

Synthesis, Characterization, and Antibacterial Activity Study of Novel Curcuminoids Derivatives

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Abstract

Curcumin is a well-known compound with broad spectrum biological activities, but unfortunately rapid degradation properties and low bioavailability. Therefore, several attempts have been made to overcome these obstructions through chemical modifications. This investigation aimed to prepare several curcuminoids and their pyrazole derivatives and study their antibacterial activity. Curcuminoids were prepared through condensation reaction of aromatic aldehyde and 2,4-pentanedione under ultrasound irradiation and in the presence of trimethyl borate and monoethanolamine, while their pyrazole derivatives were synthesized by reacting the previously prepared curcuminoids with hydrazine hydrate under reflux in the presence of acetic acid, then they were identified through melting point and their chemical structures were identified from their mass, ¹H NMR and ¹³C NMR spectra, and evaluated an *in vitro* study of the antibacterial activity of these compounds against the gram-negative *Escherichia coli* and the gram-positive *Staphylococcus aureus* using disc diffusion method. No compound has recorded any activity against *Escherichia coli*, while compounds 1-9 have shown anti-staphylococcus activity, compounds 11-14 didn't present any activity, and compound 10 shows activity at lowest studied concentration only. These findings suggest that the pyrazole substitution on curcumin derivatives that do not bear an aromatic hydroxyl group would diminish their anti-staphylococcal activity. More antibacterial activity studies are suggested regarding compound 10 activity.

Keywords Antibacterial activity; Chemical synthesis; Curcumin; Curcuminoids; Pyrazole.

تصنيع , تشخيص و تقييم الفعالية البيولوجية المضادة للبكتريا لمشتقات كركمية جديدة

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الخلاصة

يعتبر الكركمين من المركبات المعروفة بفعاليتها البيولوجية واسعة المدى، ولكن للأسف يعرف أيضا بسرعة تفككه وانخفاض توافره الحيوي ولهذا تم عمل العديد من التحويرات الكيميائية لمركب الكركمين لأجل تجاوز هذه العوائق. تهدف هذه الدراسة الى تحضير عدد من الكركميدات و مشتقاتها البيروزولية و دراسة فعاليتها المضادة للبكتريا. تم تحضير الكركميدات عبر تفاعل التكثيف بين الالدهايد الاروماتي و بين ٢,٤-بينتا دايون باستعمال جهاز الرنين الصوتي و بوجود البورات ثلاثية المثل و الأمين احادي الايثانول. بينما تم تصنيع مشتقاتها البيروزولية عبر مفاعلة تلك الكركميدات مع الهيدرازين هايدرات بوجود حمض الخليك، ثم تم تشخيص الكركميدات المحضرة عبر نقطة الانصهار، مطيافية الكتلة، طيف الرنين النووي المغناطيسي للبروتون ¹H NMR و طيف الرنين النووي المغناطيسي للكربون ¹³C NMR، تلاه دراسة الفعالية المضادة للبكتريا خارج خلوية لتلك المركبات على البكتريا سالبة صبغة الغرام الاشريكية القولونية *Escherichia coli* و على البكتريا العنقودية ذات صبغة الغرام الموجبة *Staphylococcus aureus* باستعمال طريقة تفشي القرص Disc diffusion method. لم يظهر الاختبار أي فعالية لأي مركب على بكتريا الاشريكية القولونية، بينما أظهرت المركبات المصنعة تنوعا في فعاليتها تجاه بكتريا المكورات العنقودية: فالكركميدات ١-٩ أظهرت فعالية في تقليل نمو المكورات العنقودية، اما الكركميدات ١١-١٤ فلم تظهر أي فعالية بينما كان للمركب ١٠ تأثير سلبي على نمو البكتريا في اقل تركيز درس له فقط. استنتجنا من دراستنا ان تعويض مجموعة البيروزول على مشتقات الكركمين لم يحسن من فعاليتها ضد بكتريا الاشريكية القولونية كما مركبات الكركميدات المحضرة الحاوية على مجموعة بايروزول والتي لا تملك أي مجاميع هيدروكسيل أرومته ليس لها أي فعالية على نمو بكتريا المكورات العنقودية. تقترح نتائج دراستنا أيضا إجراء دراسة موسعة أكثر للفعالية المضادة للبكتريا لمشتق الكركمين ١٠.

الكلمات المفتاحية: كركمين، بايروزول، كركميدات، الفعالية المضادة للبكتريا، التصنيع الكيميائي.

Introduction

Despite being a wealthy source of beneficial compounds, nature does not offer these to us on a golden platter; rather, it is entirely up to us to figure out how to use and modify them for our advantage, and the diferuloyl methane (or

Curcumin) is considered to be as one of the most health-benefiting goldmine natural compounds. Curcumin (Figure 1) is regarded to be the primary active component within the rhizomes of the golden Indian spice; Turmeric (*Curcuma longa*)⁽¹⁻⁴⁾.

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It is a polyphenolic compound with hydrophobic characteristics and miscellaneous biological activities including antioxidant, anti-inflammatory, anticancer, anti-microbial, anti-cardiovascular hepatoprotective activities, and despite its limited human toxicity its low bioavailability and rapid degradation, however, restrict its clinical applications^(1,5-8).

Curcumin can exist in solution in two tautomeric forms, the keto and enol forms as shown in Figure 1^(9,10). Many *in vitro* and *in vivo* studies reveal that the instability of curcumin is attributed to the enolic OH group^(10,11). Several structural alterations of curcumin were mentioned in literature such as conjugation of curcumin with gold, silver, PEG, and β -diglucoside^(10, 12). The biological activity of curcumin was also increased by modification of β -diketone moiety⁽⁴⁾.

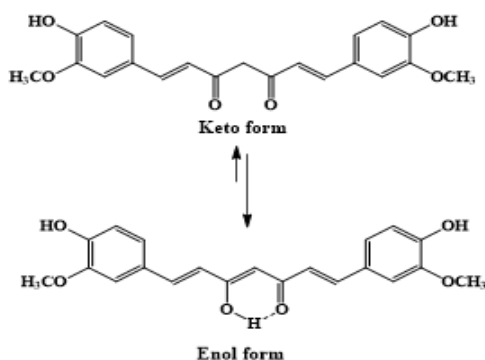


Figure1. The keto and enol forms of curcumin.

Pyrazole on the other hand, is a five-membered heterocyclic molecule with two nitrogen atoms and high biological activity including anticancer, antimicrobial, anti-inflammatory, and analgesic effect⁽¹³⁾. Attributed to the enormous activities of both curcumin and pyrazole nucleus, there is an increased need for designing novel derivatives of them.

The aim of this study is the synthesis of some pyrazoles based on curcuminoids and *in vitro* evaluation of their antibacterial activity.

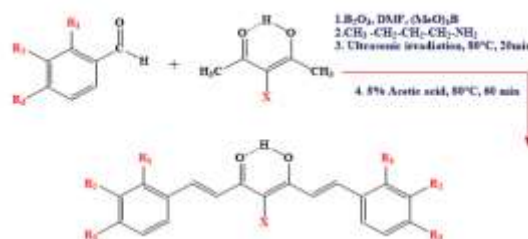
Materials and Methods

The starting materials and solvents were purchased from Sigma-Aldrich company without further purification. Melting points were measured without correction in a capillary tube with Stuart SMP30 electro thermal melting point apparatus. The reactions were followed by thin-layer chromatography. The NMR spectra were recorded with Bruker Avance Neo 400 MHz using deuterated DMSO as a solvent, and tetramethylsilane (TMS) as an internal standard. The ESI-MS of compounds 8-14 were measured with Waters Alliance 2695 Mass Spectrometer: Micromass Quattro micro-API, using two mobile phases, 80% (MeOH + 0.1 % Formic Acid) and 20% (H₂O + 0.1 % Formic Acid).

Chemical synthesis

General procedure for Synthesis of curcuminoids 1-7

The curcuminoids 1-7 were prepared according to the procedure described in the literature¹⁴, as shown in scheme 1.



	X	R1	R2	R3	Chemical structure
1	H	H	OCH3	OH	
2	CH3	H	H	OCH3	
3	CH3	H	OCH3	OCH3	
4	CH3	OCH3	OCH3	OCH3	

Scheme 1. Synthetic procedures for synthesis of curcuminoids

Continued Scheme 1

5	CH ₃	H	OCH ₃	OH	
6	CH ₃	H	OH	OCH ₃	
7	CH ₃	H	H	NHCOCH ₃	

The mixture of the aromatic aldehyde (0.025 mol: 4-hydroxy-3-methoxy benzaldehyde (3.8 gm), 4-methoxy benzaldehyde (3.4 gm), 3,4-dimethoxy benzaldehyde (4.175 gm), 2,3,4-trimethoxy benzaldehyde (4.925 gm), 3-hydroxy-4-methoxy benzaldehyde (3.8 gm), 4-acetamide benzaldehyde (4.075 gm)) and 3-methyl-2,4-pentadion (1.5 mL, 0.013 mol) were added to a mixture of boric oxide (0.9g, 0.013 mol), dimethylformamide (6 mL), trimethyl borate (2 mL) and butylamine (0.6mL, 0.006 mol) in 150 mL round bottomed flask. The reaction mixture was placed in the ultrasonic cleaner (40kHz, 500W) and irradiated for 20 min at 80°C. warm 5% acetic acid (100 mL) was added to the reaction mixture and stirred for 1 hour at 80°C. The crude product was filtered and washed several times with hot distilled water. Post-dried, the solid product was isolated by column chromatography on 200-300 mesh silica gel using a mixture of 3:1 ethyl acetate: hexane as an eluent.

1,7-bis (4-hydroxy-3-methoxy phenyl)-1,6-heptadiene-3,5-dione (**curcumin 1**). Yellow crystals; Yield 80%; mp. 179-181 °C (lit. ⁽¹⁴⁾ 180-182°C). ¹H NMR (DMSO-d₆, 400MHz), δ: 3.85(6H, s, OCH₃), 6.06 (1H,s,vinylic proton), 6.77(2H, d, *J*= 15.6 Hz, CH=C), 6.84(2H, d, *J*= 8 Hz, Ar-H), 7.16(2H, d, *J*= 8 Hz, Ar-H),7.33(2H, s, Ar-H) 7.57 (2H, d, *J*= 15.6 Hz, CH=C), 9.70 (2H, s, phenolic OH), ¹³C NMR (DMSO-d₆, 100 MHz) δ: 56.1, 101.4, 111.8, 116.2, 121.5, 123.6, 124.1, 124.2, 126.2, 126.8, 141.2, 145.1, 148.5, 149.8, 150.2, 184, 195.1. Elemental analysis: Calculated for C₂₁H₂₀O₆: C, 68.47; H, 5.47. Found C, 68.43; H, 5.43

4-Methyl-1,7-bis(4-methoxyphenyl)-1,6-heptadiene-3,5-dione (**compound 2**). Dark orang powder; Yield 77%; mp. 174-176 °C (lit. ⁽¹⁵⁾ 175-177 °C). As a tautomeric mixture's of diketo and enol forms, ¹H NMR (DMSO-d₆, 400MHz), δ:1.29 (3H, d, *J*=6 Hz, CH₃ diketo form) 2.19(3H, s, CH₃ enol form), 3.80(12H, OCH₃),4.6 (1H, q, *J*= 7.1, CH enol form), 6.91(4H, d, *J*= 16 Hz, CH=C), 6.99-7.77(20 H, m, olefinic and Ar-H). ¹³C NMR (DMSO-d₆, 100 MHz), δ: 12.0, 13.6, 55.7, 55.8, 106.5, 114.9, 115.0, 119.2, 123.4, 127.2, 128.2, 130.8, 131.0, 141.2, 143.6, 161.5, 161.9, 182.8,

196.9. Elemental analysis: Calculated for C₂₂H₂₂O₄: C, 75.41; H, 6.33. C, 75.35; H, 6.28.

4-Methyl-1,7-bis(3,4-dimethoxyphenyl)-1,6-heptadiene-3,5-dione (**3**). Dark orang powder; Yield 77%; mp. 149-151 °C (lit. ⁽¹⁶⁾ m.p. not found); As a tautomeric mixture's of diketo and enol forms, ¹H NMR (DMSO-d₆, 400MHz), δ:1.34(3H, d, *J*=6.8 Hz, CH₃ ketoform), 2.26(3H, s, CH₃ enol form), 3.40-3.88 (24H, OCH₃), 4.64(1H,q, *J*= 6.8Hz, CH ketoform), 7.01-7.67 (20H, m, olefinic and Ar-H). ¹³C NMR (DMSO-d₆, 100 MHz), δ: 12.0, 13.6, 55.7, 56.0, 56.2, 56.3, 106.6, 110.9, 111.1, 111.7, 112.0, 119.3, 123.7, 123.7, 124.1, 127.4, 128.4, 141.8, 144.0, 149.5, 149.5, 151.4, 151.8, 182.9, 196.9. Elemental analysis: Calculated for C₂₄H₂₆O₆: C, 70.23; H, 6.38. Found: C, 70.27; H, 6.32.

4-Methyl-1,7-bis(3,4,5-trimethoxyphenyl)-1,6-heptadiene-3,5-dione (**4**). Dark yellow powder; Yield 67%; mp. 111-113 °C; As a tautomeric mixture of diketo and enol forms, ¹H NMR (DMSO-d₆, 400MHz), δ: 1.33 (3H, d, *J*=6.9 Hz, CH₃ keto form), 2.26 (3H, s, CH₃),3.70- 3.86(36H, OCH₃), 4.63(1H, q, *J*= 6.9Hz, CH keto form), 7.06-7.65(16H, m, olefinic and Ar-H). ¹³C NMR (DMSO-D₆, 100 MHz), δ: 12.0, 13.4, 55.9, 56.5, 56.6, 60.6, 106.7, 107.1, 120.9, 125.3, 130.3, 131.1, 139.9, 140.2, 142.0, 143.9, 153.6, 153.6, 153.8, 182.9, 197.0. Elemental analysis: calculated for C₂₆H₃₀O₈: C, 66.37; H, 6.43. Found: C, 66.30; H, 6.38.

4-Methyl-1,7-bis(4-Hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (**5**). Dark orang powder; Yield 74%; mp. 183-185 °C (lit. ⁽¹⁵⁾ 186-187 °C). As a tautomeric mixture of diketo and enol forms, ¹H NMR (DMSO-d₆, 400MHz), δ:1.29(3H,d, *J*= 6.4, CH₃ keto form), 2.21(3H, s, CH₃ enol form),3.51-3.86(12H, OCH₃), 4.58(1H, q, *J*=6.8, CH keto form),6.90 (4H, d, *J*=16, CH=C), 7.60 (4H, d, *J*=15.6, CH=C), 6.80-7.62(12H, m, Ar-H), 9.70(4H, s, phenolic OH). ¹³C NMR (DMSO-d₆, 100 MHz) δ: 12.01, 13.6, 55.6, 56.1, 56.3, 106.22, 111.8, 112.0, 116.1, 118.3, 122.7, 123.9, 124.2, 126.2, 127.2, 142.0, 144.4, 148.4, 149.8, 150.2, 182.8, 196.8. Elemental analysis: calculated for C₂₂H₂₂O₆: C, 69.10; H, 5.80. Found C, 73.10; H, 5.75.

4-Methyl-1,7-bis(4-methoxy-3-hydroxyphenyl)-1,6-heptadiene-3,5-dione (**6**). Orang powder; Yield 70%; mp. 191-193 °C. As a tautomeric mixture of diketo and enol forms, ¹H NMR (DMSO-d₆,

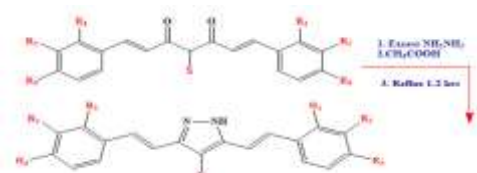
400MHz) δ : 1.30 (3H, d, $J=6.8$, CH₃ diketo form), 2.19 (3H, s, CH₃ enol form), 3.49 -3.85(12H, OCH₃), 4.63(1H, q, $J=7$, CH keto form), 6.80(4H, d, $J=16$, CH=C), 7.59 (4H, d, $J=16$, CH=C), 6.98-7.56 (12H, m, Ar-H) 9.25 and 9.30(4H, s, phenolic OH). ¹³C NMR (DMSO-d₆, 100 MHz) δ : 11.9, 13.6, 55.6, 56.07, 56.09, 106.4, 112.4, 114.7, 114.8, 118.9, 122.1, 122.4, 123.2, 127.4, 128.5, 141.7, 144.1, 147.2, 150.5, 150.9, 182.7, 196.9. Elemental analysis: Calculated for C₂₂H₂₂O₆: C, 69.10; H, 5.80. Found: C, 69.17; H, 5.78.

4-Methyl-1,7-bis(4-acetamidophenyl)-1,6-heptadiene-3,5-dione (**compound 7**). Dark orange powder; Yield 65%; mp. 245-247 °C (lit. ⁽¹⁵⁾ 248-249 °C). As a tautomeric mixture's of diketo and enol forms, ¹H NMR (DMSO-d₆, 400MHz) δ : 1.29

(3H, d, $J=7.0$, CH₃ diketo form), 2.07(12H, s, COCH₃) 2.17(3H, s, CH₃ enol form), 4.6 (1H, q, $J=6.6$, CH keto form), 6.91(4H, d, $J=16$ CH=C), 7.23(4H, d, $J=15.6$, CH=C), 7.56-7.72(16H, m, Ar-H) 10.16(4H, s, NH). ¹³C NMR (DMSO-d₆, 100 MHz) δ : 11.9, 13.6, 24.6, 55.7, 106.8, 119.4, 119.8, 124, 129.2, 129.9, 130.1, 141.1, 141.7, 142.1, 143.4, 169.2, 182.8, 197.0. Elemental analysis: Calculated for C₂₄H₂₄N₂O₄: C, 71.27; H, 5.98; N, 15.82. Found: C, 71.31; H, 5.95; N, 15.80

General procedure for synthesis of pyrazole derivatives of curcuminoids derivatives (8-14)

The pyrazole derivatives (8-14) were prepared according to the procedure described in the literature ⁽¹⁴⁾ with mild modifications, as shown in Scheme 2.



	X	R1	R2	R3	Chemical structure
8	H	H	OCH3	OH	
9	CH3	H	OH	OCH3	
10	CH3	H	OCH3	OH	
11	CH3	H	OCH3	H	
12	CH3	OCH3	OCH3	OCH3	
13	CH3	H	OCH3	OCH3	
14	CH3	H	H	NHCOCH3	

Scheme 2. Synthetic procedures for synthesis of pyrazole derivatives of curcuminoids

To a 25 mL round-bottomed flask containing 5 mL solution of the appropriate curcuminoids (0.239 g (0.65 mmol)) in glacial acetic acid, hydrazine hydrate (0.24 mL, 5 mmol) was added. The reaction solution was refluxed with stirring for 1-2 hour, until the reaction was completed as monitored by change of the color from dark orange to pale yellow. After evaporated the solution distilled water (2 mL) was added to dissolve the remaining hydrazine and the product was extracted three times with ethyl acetate (20 mL). The combined organic layers were dried over anhydrous magnesium sulfate, and the solvent was removed in rotary evaporator. The residue was purified by column chromatography on 200-300 mesh of silica gel using a mixture of 1:2 ethyl acetate: hexane as an eluent, to give off white powder of compounds (8-14).

4,4'-((1E,1'E)-(1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol) (compound 8). Off white powder; Yield 45%; mp. 214-216 °C; ¹H NMR (DMSO-d₆, 400MHz) in ppm: 3.85 (6H, s, OCH₃), 6.65 (1H, s, vinylic proton), 6.78 (2H, d, J=8, Ar-H), 6.94 (4H, m, olefinic- H and Ar-H), 7.05 (2H, d, J=16, olefinic- H), 7.15 (2H, s, Ar-H), 9.22 (2H, s, OH), 12.89 (1H, s, NH-pyrazole). ¹³C NMR (DMSO-d₆, 100 MHz) δ: 56.1, 99.8, 110.0, 116.1, 120.6, 128.8, 130.1, 147.3, 148.4. C₂₁H₂₁N₂O₄ ESI MS: m/z Calculated 365.0, found: 366.0 [M +1⁺].

4,4'-((1E,1'E)-(4-methyl-1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol) (compound 9). Off white powder; Yield 48%; mp. 238-240 °C; ¹H NMR (DMSO-d₆, 400MHz) in ppm: 2.23 (3H, s, CH₃), 3.79 (6H, s, OCH₃), 6.87 - 7.06 (10H, m, olefinic- H and Ar-H), 9.04 (2H, s, OH), 12.70 (1H, s, NH-pyrazole). ¹³C NMR (DMSO-d₆, 100 MHz) δ: 8.9, 56.1, 112.0, 112.7, 113.2, 115.2, 118.7, 128.5, 130.6, 147.1, 148.1. C₂₂H₂₂N₂O₄ ESI MS: m/z Calculated 379.0, found: 380.0 [M +1⁺].

5,5'-((1E,1'E)-(4-methyl-1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol) (compound 10). Off white powder; Yield 54%; mp. 263-269 °C; ¹H NMR (DMSO-d₆, 400MHz) in ppm: 2.26 (3H, s, CH₃), 3.85 (6H, s, OCH₃), 6.78 (2H, d, J= 8.4, Ar-H), 6.98-6.94 (4H, m, olefinic- H and Ar-H), 7.06 (2H, d, J=16.4, olefinic- H), 7.20 (2H, s, CH), 9.16 (2H, s, OH), 12.72 (1H, s, NH-pyrazole). ¹³C NMR (DMSO-d₆, 100 MHz) δ: 9.1, 56.1, 110.0, 111.7, 115.1, 116.1, 120.4, 128.8, 129.2, 147.1, 148.4. C₂₂H₂₂N₂O₄ ESI MS: m/z Calculated 379.0, found: 380.0 [M +1⁺].

3,5-bis((E)-4-methoxystyryl)-4-methyl-1H-pyrazole (compound 11). Off white powder; Yield 57 %; mp 213-216 °C; ¹H NMR (DMSO-d₆, 400MHz) in ppm: 2.26 (3H, s, CH₃), 3.79 (6H, s, OCH₃), 6.85 (2H, d, CH), 6.96- 7.56 (12H, m, olefinic- H and Ar-

H), 12.87 (1H, s, NH-pyrazole). ¹³C NMR (DMSO-d₆, 100 MHz) δ: 8.9, 55.6, 112.1, 114.7, 128.0, 129.1, 130.2, 159.4. C₂₂H₂₃N₂O₂ ESI MS: m/z Calculated 347.0, found: 348.0 [M +1⁺].

4-methyl-3,5-bis((E)-3,4,5-trimethoxystyryl)-1H-pyrazole (compound 12). Off white powder; Yield 43%; m.p. 171-174 °C; ¹H NMR (DMSO-d₆, 400MHz) in ppm: 2.30 (3H, s, CH₃), 3.69-3.85 (18H, OCH₃), 6.91 - 7.14 (8H, m, olefinic- H and Ar-H), 12.60 (1H, s, NH-pyrazole). ¹³C NMR (DMSO-d₆, 100 MHz) δ: 9.1, 56.4, 60.5, 104.1, 112.8, 117.4, 128.7, 133.3, 137.8, 153.58. C₂₆H₃₁N₂O₆ ESI MS: m/z Calculated 367.0, found: 368.0 [M +1⁺].

3,5-bis((E)-3,4-dimethoxystyryl)-4-methyl-1H-pyrazole (compound 13). Off white powder; Yield 52%; mp. 172-175 °C; ¹H NMR (DMSO-d₆, 400MHz) in ppm: 2.92 (3H, s, CH₃), 3.77 (6H, s, OCH₃), 3.85 (6H, s, OCH₃), 6.94 (2H, d, J= 8, Ar-H), 7.06 -7.10 (6H, m, olefinic- H and Ar-H), 7.25 (2H, s, Ar-H), 12.83 (1H, s, NH-pyrazole). ¹³C NMR (DMSO-d₆, 100 MHz) δ: 9.1, 56.0, 109.4, 112.2, 120.2, 128.5, 130.5, 149.2, 149.5. C₂₄H₂₇N₂O₄ ESI MS: m/z Calculated 407.0, found: 408.0 [M +1⁺].

N,N'-(((1E,1'E)-(4-methyl-1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl))bis(4,1-phenylene))diacetamide (compound 14). Off white powder; Yield 49%; mp. 329-331 °C; ¹H NMR (DMSO-d₆, 400MHz) in ppm: 2.08 (6H, s, CH₃C=O), 2.27 (3H, s, CH₃), 7.04 -7.16 (4H, m, olefinic- H), 7.55 (4H, d, J= 8.4 Ar-H), 7.64 (4H, d, J= 8.4, Ar-H), 10.05 (2H, s, NH-C=O), 12.93 (1H, s, NH-pyrazole). ¹³C NMR (DMSO-d₆, 100 MHz) δ: 8.9, 24.5, 112.5, 119.6, 127.2, 128.1, 132.3, 139.3, 168.8. C₂₄H₂₅N₄O₂ ESI MS: m/z Calculated 401.0, found: 402.0 [M +1⁺].

***In vitro* antibacterial activity assay for curcuminoids and its studied pyrazole derivatives**

The test organisms were obtained from biology department/ College of Sciences/ University of Basrah. Organisms included: The Gram-negative bacteria *Escherichia coli* and the Gram-positive bacteria *Staphylococcus aureus*. The *invitro* assessment of antimicrobial activity, was performed following modified protocol for the disc diffusion method^(19, 20). For each of the studied compounds, three concentrations (0.5, 0.25 and 0.1 mg/mL) were prepared using DMSO as a solvent. A diluted suspension of each of Activated bacterial strains was prepared using 0.9% NaCl, and their turbidity was justified against 0.5 McFarland standard. 10 μL of the bacterial inoculum was spread over Mueller – Hinton agar plate surface, then sterile filter paper discs (0.6mm in diameter) loaded with 3 μL of each concentration of studied compounds were placed on the surface of seeded agar plates, and

the agar plates were incubated for 18-24 hours at 37°C in the incubator (Binder, FD23, USA). All samples were tested in triplicate. Selected antibiotic discs along with a control disc loaded with DMSO were introduced into other agar plates inoculated with similar bacterial strains to test bacterial sensitivity to them.

Results and Discussion

curcuminoids (1–7)

Curcuminoids (1–7) were synthesized by ultrasonic irradiation of the reaction mixture containing acetyl acetone or 3-methylpentane-2,4-dione, substituted aromatic aldehyde, boric oxide, trimethyl borate, butyl amine and dimethylformamide for 40 minutes as shown in scheme 1⁽¹⁴⁾. The prepared compounds were analyzed by their ¹H NMR and ¹³C NMR. Curcumin exists mainly as an enolic form in solution, so its ¹H NMR was characterized by the appearance of a vinylic proton (proton directly attached to an alkene carbon) at 6.06 ppm and the absence of the methylene protons signal due to keto form. The α -methyl analogs of curcumin 2-7, which contain the methyl group at the 4- position, exist as a racemic mixture of keto and enol forms. This was confirmed by two notes in the ¹H NMR spectra. The first one was the presence of the singlet signal at 2.2 ppm due to the enol form of the methyl proton directly attached to the alkene carbon. The second is the presence of two signals one doublet at 1.3 ppm and the second was quartet at 4.6 ppm due to keto form methyl protons directly attached to the α -carbon adjacent to the carbonyl groups and methine proton respectively which in agreement with previous results^(16,17, 21). The ¹³C NMR spectra show signals for both keto and enol forms^(21,22).

Pyrazole derivatives of curcumin 8-14

The compounds were prepared by the conventional cyclocondensation of hydrazine with carbonyl system method which is based on the reaction between curcuminoids (1-7) and hydrazine hydrate in the presence of glacial acetic acid as solvent and catalyst at reflux temperature (118 °C) for 1-2 hours as shown in scheme 2⁽¹⁸⁾. The validity of the synthesized compounds was confirmed by ¹H NMR and ¹³C NMR and ESI-MS. The structures of prepared compounds were confirmed by the

presence of proton signals (12.60 -12.93) and carbon-13 signals (139.3- 159.4) which belong to the NH group and C=N of pyrazole ring respectively⁽²²⁾. The molecular weights of compounds 8-14 were determined as [M +1⁺] - 1 using ESI MS. The results show that there is a difference between the calculated [M +1⁺] values for the studied compounds and experimental values for about 1 point. The differences between experimental and calculated values could be attributed to the protonation of compounds by formic acid present in the aqueous solution of methanol, which is used as a mobile phase in this analysis¹⁸.

In vitro antibacterial activity assay for curcuminoids and its studied pyrazole derivatives

In vitro antibacterial activity assay results were shown in Table 1. At tested concentrations, neither curcumin nor its studied derivatives have shown any activity against *Escherichia coli*. These findings of curcumin correspond with previous studies^{18,24}. However, a varying degree of anti-staphylococcal activity was shown by the tested compounds^{24,25}. Previous studies have correlated the antibacterial activity of curcumin to the presence of aromatic hydroxyl groups within its chemical structure and/or to the unsaturated ketone group^{26, 27, 28}.

Positive results have been detected for both curcumin and its pyrazole derivatives (compound 8) despite the absence of an unsaturated ketone group within the latter, which means that their anti-staphylococcal activity is mainly referred to the aromatic hydroxyl groups, and the same explanation is applicable to the compounds 5 and 6 and their pyrazole derivatives (compounds 9 and 10 respectively). Meanwhile, compounds 2,3,4, and 7 (all lack aromatic hydroxyl groups) have shown anti-staphylococcal activity, while their pyrazole derivatives 11, 13, 12, and 14 respectively, didn't show any activity, which suggests that the anti-staphylococcal activity of these curcuminoids 2,3,4 and 7 is referred to the α , β -unsaturated ketone and replacement of this group with pyrazole group inhibit their activity.

It's important to mention that compound 10 has shown its anti-staphylococcal activity at the lowest concentration tested (0.1 mg/mL) only.

Table 1. The In vitro antibacterial activity assay results of curcuminoids and their pyrazole derivatives

Compound	Conc. (mg/mL)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Curcumin (1)	0.5	-**	+*
	0.25	-	+
	0.1	-	+
Compound 2	0.5	-	+
	0.25	-	+
	0.1	-	+

Continued table 1.

Compound 3	0.5	-	+
	0.25	-	+
	0.1	-	+
Compound 4	0.5	-	+
	0.25	-	+
	0.1	-	+
Compound 5	0.5	-	+
	0.25	-	+
	0.1	-	+
Compound 6	0.5	-	+
	0.25	-	+
	0.1	-	+
Compound 7	0.5	-	+
	0.25	-	+
	0.1	-	+
Compound 8	0.5	-	+
	0.25	-	+
	0.1	-	+
Compound 9	0.5	-	+
	0.25	-	+
	0.1	-	+
Compound 10	0.5	-	-
	0.25	-	-
	0.1	-	+
Compound 11	0.5	-	-
	0.25	-	-
	0.1	-	-
Compound 12	0.5	-	-
	0.25	-	-
	0.1	-	-
Compound 13	0.5	-	-
	0.25	-	-
	0.1	-	-
compound 14	0.5	-	-
	0.25	-	-
	0.1	-	-

*Positive results (the tested compound have shown antibacterial activity)

** Negative results (the tested compound did not show any antibacterial activity)

Conclusion

Curcumin and some of its derivatives were synthesized, characterized, and their antibacterial activity was studied against *E. coli* and *Staphylococcus aureus*. Neither curcumin nor any of its derivatives have shown any activity against *E. coli*. While the anti-staphylococcal activity, on the other hand, has been shown by compounds 1-9, compounds 11-14 didn't present any activity, and compound 10 shows activity at the lowest studied concentration only. These findings suggest that the pyrazole substitution on curcumin derivatives that do not bear an aromatic hydroxyl group would result

in diminishing their anti-staphylococcal activity. More antibacterial activity studies are suggested regarding compound 10 activity. help.

Conflicts of Interest

The authors have no conflict of interest to declare

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Ethics Statements

This article was approved by the scientific and ethical committees in College of Pharmacy/ University of Basrah.

Author Contribution

Mustafa M. AL-Hakiem, Mustafa Q. Alderawy and Rita S Elias contribute in the synthesis and characterization of the synthesized curcumin derivatives. Reham A. AL-Anssari contribute in the in vitro antibacterial activity study. All the authors contributed in revision and rearrangement of the article.

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