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Ultrasonic Assisted Synthesis of 1,5-*Bis*(3′-ethoxy-4′-hydroxyphenyl)-1,4-pentadiene-3-one: Effect of Time and Temperature

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The compound 1,5-*bis*(3′-ethoxy-4′-hydroxyphenyl)-1,4-pentadiene-3-one (EHP), an analog of curcumin, displays significant antioxidant, anti-inflammatory and antibacterial properties. Synthesized through an aldol condensation reaction, EHP modifies the structure of curcumin by substituting methoxy groups with ethoxy groups and converting diketones to monoketone. This study explores the influence of time and temperature on the ultrasonic-assisted synthesis of 1,5-*bis*(3′-ethoxy-4′-hydroxyphenyl)-1,4-pentadiene-3-one (EHP). The synthesis process involved precise measurements and controlled conditions to achieve the desired compound. Through a series of experiments conducted at varying temperatures and irradiation times, the synthesis yield and purity of EHP were evaluated. Analysis techniques including thin layer chromatography (TLC), densitometry, ultraviolet-visible (UV-vis) spectrophotometry, Fourier-transform infrared spectroscopy (FTIR), liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy were employed to characterize the EHP compound. The results indicate the sensitivity of EHP synthesis to reaction conditions, it reduce synthesis time (10 min) with improve the product yield (87.17%) at moderate temperature (30 ºC). The findings provide valuable insights into optimizing synthesis conditions for enhanced product quality.

Keywords: Aldol condensation, Curcumin, Ultrasonication, Time, Temperature.

INTRODUCTION

Indonesia's abundant biodiversity offers a vast reservoir of potential medicinal resources. Among these, curcumin, a compound derived from *Curcuma longa*, has garnered significant attention and research. Notably, curcumin stands out for its yellow colour and versatile applications as a food coloring, spice and medicine, devoid of adverse effects [1]. Its multifaceted activities, including antioxidant, anti-inflammatory and antibacterial properties, have fueled extensive exploration aimed at uncovering new, more potent medicinal compounds [2]. Therefore, various compounds are reported such as 1,5-*bis*(3′-ethoxy-4′-hydroxyphenyl)-1,4-pentadiene-3-one (EHP) represents a curcumin analog with superior antioxidant, anti-inflammatory and antibacterial effects [3,4] compared to curcumin itself. Exhibiting enhanced activity over curcumin, EHP displays potent antibacterial effects, surpassing conventional antibiotics like amoxicillin and cefadroxil in combating Gram-positive and Gram-negative bacteria [3].

Prior investigations into EHP synthesis predominantly relied on the conventional methods such as magnetic stirring in reverse cooling and reflux. The aldol condensation method utilizing ethyl vanillin, acetone, methanol and hydrochloric acid as catalyst under specific conditions. However, these methods entail prolonged reaction times and substantial solvent usage, necessitating the exploration of alternative synthesis routes [5].

The efficient synthesis of organic compounds is a fundamental aspect of modern chemical research, driven by the demand for novel materials with tailored properties. Ultrasonic-assisted synthesis has emerged as a promising technique, offering enhanced reaction rates and yields compared to the traditional methods [6,7]. Therefore, utilization of the ultrasonication method for EHP synthesis, employing acoustic waves at a frequency of 40 kHz. By capitalizing on the acoustic properties of these waves, a significant improvement in the synthesis efficiency of EHP is anticipated, potentially yielding higher purity and quantity of the compound.

Previous work reports the efficacy of ultrasonication in enhancing the yield of curcumin and its analogs, suggesting its potential for optimizing EHP synthesis. Studies have reported substantial increases in yield with prolonged ultrasonic extraction times, further underscoring the promise of this method [8,9]. Moreover, the application of ultrasonication in condensation reactions has yielded high yields of curcumin analogs, displaying its versatility and effectiveness in synthetic chemistry [10,11]. However, despite the growing interest in ultrasonic-assisted synthesis, there remains a gap in knowledge regarding the specific effects of time and temperature on the synthesis of 1,5-*bis*(3′-ethoxy-4′-hydroxyphenyl)-1,4-pentadiene-3-one using this methodology.

This study aims to address this gap by systematically investigating the impact of time and temperature on the ultrasonic-assisted synthesis of 1,5-*bis*(3′-ethoxy-4′-hydroxyphenyl)-1,4-pentadiene-3-one. Through a series of experiments, the optimal conditions are elucidated for maximizing product yield and purity while minimizing undesirable byproducts. The insights gained from this research will not only contribute to the fundamental understanding of sonochemical reactions but also offer practical guidelines for the scalable synthesis of this valuable compound for various applications.

EXPERIMENTAL

Ethyl vanillin (3-ethoxy-4-hydroxybenzaldehyde) of highest grade, acetone, concentrated hydrochloric acid, 96% ethanol, methanol, *n*-hexane and ethyl acetate were procured from Sigma-Aldrich, USA with 98% purification. All the chemical reagents were employed without additional purification.

Synthesis of 1,5-*bis*(3′-ethoxy-4′-hydroxyphenyl)-1,4-pentadiene-3-one (EHP): In a dried beaker, ethyl vanillin (4.8 g) was carefully added to 7.2 mL acetone while stirring until a homogeneous mixture achieved. Following this, 100 µL of HCl was carefully introduced into the solution. The resulting mixture was sonicated at different temperatures of 30, 40 and 50 ºC for 10, 20 and 30 min, respectively, utilizing a power of 36 KHz. Upon completion of the reaction, the product was purified through the column chromatography and then recrystallized with ethanol-water (1:2) and finally allowed to cool for a day. The precipitated crystals were subsequently filtered and dried thoroughly to prepare them for further analysis.

Purification of EHP by column chromatography: For purification of synthesized EHP compound to prepare a column, a small bead of cotton was added as a filler at the bottom, followed by the addition of 230-400 mesh silica gel powder until it reached three-quarters of the column's height. Firstly, column must be saturated packed with *n*-hexane-ethyl acetate (3:2) eluent and to ensure the air voids are absent into a packed column after that column must be closed by stoper. Then, the synthesized compound was dissolved in *n*-hexane-ethyl acetate (3:2) and introduced into the column. Subsequently, the eluent was continuously added and separated fractions were collected in vials until all compounds were isolated. Each fraction collected underwent analysis *via* thin layer chromatography (TLC). EHP compound was dissolved in ethanol (96%) followed by water in order to obtain a precipitate and the remaining solvent was allowed to evaporate and finally collected the dry product.

Characterization: The Fourier transform infrared spectrum was obtained using a Bruker TFS-66 V/S FT-IR spectrometer, scanning in the range of 4000-600 cm^{-1} using the KBr pellet. The maximum wavelength (λ_{max}) of the synthesized EHP was determined using a TU-1810PC UV-vis spectrophotometer across a range of 200-800 nm. Liquid chromatography analysis utilized a Finnigan Surveyor MS system with a Phenomenex Synergi Fusion-RP column operating at 25 ºC, injecting 10 µL of sample. LC-MS/MS employed a mobile phase of 15 mM ammonium formate and formic acid (pH = 3.3) in water (A) and methanol (B) at 150 µL/min, with gradient elution over 0-15 min. Mass spectral analysis employed a LCQ ion-trap mass detector in positive ion mode with specific settings. Preliminary tuning involved 2-dimethylaminoethanol, optimizing the parameters with TunePlus software. Amine characterization included retention times and full scan MS/MS data (±standard deviation, n = 20).

RESULTS AND DISCUSSION

The compound 1,5-*bis*(3′-ethoxy-4′-hydroxyphenyl)-1,4-pentadien-3-one (EHP) represents a modification to the gamavuton structure, achieved by substituting the methoxy group with an ethoxy group through an aldol condensation reaction. Aldol condensation involves the combination of two distinct carbonyl compounds, typically ketones or aldehydes, catalyzed by either an acid or a base [5]. In this synthesis, ethyl vanillin ($C_9H_{10}O_3$) serves as the primary component, while acetone (C_3H_6O) functions as both a solvent and a reagent, facilitating the connection of two ethyl vanillin molecules by forming a conjugated double bond with an aromatic ring. HCl serves as the catalyst, enhancing reaction efficiency by acidifying the environment (pH ± 2) and thereby accelerating product formation due to an increased concentration of hydrogen ions. This acidity promotes the generation of a stable enolate, heightening the reactivity of the enolate's α-carbon towards the carbonyl carbon of ethyl vanillin. Acid-catalyzed reactions proceed *via* enolate formation, which represents the rate-determining step. Subsequently, the enolate's carbon-carbon double bond undergoes electrophilic addition to yield a more stable carbocation. Thin layer chromatography (TLC) employing *n*-hexane-ethyl acetate eluent (3:2) facilitated compound identification, followed by recrystallization in ethanol-water (1:2) and cooling to the induce crystal formation, ensuring product purity.

Table-1 presents the R_f values of both the synthesized compounds and ethyl vanillin, along with the number of spots observed for each synthesized compound using TLC. These findings demonstrate that EHP synthesis *via* ultrasonication is rapid; however, the presence of multiple visible spots indicates the need for further treatment, specifically purification through column chromatography.

The percentage yield results from the synthesis of EHP compounds are shown in Table-2, revealing the minimal variation in yield across different time units. The synthesized EHP

TABEL-2
PERCENTAGE OF YIELD

Temp. (°C)	Time (min)	Initial ethyl vanillin		Mass of EHP		Yield (%)
		gram	mol	Theoritical	Experiment	
30	10	4.8076	0.0289	5.1120	4.4563	87.17
	20	4.8080	0.0289	5.1120	4.1802	81.77
	30	4.8082	0.0289	5.1120	4.3991	86.05
40	10	4.8130	0.0290	5.1475	4.3891	85.27
	20	4.8140	0.0290	5.1475	4.0569	78.81
	30	4.8137	0.0290	5.1475	3.8540	74.87
50	10	4.8021	0.0289	5.1120	4.0054	78.35
	20	4.8048	0.0289	5.1120	4.1240	80.67
	30	4.8055	0.0289	5.1120	4.2357	82.80

TABLE-1
RETARDATION FACTOR OF COMPOUNDS MIXTURE (SYNTHESIS RESULT) ON THE TLC PLATE

Temp. (°C)	Time (min)	Number of node	Retardation factor (R_f)		R_f of ethyl vanillin
			Node 1	Node 2	
30	10	3	0.41	0.51	0.65
	20	3	0.41	0.51	0.65
	30	3	0.40	0.50	0.64
40	10	3	0.40	0.50	0.65
	20	3	0.40	0.50	0.65
	30	3	0.40	0.50	0.65
50	10	3	0.40	0.51	0.65
	20	3	0.40	0.51	0.65
	30	3	0.40	0.51	0.66

TABLE-3
MELTING POINT RANGE ON ETHYL VANILLIN AND EHP COMPOUNDS

Temp. (°C)	Time (min)	Melting point (°C)		
		EHP before coloumn chomatography	EHP after coloumn chomatography	Ethyl vanillin
30	10	72.2-73.5	79.2-79.6	77.7-78.5
	20	72.5-73.9		
	30	72.9-74.2		
40	10	73.5-74.8		
	20	73.7-75.1		
	30	73.8-75.9		
50	10	75.1-76.5		
	20	75.3-76.7		
	30	75.5-77.1		

compound appears as a greenish-yellow powder with a distinct aromatic odour and exhibits a lower melting point compared to the reference EHP compound. Significantly, its melting range is almost matched with that of ethyl vanillin, suggesting the incomplete reaction conversion. Conversely, purified EHP displays a slightly elevated melting range of 79.2-79.6 ºC (Table-3) indicating enhanced purity compared to ethyl vanillin and confirming successful purification efforts.

Densitometry: Densitometry was employed to determine accurately the actual percentage yield of EHP compounds synthesized. Utilizing a maximum absorption wavelength of 283 nm, the densitometry test yields the results. Table-4 illustrates that variations in temperature and duration exert an influence on the synthesis yield of EHP compounds. Reduced yields may stem from reversible reactions, hindering complete conversion of reactants to products. The complexity of the aldol condensation reaction mechanism further complicates matters, as intermediate products may arise alongside the desired EHP compound. These intermediates possess the potential to undergo further reactions, leading to the formation of undesired by-products. Thus, elucidating the intricacies of the reaction pathway becomes crucial for optimizing synthesis conditions and enhancing product yield.

UV-Visible studies: Ultraviolet-visible (UV-vis) spectrophotometry, which measures the electromagnetic radiation absorption within a specific wavelength range, serves as a pivotal tool for characterizing synthesized compounds. Through UV-vis spectrophotometry, the wavelength at which EHP compounds exhibit the maximal absorption can be identified (Fig. 1). Analysis of the UV-visible spectrum helps in identifying chromophore groups, such as benzene rings or conjugated double bonds. Using the Woodward-Fieser rule, the theoretical maximum absorption wavelength of EHP compounds is 281 nm. The experi-

TABLE-4
DATA ON THE PERCENTAGE YIELD OF ETHYL VANILLIN AND EHP COMPOUNDS USING DENSITOMETRY ANALYSIS

Temp. (°C)	Time (min)	R_f		Area (%)		Percentage of EHP in yield
		EHP	Ethyl vanillin	EHP	Ethyl vanillin	
30	10	0.41	0.65	12.16	33.44	10.60
	20	0.41	0.65	17.49	42.87	14.30
	30	0.41	0.64	13.16	29.32	11.32
40	10	0.42	0.64	7.22	70.10	6.16
	20	0.41	0.64	10.33	59.42	8.14
	30	0.42	0.65	11.38	32.81	8.52
50	10	0.51	0.65	2.31	57.16	1.81
	20	0.52	0.73	2.09	34.21	1.69
	30	0.45	0.66	8.55	38.72	7.08

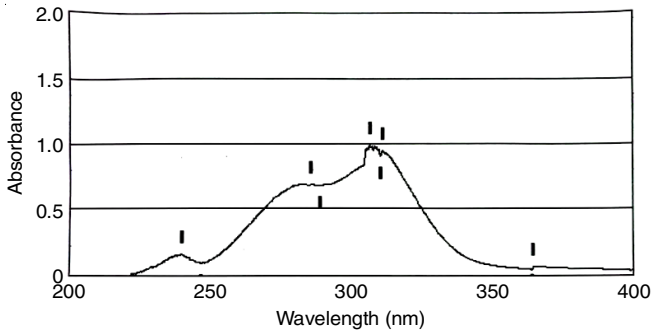

Fig. 1. Absorbance spectrum of sythesized result compounds

mental findings revealed that the EHP compounds synthesized *via* ultrasonication exhibit a maximum absorption wavelength of 286.30 nm, with the appearance of the additional absorption peaks at 311.80 nm and 307.20 nm possibly attributable to side product compounds. Further, EHP compounds exhibit absorption peaks at 389.00 nm and 283.00 nm (Table-5), with the latter aligning closely with the wavelength observed for EHP synthesized *via* ultrasonication. Discrepancies between calculated and observed maximum wavelength values typically range from 0 to 4 nm according to the Woodward-Fieser theory, indicating relatively stable synthesis conditions for the compound.

TABLE-5
MAXIMUM WAVELENGTH OF SYTHESIZED COMPOUNDS

Compound	Maximum wavelength (nm)
Ethyl vanillin	365.10, 226.50
EHP	311.80, 307.20, 286.30

FTIR studies: The FTIR spectrum (Fig. 2) reveals the significant characteristics of the synthesized EHP compound. An absorption band at 3348.74 cm^{-1} signifies the presence of hydroxy groups (-OH), indicative of robust hydrogen bonding with adjacent groups, resulting in the distinct OH absorption. The conjugated carbon groups (-C=C-) within the aromatic benzene nucleus manifest at 1571.69 and 1503.23 cm^{-1}. Additionally, C-H stretching bands were observed at 2935.14 and 2729.77 cm^{-1}. The sharp stretching vibration at 1674.37 cm^{-1} indicates the presence of a ketone group (C=O). An absorption band at 1101.04 cm^{-1} suggests the carbon-oxygen interaction within the ethoxy group. Furthermore, wavenumbers at 827.20 and 630.39 cm^{-1} denote the aromatic ring substitution. These spectral features collectively provide the valuable insights into the molecular structure and functional groups present in the synthesized EHP compound.

LC-MS analysis: The liquid chromatography-mass spectrometry (LC-MS) is pivotal in characterizing EHP compounds, facilitating identification based on the molecular weight and fragmentation patterns resulting from electron beam bombardment. The chromatogram distinctly illustrates the retention times of ethyl vanillin and synthesized EHP compounds. Ethyl vanillin exhibits a molecular weight of 166.18 g/mol, whereas EHP weighs 354.40 g/mol. The chromatograms of the synthesized compounds reveal peaks at retention times of 6.48, 7.85, and 10.07 min, indicating impurity due to the absence of a singular peak. Mass spectrometer analysis identifies fragments corresponding to each retention time (Fig. 3). Remarkably, the compound EHP was detected at a retention time of 10.07 min with a molecular weight represented by an *m/z* value of 355.15.

^{1}H NMR studies: The ^{1}H NMR spectrum (Fig. 4) reveals the distinct chemical shifts at δ_H 1.3248 and 2.5735 ppm, indicative of methyl groups; δ_H 6.7642 and 7.2662 ppm, representing aromatic groups containing C=C and -O-C bonds; and δ_H 7.6244 ppm, corresponding to an ethylene group with a C=C bond. These chemical shifts also provide valuable insights into the molecular composition and structural elements present within the analyzed compound.

Conclusion

In summary, this study elucidates the intricate relationship between reaction conditions and the synthesis of 1,5-*bis*(3′-ethoxy-4′-hydroxyphenyl)-1,4-pentadiene-3-one (EHP) *via* ultrasonication. The experimental results underscore the importance of temperature and duration in influencing synthesis yield and purity. Through comprehensive analysis utilizing various spectroscopic and chromatographic techniques, the molecular structure and functional groups of the synthesized compound were elucidated. Challenges such as impurities and incomplete conversion were identified, emphasizing the need for further optimization of the synthesis conditions. Overall, this research contributes to the understanding of reaction kinetics and optimization strategies for the synthesis of EHP and related compounds, with potential applications in pharmaceuticals, materials science and organic chemistry.

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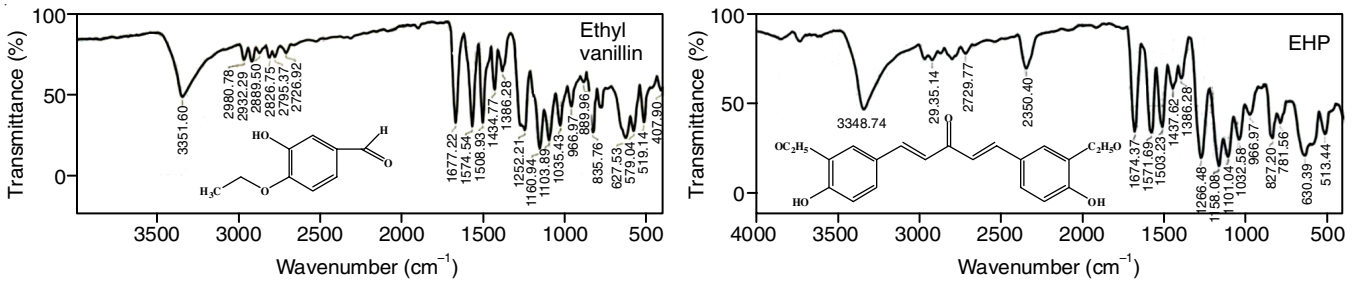

Fig. 2. FTIR spectrum of sythesized result compounds

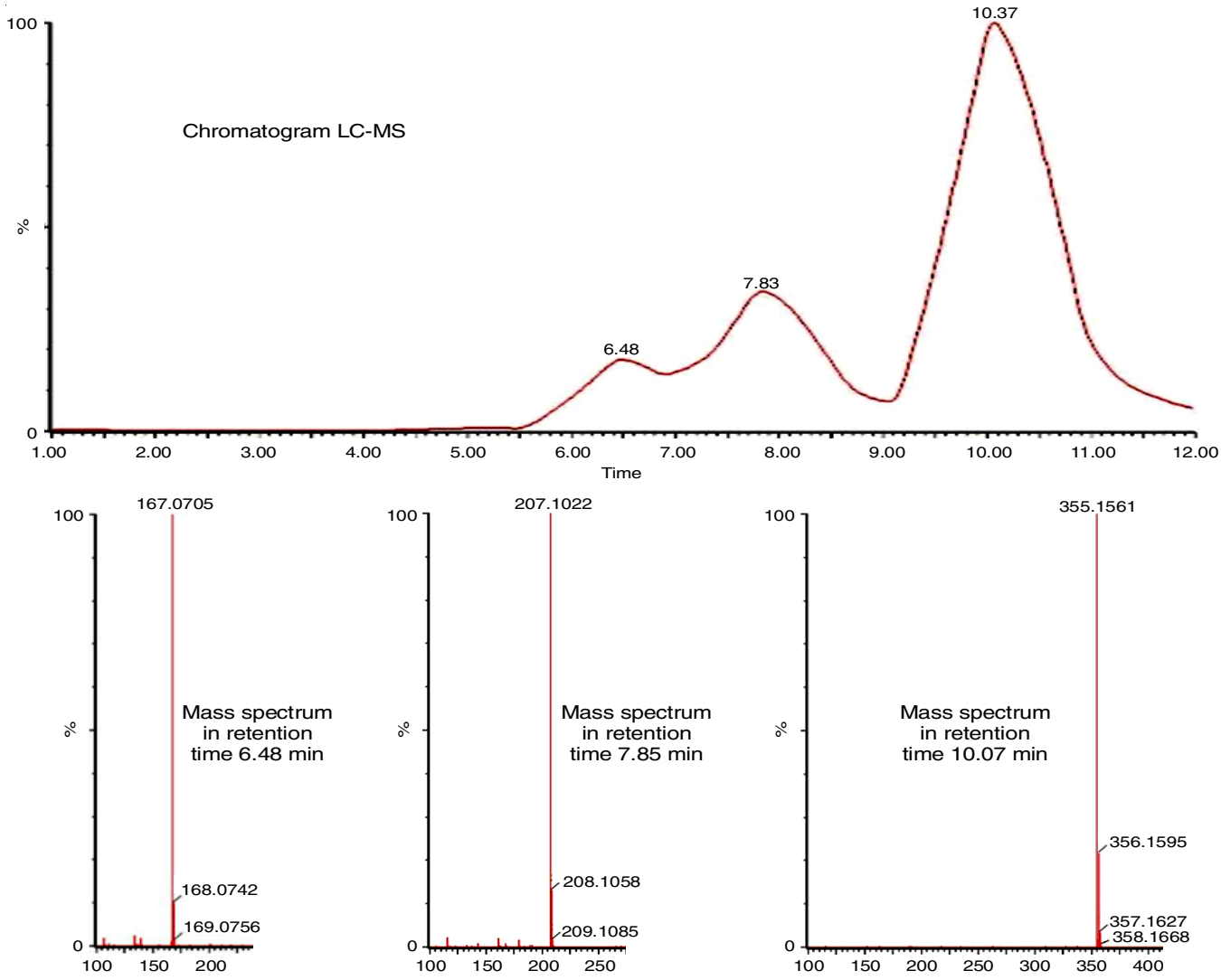

Fig. 3. Chomatogram of sythesized result compounds

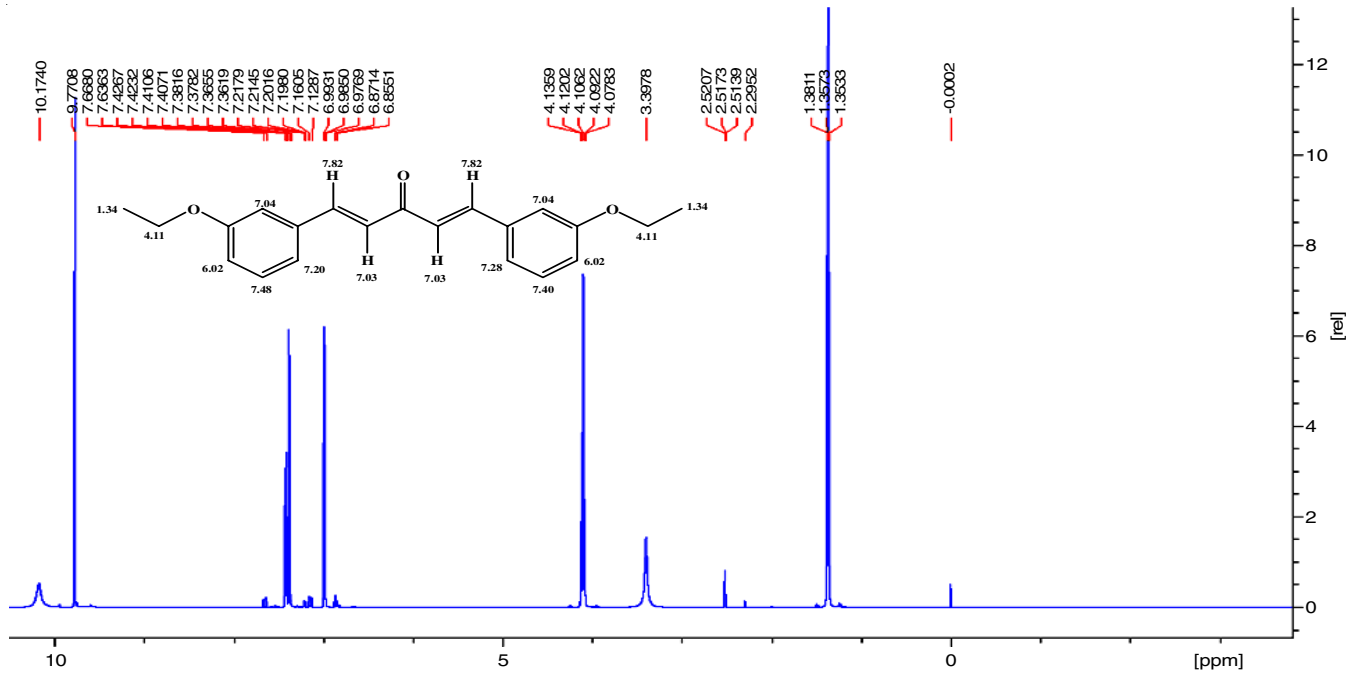

Fig. 4. H NMR spectrum of sythesized result compounds

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. M.T. El-Saadony, T. Yang, S.A. Korma, M. Sitohy, T.A.A. El-Mageed, S. Selim, S.K. Al Jaouni, H.M. Salem, Y. Mahmmod, S.M. Soliman, S.A.A. Mo'men, W.F.A. Mosa, N.A. El-Wafai, H.E. Abou-Aly, B. Sitohy, M.E. Abd El-Hack, K.A. El-Tarabily and A.M. Saad, *Front. Nutr.*, **9**, 1040259 (2023); https://doi.org/10.3389/fnut.2022.1040259
2. J. Sharifi-Rad, Y. El-Rayess, A.A. Rizk, C. Sadaka, R. Zgheib, W. Zam, S. Sestito, S. Rapposelli, K. Neffe-Skocinska, D. Zielinska, B. Salehi, W.N. Setzer, N.S. Dosoky, Y. Taheri, M. El-Beyrouthy, M. Martorell, E.A. Ostrander, H.A.R. Suleria, W.C. Cho, A. Maroyi and N. Martins, *Front. Pharmacol.*, **11**, 01021 (2020); https://doi.org/10.3389/fphar.2020.01021
3. A. Purwanggana, E. Mumpuni and E. Mulatsari, *Int. J. Pharm. Pharm. Sci*., **10**, 70 (2018); https://doi.org/10.22159/ijpps.2018v10i5.25143
4. G. Liang, S. Yang, L. Jiang, Y. Zhao, L. Shao, J. Xiao, F. Ye, Y. Li and X. Li, *Chem. Pharm. Bull.*, **56**, 162 (2008); https://doi.org/10.1248/cpb.56.162
5. R. Mestres, *Green Chem*., **6**, 583 (2004); https://doi.org/10.1039/B409143B
6. M.A. Ahmed and A.A. Mohamed, *iScience*, **27**, 108583 (2024); https://doi.org/10.1016/j.isci.2023.108583
7. L. Shen, S. Pang, M. Zhong, Y. Sun, A. Qayum, Y. Liu, A. Rashid, B. Xu, Q. Liang, H. Ma and X. Ren, *Ultrason. Sonochem*., **101**, 106646 (2023); https://doi.org/10.1016/j.ultsonch.2023.106646
8. D. Zelelew, M. Endale, Y. Melaku, T. Geremew, R. Eswaramoorthy, L.T. Tufa, Y. Choi and J. Lee, *Adv. Appl. Bioinform. Chem*., **16**, 61 (2023); https://doi.org/10.2147/AABC.S403413
9. F.E. Hawaiz and D.A.S. Omer, *ARO-Sci. J. Koya Univ*., **5**, ARO.10149 (2017); https://doi.org/10.14500/aro.10149
10. M.A. Obregón-Mendoza, W. Meza-Morales, Y. Alvarez-Ricardo, M.M. Estévez-Carmona and R.G. Enríquez, *Molecules*, **28**, 289 (2023); https://doi.org/10.3390/molecules28010289
11. L. Mardiana, B. Ardiansah, A. Septiarti, R. Bakri and G. Kosamagi, *AIP Conf. Proc*., **1862**, 030096 (2017); https://doi.org/10.1063/1.4991200