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Study of Curcumin Degradation Using UV-Vis Spectrophotometry and Density Functional Theory

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Abstract. The study of curcumin degradation has been carried out by optimizing the effect of pH and solvents using the UV-Vis spectrophotometric method. The stability of curcumin decreased as pH increased. Curcumin was degraded rapidly in alkaline solutions. Curcumin has different maximum wavelengths in the different solvents. The UV spectrum of curcumin shifted as the pH increased. The UV spectra of curcumin shifted towards a smaller wavelength in alkaline solution. The shift in the UV spectra was due to curcumin degradation in an alkaline solution. In addition, Density functional Theory (DFT) calculation was carried out to simulate the curcumin and its degradation product ground states and excited states. The absorption spectra calculated using the TDDFT method showed that the maximum wavelength of curcumin and its degradation products agreed with the experimental data.

INTRODUCTION

Curcumin has been widely reported for its pharmacological activities such as anti-inflammatory, antibacterial, and antiviral, as well as its therapeutic potential for cancer and Alzheimer's disease [1]. However, it was reported that curcumin has poor bioavailability due to its hydrophobic properties [2]. Curcumin was not found in tissues after oral administration for 29 days at a dose of 440-2200 mg/day [3]. According to [4], there was a possibility that the pharmacological effect of curcumin came from its degradation product which has higher water solubility.

Several studies on curcumin degradation reported that more than 90% of curcumin was degraded rapidly under high pH [5]. The degradation products of curcumin were formed after 10 minutes at pH 8.5. The degradation products were feruloyl methane and ferulic acid which degraded further to vanillin. UV-Vis spectrophotometry method can be used to observe the degradation of curcumin, not only because of more cost-effective but also because it can be used for qualitative as well as quantitative analysis [6].

Computational science is a link between theory and experiment that can be used as a guide for scientists to minimize the possibility of errors occurring when conducting experiments. Density Functional Theory (DFT) is a method of calculating energy using charge density. This method has an advantage over previous methods such as ab initio and semi-empirical because it can calculate a complex compound more simply and quickly with reliable results.

In this study, the degradation of curcumin and its degradation product was studied by calculating geometric optimization, optimizing the effect of pH (8, 10, 12, and 14), and solvents (ethanol and methanol) using the UV-Vis spectrophotometry method and confirmed using DFT calculation.

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MATERIALS AND METHOD

Materials and Instrument

The materials used in this study were curcumin 99% (Sigma Aldrich), vanillin 99% (Sigma Aldrich), methanol (Smartlab), ethanol (Smartlab), aqua DM, NaOH (Sigma Aldrich), and using a UV-Vis Spectrophotometer instrument Genesys 10S (Thermo Scientific).

Preparation of stock solution and working solution of curcumin

The research procedure was initiated by making a $1x10^{-3}$ M curcumin stock solution. The curcumin stock solution was prepared by dissolving 9.3 mg of curcumin with 25 mL of ethanol and methanol, respectively. Then proceed with making a 1.2 x 10^{-5} M curcumin working solution. The curcumin working solution was prepared by diluting 3 mL of curcumin stock solution with 250 mL of ethanol and methanol solvents. The working solution of curcumin 1.2 x 10-5 M was added with 0.1 N NaOH solution and measured with a pH meter until it reached the predetermined pH, with variations in pH 8, 10, 12, and 14.

UV-Vis Spectrophotometry

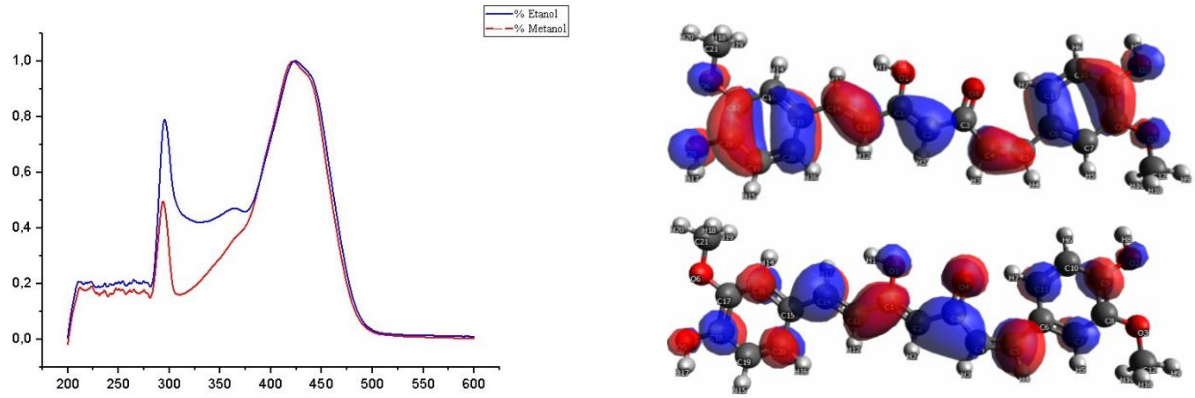
The absorbance was measured and determined at maximum wavelength using a UV-Vis spectrophotometer. Tests were carried out after 10 minutes, 1 hour, 3 hours, 9 hours, 24 hours, 48 hours, and 192 hours.

Computational Method

The computational optimization was carried out at the B3LYP level with the ORCA 4.0.1 program. The ORCA input file was created using Avogadro 1.2.0 software. Computational calculations using the DFT method provide output in the form of optimized chemical structure, energy in the ground state, and chemical compound coordinates for the next calculation, namely the TD-DFT calculation. TD-DFT calculations provide data in the form of UV-Vis spectra, energy gap, and electron density of the HOMO/LUMO orbitals.

DISCUSSION

Figure 1 showed that curcumin has a maximum wavelength of 420 nm in methanol solvent [7]. While curcumin in ethanol solvent has a maximum wavelength of 425 nm [6]. The excited state orbital of curcumin in ethanol has lower excitation energy levels. So that absorption occurs at a larger wavelength.



(a) (b)

FIGURE 1. (a) UV Spectra of Curcumin in Methanol and Ethanol (b) HOMO and LUMO of curcumin

The maximum absorption that occurs at 420 nm and 425 nm in curcumin spectra was caused by the electron transition from HOMO to LUMO, namely the electron transition from n–π* which occurs due to the presence of auxochrome groups in the curcumin structure (Fig 1). The DFT calculation of the excitation energy required for the n–π* transition was 2.727 eV, with a maximum wavelength of 455 nm (Table 1). While the maximum absorption at a wavelength of 363 nm was caused by the excitation of electrons from HOMO-2 to LUMO, namely the π-π* electron transition. The π-π* transition indicates the presence of a conjugated C=C chromophore group. The DFT calculation states that the excitation energy required for the transition from π-π* was 3.584 eV which was equivalent to 345 nm. The experimental results did not show a significant difference with the DFT calculation.

TABLE 1. Comparison of Experimental and Theoretical Curcumin Spectra

Solvent	Experiment λ (nm) max	TDDFT Method λ (nm) max
Methanol	420	455
Ethanol	425	454

Figure 2 shows that, at alkaline pH, the maximum wavelength of curcumin was shifted in a similar trend. In the initial 24 hours, curcumin's maximum wavelength was shifted to a larger wavelength from 470 nm to 500 nm. This was caused by the deprotonation in the structure of curcumin in alkaline pH [8]. Furthermore, after 24 hours, curcumin's maximum wavelength was shifted to a lower wavelength caused by the degradation of curcumin.

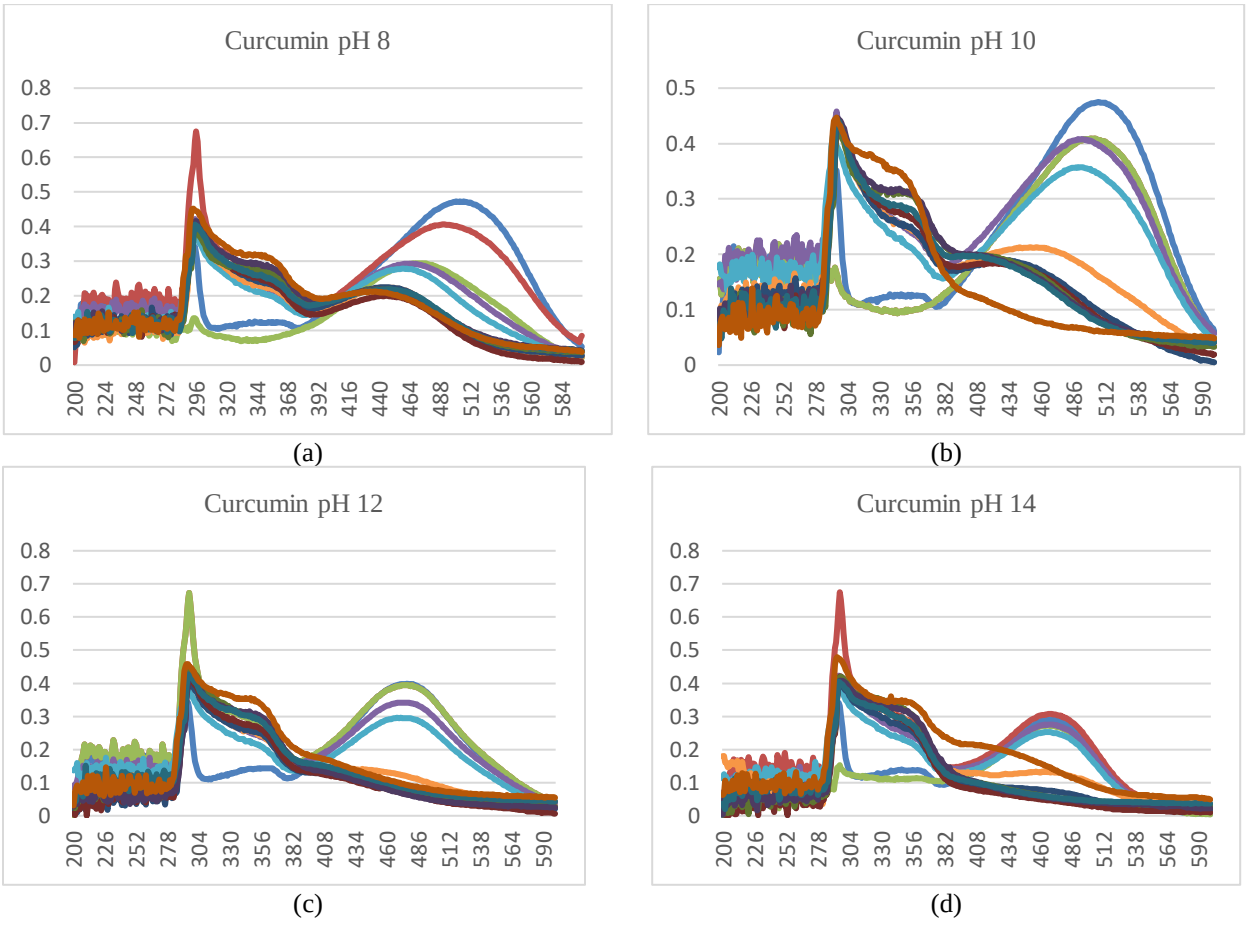


FIGURE 2. (a) UV Spectra of Curcumin at pH 8, (b) pH 10, (c) pH 12, and (d) pH 14

Table 2 and Table 3 showed that in methanol, curcumin degrades faster than in ethanol. This indicated that curcumin has higher stability in ethanol solvents [6]. This was due to ethanol polarity that was close to curcumin [9]. In addition, in each solvent, the higher the pH, the more curcumin was degraded [10]. At high pH, more OH- attack

the carbonyl group of curcumin through alkaline hydrolysis reactions [11]. During the hydrolysis reaction, the curcumin base was degraded into smaller compounds. Therefore, curcumin levels decreased as the pH increased.

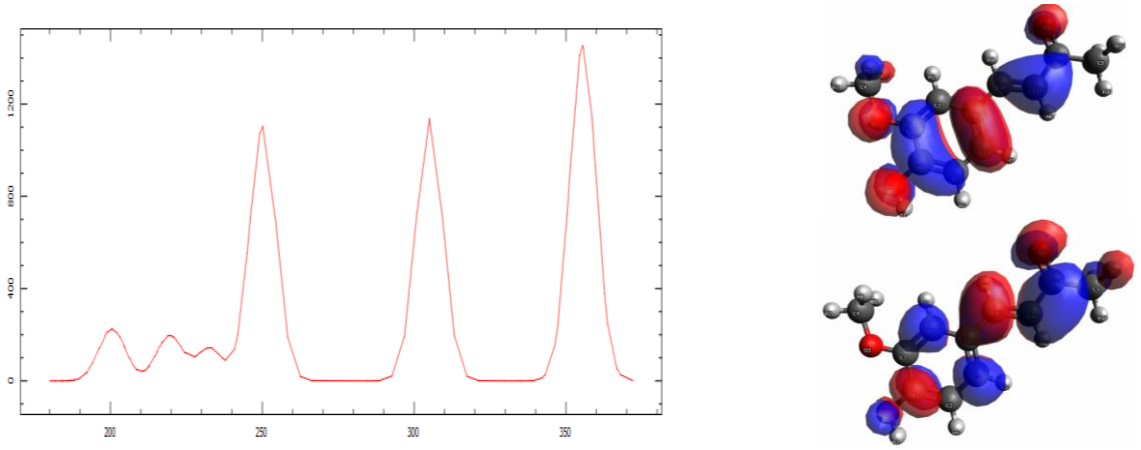
TABLE 2. The Decrease of Curcumin Concentration in Methanol After 10 Minutes

pH	Absorbance	Concentration	Degraded Curcumin
neutral	0.61	1.217460317	0
8	0.228	0.813227513	41.13%
10	0.220	0.808994709	41.44%
12	0.200	0.804761905	41.74%
14	0.160	0.741269841	46.34%

TABLE 3. The Decrease of Curcumin Concentration in Ethanol After 10 Minutes

pH	Absorbance	Concentration	Degraded Curcumin
neutral	0.765	1.545295	0
8	0.545	1.369856	3.646%
10	0.456	1.298884	8.638%
12	0.371	1.2311	13.405%
14	0.293	1.1689	17.7809%

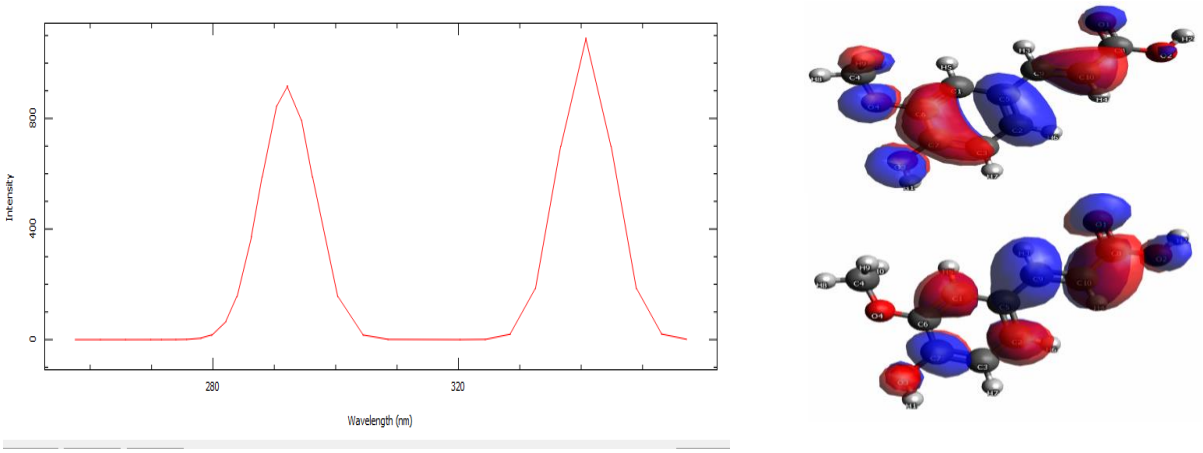
After 10 minutes, curcumin changed its color to red at pH 8 and brick red at pH 10, 12, and 14. The change in the curcumin color indicated a change in the curcumin chemical structure due to alkaline hydrolysis. The brown color that appears on curcumin indicates the formation of curcumin degradation products, feruloyl methane. Feruloyl methane quickly forms a brownish yellow condensation product. After 24 hours, the curcumin solution become colorless and became clearer over time. This indicates that the degradation products of curcumin, ferulic acid, and vanillin were starting to be formed.



(a) (b)

FIGURE 3. (a) TDDFT Spectra of Feruloylmethane (b) HOMO and LUMO Feruloylmethane

The absorption spectra of feruloyl methane calculated using the DFT method on the B3LYP theory and the def2-TZVP basis set produced several peaks that appeared with various intensities (Fig 3). The highest peak that appears in the absorption spectra of feruloyl methane was at 356.12 nm produced from the n-π* electron transition with a predicted energy gap (Eg) of 3.482 eV. While the second peak at 305 nm was the of the π-π* electron transition with an energy gap of 4.065 eV which indicates the presence of a conjugated C=C chromophore group [11]. The DFT method did not show a significant difference with the experimental results, so it can be concluded that the peak that appears at 355 nm in the curcumin spectra was the peak of a curcumin degradation product, feruloyl methane.

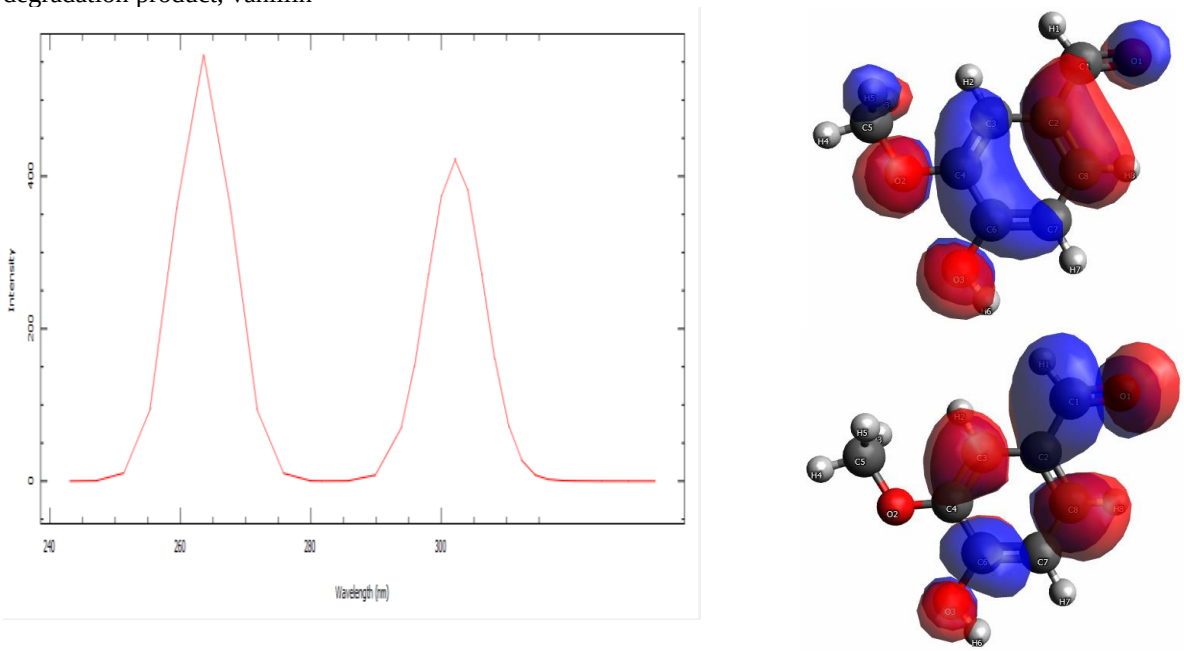


(a) (b)

FIGURE 4. (a) TDDFT Spectra of Ferulic Acid (b) HOMO and LUMO Ferulic Acid

Figure 4 shows the spectra from TDDFT on the theory of B3LYP def2-TZVP for ferulic acid compounds. The peak at 340 nm was produced from carbonyl n→π* electron transition (Fig. 4). The energy gap (Eg) from the DFT method for the n→π* transition was 3.642 eV (340.41 nm). The second peak at 291.92 was the π→π* electrons transition from ferulic acid double bonds with an energy gap of 4.247 eV. The DFT study did not show any significant differences, so the peaks that appeared at 340 nm and 292 nm in the experimental curcumin spectra were the peaks of the degradation product of curcumin, ferulic acid.

The absorption peak at 307 nm was produced from vanillin HOMO→LUMO electron excitation. The n-π* electron transition with an energy gap (Eg) of 3.915 eV was calculated using B3LYP/def2 -TZVP basis set (Fig 5). While the second peak that appears at 263 nm was due to π-π* electron transition. The excitation energy generated from the π-π* transition was 4.704 eV. The results obtained from the DFT method did not show a significant difference from the experimental results, so the peak that appeared at 307 nm was the peak of the curcumin degradation product, vanillin



(a) (b)

FIGURE 5. (a) TDDFT Spectra of Ferulic Acid (b) HOMO and LUMO Ferulic Acid

CONCLUSION

The higher the solution pH, the faster the degradation of the curcumin. In methanol solvent, curcumin degraded faster than in ethanol solvent. After the first 10 minutes, the degradation products of curcumin were ferulic acid and feruloyl methane which appeared at maximum wavelengths of 355 nm and 340 nm. In addition, feruloyl methane underwent further degradation to vanillin after 9 hours with a maximum wavelength of 307 nm. This was confirmed by the DFT calculation. The DFT calculation was in accordance with the literature and experiments.

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