, Ninh Binh	2/2021	8.43	501.1	0.358	59.41	**58.47**	1.93
				500.8	0.354	58.78		
				499.9	0.344	57.22		
B4	Na Hang, Tuyen Quang	10/2021	8.48	500.9	0.383	63.64	**63.54**	1.98
				500.1	0.389	64.74		
				500.1	0.374	62.24		
B5	My Duc, Ha Noi	7/2021	8.22	500.2	0.402	66.71	**65.44**	1.88
				501.2	0.388	64.25		
				500.3	0.394	65.36		
B6	Dai Tu, Thai Nguyen	2/2021	8.21	500.6	0.371	61.49	**61.36**	1.72
				501.3	0.364	60.24		
				500.5	0.376	62.34		
B7	Quang Xuong, Thanh Hoa	2/2021	8.42	501.7	0.341	56.51	**56.83**	1.66
				501.2	0.349	57.90		
				500.9	0.338	56.10		
B8	Tam Dao, Vinh Phuc	10/2021	8.41	500.5	0.322	53.47	**53.62**	1.77
				500.9	0.318	52.76		
				500.5	0.329	54.64		
B9	Huu Lung, Lang Son	6/2021	8.45	500.9	0.371	61.62	**61.69**	1.57
				501.1	0.366	60.76		
				500.3	0.377	62.69		
B10	Nguyen Binh, Cao Bang	6/2021	8.19	500.8	0.403	66.77	**65.46**	1.75
				500.0	0.391	64.88		
				500.0	0.390	64.72		

4. Discussion

Quantity of total polyphenol content by using UV-Vis spectroscopy was guided in Appendix 12.6 of Vietnamese Pharmacopoeia V [13], and was applied in evaluating polyphenol content in numerous different samples such as *Phyllanthus emblica* [11], *Hibiscus sapdariffa* L. [12]. This research consulted the Vietnamese Pharmacopoeia V's guidelines and a number of earlier publications to develop the quantitative method for determining total polyphenol content in TC leaves. The validated method was suitable for measuring the TC leaves' total phenol content with good repeatability, precision, and accuracy and was suitable to measure the total polyphenol content in the sample. The results of 10 samples of TC leaves including young, mature, and old leaves showed results with total polyphenol content ranging from 53.62 to 65.46 mg GAE/g; young leaf samples showed results from 56.83 to 61.36; mature leaf samples showed results from 59.94 to 65.46 mg GAE/g; two old leaf samples showed results ranging from 53.62 to 63.54 mg GAE/g. However, to evaluate the influence of each property (climate, the age of the tree, or soil properties…) on total polyphenol content in TC leaves, it is necessary to quantify more representative samples. Until now, no previous publication presented total polyphenol content in the leaves of TC. However, a number of researchers have published total polyphenol content in the extract from TC leaves: the research of O. Q. Allyn showed that the ethanol 95% extract contained 208.722 mg GAE/g [7] and according to the publication of H. V. Annegowda, ethanol extract from dried leaves contained 238.36 ± 1.12 mg GAE/g of total polyphenol content [10]. In which, the study of O. Q. Allyn used 5 ml of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 4 ml of sodium carbonate 4M (about 10%) for 0.5 ml of diluted extract, stood for 15 min and measured the absorbance at 765 nm [7]. Thus, it can be seen that the volume and concentration of reagents were equivalent to this study. The selected wavelength for measurement was different due to the different standards, chemicals, and equipment leading to the change of absorption maximum wavelength.

5. Conclusion

The method for quantifying total polyphenol content in *Terminalia catappa* L. leaves was developed by using UV-Vis spectroscopy and the coloring reaction between the sample solution and Folin-Ciocalteu reagent before photometric measurement at wavelength 730 nm. The method was validated to show good linearity (R^2 = 0.9957), precision (% relative standard deviation < 2%), accuracy (92.28% - 93.64%), limits of detection (0.033 µg/ml) and quantification (0.100 µg/ml). The results of analyzing 10 leaf samples performed total polyphenol content ranging from 53.62 to 65.46 mg GAE/g.

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EFFICACY OF DIHYDROQUERCETIN NANOEMULSION IN TYLOXAPOL-INDUCED HYPERLIPIDEMIC MICE

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Summary

Efficacy of Dihydroquercetin Nanoemulsion in Tyloxapol-Induced Hyperlipidemic Mice

Dihydroquercetin (DHQ), also called as taxifolin, has been reported to have many beneficial properties for human health. However, its low solubility and bioavailability are major obstacles for the biomedical application of the flavonoid. This study aims to evaluate the effects of dihydroquercetin (DHQ) and dihydroquercetin loaded self-nano-emulsifying drug delivery systems (NanoDHQ) containing 8% DHQ on lipid profiles in tyloxapol-induced hyperlipidemic mice. *Swiss albino* mice were daily treated with DHQ (24 and 48 mg/kg, p.o) or NanoDHQ (37.5, 75 and 150 mg/kg) or fenofibrate (100 mg/kg, p.o), a reference drug, two week before tyloxapol injection (Triton WR 1339, 250 mg/kg, i.v). After 16-hour administration of tyloxapol, blood was collected and the levels of serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), non-high-density lipoprotein-cholesterol (non-HDL-C), and low-density lipoprotein-cholesterol (LDL-C) were measured. Our results showed that the levels of TC, TG, LDL-C, non-HDL-C in the model group were significantly increased, while the HDL-C level of the animals were markedly decreased in comparing with the control group. The treatment of DHQ at the dose of 48 mg/kg significantly decreased TG level but not altered the other lipid indexes as compared to the model group. Interestingly, the administration of NanoDHQ (75 and 150 mg/kg) significantly and dose-dependently attenuated the elevation of TC and TG level in the tyloxapol-treated mice. Moreover, the NanoDHQ treatment