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Synthesis of Substituted Chalcones and Investigation of Their Biological Activities

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Abstract

The synthesis of highly antioxidants and antimicrobial agents has attracted great attention due to their high importance to human health. Chalcones are known to exhibit wide spectrum of biological activities such as anticancer, antibacterial and antioxidant. We synthesized chalcones **C1-C7** by using aldol condensation of substituted aldehydes and substituted acetophenone under basic conditions. The antioxidant activity was investigated *via* DPPH method and the antibacterial activity was measured against four bacterial strain; two Gram (+) and two Gram (-) bacteria. Chalcone **C1** showed the highest antioxidant activity with IC_{50} value of 0.364μmol/mL and **C5** revealed the least antioxidant effect with IC_{50} value of 0.883μmol/mL. The antibacterial activity was tested for chalcones **C1**-**C6** and the results revealed that Gram (+) bacteria are more susceptible to the tested chalcones than Gram (-) bacteria. Chalcones **C5** and **C6** showed the highest antibacterial activity against *B. subtilis* with inhibition zone of 18.33 ± 0.58 and 17.33 ± 0.58 mm, respectively. **C4** also exhibited appreciable antibacterial effect against *E. coli* with 17.67 ± 1.53 mm inhibition zone. The inhibitory effect of Chalcones **C1**, **C2** and **C3** varied from as low as 10.67±0.58 mm to as high as 14.67±0.58 mm inhibition zone.

Keywords: Chalcones, Aldol condensation, antioxidant activity, antibacterial activity.

1. Introduction

Chemists are continuously looking for new agents that exhibit high biological activities such as anticancer, antioxidant and antimicrobial. Chalcones are known for their wild biological activities especially anticancer [1], antimicrobial [2] and antioxidant [3]. Free radicals such as superoxide ($O_2^{\cdot-}$), hydrogen radical ($H^{\cdot}$) and hydrogen peroxide H_2O_2, which are produced either from biochemical processes in the body or from the increase exposure to environmental xenobiotics, are responsible for a number of disease including cancer, diabetes and cardiovascular diseases [4]. Chalcones inhibit the action of the free radicals by different mechanisms such as free radical scavenging or hydrogen donation singlet oxygen quenching. When chalcones react with radicals, they are converted to the phenoxy radicals and consequently to a stable semiquinone or non stable quinone radicals. The antibacterial activity of chalcones is mainly due to the presence of phenolic hydroxyl groups which have high affinity for proteins and thus may inhibit microbial enzymes [5]. The reported studies showed that the type and positions of the substituents on the two aromatic rings of chalcones have great influence on their biological activities. Some studies revealed that the requirements for antimicrobial activity of chalcones are the presence of at least one phenolic hydroxyl group and some degree of lipophilicity [6], Vanangamudi et al. have studied the antimicrobial activity of ten synthesized substituted chalcones against five gram positive and five gram negative pathogenic strains by Kirby-Bauer method. Most of the compounds show moderate, good to excellent antimicrobial activity [7].

The antioxidant activity can be measured by determining the free radical inhibitory ability of the antioxidant agent by using very stable radical such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in ethanolic solution. The DPPH radical has deep purple color whereas the reduced form $DPPH_2$ is yellow as shown in Figure 1.

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The radical scavenging activity of antioxidant agent can be expressed by means of IC_{50} which represent the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% [8].

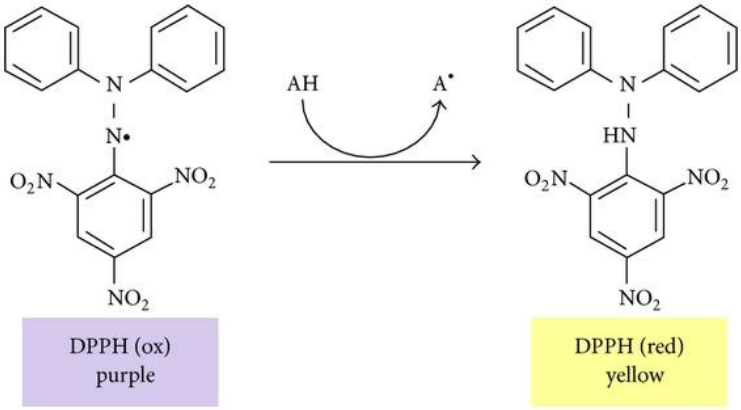


Figure 1. Mechanism of DPPH with an antioxidant having transferable hydrogen radical

Here we report the synthesis of seven substituted chalcones (**C1-C7**) using aldol condensation method. The structures of these chalcones were characterized by IR, ^{1}H NMR and ^{13}C NMR spectroscopy. We investigated the antioxidant activity of these chalcones activity by DPPH assay and their antimicrobial activity against four pathogenic bacterial strains, two are Gram (+) species and included *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 6538) and the other two are Gram (-) species and included *Escherichia coli* (ATCC 11775) and *Pseudomonas aeruginosa* (ATCC 10145).

Experimental 2

2.1 Synthesis of chalcones

All the solvents used in this study were of commercial grade and all chemicals were purchased from Aldrich and they were used without further purification. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.15-0.20 mm silica gel plates (MERCK company) using UV light or iodine champers as visualizing agents. Column chromatography was performed on Merck silica gel (60F254 aluminum plates mesh) using different concentration ratio of hexane and ethyl acetate as eluating solvent.

Nuclear Magnetic Resonance (NMR), ^{1}H & ^{13}C spectra were measured at 400 MHz (BRUKER). All ^{1}H NMR and ^{13}C NMR samples were measured in deutrated chloroform $CDCl_3$ using tetramethylsilane (TMS) as internal standards and $CDCl_3$ with the triple carbon chemical shift are at 77.36, 77.05 and 76.73 ppm was used as reference. The chemical shift values for all spectra are given in parts per million (ppm) with the coupling constant (*J*) in Hertz (Hz). Infrared (IR) spectra were recorded on a Fourier Transform Infrared (FT-IR)-restige-21spectrometer, liquid cell (demountable cell) and the cell was constructed of sodium chloride (NaCl).

General procedure for the synthesis of chalcones **C1-C7**

An equimolar mixture of substituted acetophenone and aromatic aldehyde dissolved in excess amount of (20% KOH in H_2O) and ethanol. The mixture was refluxed for overnight. The reaction mixture was cooled in ice bath and acidified with concentrated HCl solution. The formation of the product was confirmed by TLC analysis. The crude product was then purified by recrystalization from ethanol: water (2:1).

2E-(2,4-dihydroxy-phenyl)-3-phenyl-prop-2-en-1-one ***(C1)*** was obtained as crude solid by mixing (0.005 mol, 0.67g) of 2',4'-dihydroxyacetophenone with (0.005 mol, 0.53 g) of benzaldehyde ; The crude product (0.36 g, 29.7%) ; Chalcone **C1** has a m.p of (110 - 112 ^{o}C, (*lit.*150-151 [9] and *lit.*145-146 [10]) ; IR (NaCl, ν, cm^{-1}): 3583.96 (OH) , 3027.55 (C-H (sp^2)) , 1624.28 (C=O) , 1492.48 (C=C) ; ^{1}H NMR (400 Hz , $CDCl_3$) : δ 7.67 (1H, d, *J* = 8.88 Hz, Ar-H), 7.52 (2H, d, *J* = 7.2 Hz, Ar-H), 7.39 (1H, t, *J* = 7.2 Hz, Ar-H), 7.32 (2H, d, *J* = 7.2 Hz, CH=CH-Ar), 7.17 (1H, d, *J* = 16.0 Hz, -Ar-CH=CH), 6.76 (1H, d, *J* = 8.88 Hz, Ar-H), 6.58 (1H, d, *J* = 15.9 Hz, CH=CHCO), 6.43 (1H, s, Ar-H); ^{13}C NMR (400 Hz , $CDCl_3$): δ 202.6 (C) , 165.2 (C), 163.8 (C), 145.1 (CH), 136.5 (C), 132.9 (CH), 128.0 (CH), 128.0 (CH), 127.1 (CH), 126.7 (CH), 126.6 (CH), 121.5 (CH), 113.9 (C), 108.1 (CH), 103.4 (CH).

*2E-1-(5-chloro-2-hydroxy-phenyl)-3-phenyl-prop-2-en-1-one (**C2**)* was obtained as yellow solid by mixing (0.005mol, 0.85g) of 5-Chloro-2-hydroxycacetophenone with (0.005mol, 0.53g) of benzaldehyde; The crude product (0.54g, 41.9 %) has a m.p of 99-102 °C (*lit.*59-60 °C [11] and *lit.* 89-91 °C [12]); IR (NaCl ,ν, cm^{-1}): 3448.72 (OH), 1651.62 (C=O), 1469.76 & 1450.47 (C=C), 983.70 (C-Cl); ^{1}H NMR (400 Hz , $CDCl_3$): δ 7.99 (1H, d, *J* = 15.5 Hz, CH=CH-Ar), 7.92 (1H, d, *J* = 2.52, Ar-H), 7.74 (1H, m, Ar-H), 7.61 (1H, d, *J* = 15.5 Hz, -CO-CH=CH), 7.50 (5H, m, Ar-H), 7.03 (1H, d, *J* = 9.00, Ar-H); ^{13}C NMR (400 Hz, $CDCl_3$): δ 192.8 (C), 162.1 (C), 146.6 (CH), 136.2 (CH), 134.3 (CH), 131.3 (CH), 129.1 (2CH), 128.8 (2CH), 128.8 (CH), 123.6 (C), 120.6 (C), 120.3 (CH), 119.5 (CH).

*2E-1-(5-Bromo-2-hydroxy-phenyl)-3-phenyl-prop-2-en-1-one **(C3)*** was obtained as crude yellow solid by mixing (0.005 mol, 0.75 g) of 5-Bromo-2-hydroxyacetophenone with (0.005 mol, 0.53g) of benzaldehyde; The crude product (0.46 g, 30.2%) has a m.p of 81-85 °C (*lit.* 98°C [13]) ; IR (NaCl ,ν, cm^{-1}): 3448.72 (OH), 1677.83 (C=O), 1492.90 & 1450.47 (C=C Ar) . ^{1}H NMR (400 Hz, $CDCl_3$) : δ 8.06 (1H, d, *J* = 2.40 Hz , Ar-H), 7.99 (1H, d, *J* = 15.5 Hz , CH=CH-Ar), 7.74 (2H, m, Ar-H), 7.62 (1H, d, *J* = 15.3 Hz, CO-CH=CH), 7.65-7.44 (4H, m, Ar-H), 6.98 (1H, d, *J* = 8.88 Hz, Ar-H). ^{13}C NMR (400 Hz, $CDCl_3$): δ 203.85 (C), 161.32 (C), 139.11 (CH), 139.11 (C), 139.11(CH), 132.93 (4CH), 120.91 (CH), 120.50 (4CH), 110.43 (C).

*2-E-1-(2-Hydroxy-4-methoxy-phenyl)-3-phenyl-prop-2-en-1-one **(C4)*** was obtained as yellow solid by mixing (0.005 mol, 0.48 g) of 2-hydroxy-4-methoxy-acetophenone with (0.005 mol, 0.53 g) of benzaldehyde. The crude product (0.54 g, 45.4%) has a m.p of 87-90 °C (*lit.*107-108 °C [10]); IR (NaCl, ν, cm^{-1}): 3448.72 (OH), 1651.07 (C=O), 1469.76 & 1450.47 (C=C Aromatic); ^{1}H NMR (400 Hz , $CDCl_3$): δ 7.92 (1H, d, *J* = 15.5 Hz, CH=CH-Ar), 7.87 (1H, d, *J* = 8.68 Hz , Ar-H), 7.58 (1H, d, *J* = 15.5 Hz, -CO-CH=CH), 7.45 (6H, m, Ar-H), 6.48 (1H, s, Ar-H), 3.90 (3H, s, CH_3); ^{13}C NMR (400 Hz, $CDCl_3$): δ 191.86 (C), 166.74 (C), 165.29 (C), 144.42 (CH), 134.82 (C), 132.33 (CH), 130.69 (CH), 130.53 (CH), 129.02 (CH), 128.57 (CH), 128.42 (CH), 120.34 (CH), 114.12 (C), 107.64 (CH), 101.12 (CH), 55.62 (CH_3)

*2-E-3-(2-Hydroxy-phenyl)-1-phenyl-prop-2-en-1-one (**C5**)* was obtained as crude dark solid (40.23g, 90.64%) using (0.200 mol, 24.03g) of acetophenone and (0.200 mol, 24.40 g) of salicylaldehyde; the crude product was purified by recrystallization from 2:1 ethanol: water ; the pure **C5** was obtained as light yellow crystals with a m.p of 135-137 °C, (*lit.*152-153 °C [10]) ; R_f of 0.257 (in 3:1 hexane: ethyl acetate). IR (NaCl, *ν*, cm^{-1}): 3290.10 (OH), 3017.06 (C-H (sp^2)), 1599.02 (C=O), 1456.31 (C=C). ^{1}H NMR (400Hz, $CDCl_3$): δ 8.19 (1H, d, *J* = 15.9 Hz, CH=CH-Ar), 8.07 (2H, d, *J* = 7.24 Hz, Ar-H), 7.74 (1H, d, *J* = 15.9 Hz, CH=CH-CO), 7.62 (2H, t, *J* = 6.43 Hz, Ar-H), 7.55 (1H, t, *J* = 7.72 Hz, Ar-H), 7.32 (1H, t, *J* = 7.68 Hz, Ar-H), 7.27 (1H, d, *J* = 7.52 Hz, Ar-H), 7.00 (1H, t, *J* = 7.48 Hz, Ar-H), 6.95 (1H, d, *J* = 8.10 Hz, Ar-H); ^{13}C NMR (400Hz, $CDCl_3$): δ 192.0 (C), 156.0 (C), 141.0 (CH), 139.0 (CH), 133.0 (CH), 132.0 (CH), 130.0 (2CH), 129.0 (2CH), 129.0 (C), 123.0 (CH), 122.0 (C), 121.0 (CH), 117.0 (CH).

*2-E-3-(2-Hydroxy-phenyl)-1-(4-methoxy-phenyl)-prop-2-en-1-one (**C6**)* was obtained as crude dark solid (2.27g, 44.7%) using (0.0200 mol, 3.00g) of 4-methoxyacetophenone and (0.0200 mol, 2.44g) of salicylaldehyde; the crude product was purified by recrystallization from 2:1 ethanol: water, the pure **C6** was obtained as yellow crystals with a m.p of 128-130 °C ; R_f of 0.19 (in 3:1 hexane: ethyl acetate); IR (NaCl, *ν* , cm^{-1}) : 3378.83 (OH), 3017.06 (C-H (sp^2)), 1602.42 (C=O), 1456.31 (C=C); ^{1}H NMR (400Hz, $CDCl_3$): δ 8.20 (1H, d, *J* = 15.7Hz, CH=CH-Ar), 8.10 (2H, d, *J* = 8.50 Hz, Ar-H), 7.71 (1H, d, *J* = 15.7 Hz, CH=CH-CO), 7.53 (1H, d, *J* = 8.00 Hz, Ar-H), 7.24 (1H, t, *J* = 7.34 Hz, Ar-H), 7.11 (1H, d, *J* = 7.26 Hz, Ar-H), 6.98 (2H, d, *J* = 7.90 Hz, Ar-H), 6.90 (1H, t, *J* = 8.30 Hz, Ar-H), 3.90 (3H, s, OCH_3); ^{13}C NMR (400Hz, $CDCl_3$): δ 188.0 (C), 163.0 (C), 156.0 (C), 141.0 (C), 131.5 (CH), 131.3 (2CH), 131.0 (CH), 130.0 (C), 122.0 (CH), 120.5 (CH), 117.0 (CH), 116.5 (CH), 114.0 (2CH), 58.0 (CH_3).

*2-E-1-(4-Chloro-phenyl)-3-(2-hydroxy-phenyl)-prop-2-en-1-one **(C7)*** was obtained as crude dark solid (4.50g, 87.1%) using (0.0200 mol, 3.09 g) of 4-chloroacetophenone and (0.0200 mol, 2.44 g) of salicylaldehyde; the crude product was purified by recrystallization from 2:1 ethanol: water, the pure **C7** was obtained as red crystals with a m.p of 115-120°C; R_f of 0.27 (in 3:1 hexane: ethyl acetate); IR (NaCl, ν, cm^{-1}) : 3590.44 (OH), 3017.06 (C-H (sp^2)), 1588.83 (C=O), 1486.89 (C=C); ^{1}H NMR (400Hz, $CDCl_3$): δ 8.13 (1H, d, *J* = 15.8 Hz, CH=CH-Ar), 7.93 (2H, d, *J* = 8.40 Hz, Ar-H), 7.68 (1H, d, *J* = 15.8 Hz, CH=CH-CO), 7.45 (2H, d, *J* = 8.40 Hz, Ar-H), 7.20 (1H, d, *J* = 7.76 Hz, Ar-H), 7.15 (1H, t, *J* = 7.28 Hz, Ar-H), 6.96 (1H, t, *J* = 7.20 Hz, Ar-H), 6.90 (1H, d, *J* = 8.3 Hz, Ar-H); ^{13}C NMR (400Hz, $CDCl_3$): δ 189.0 (C), 156.0 (C), 141.0 (C), 140.0 (CH), 135.0 (C), 130.0 (2CH), 129.0 (2CH), 128.0 (CH), 127.0 (CH), 122.0 (C), 121.0 (CH), 120.5 (CH), 116 (CH).

2.2 Antioxidant test for chalcones C1-C7

Four different concentrations (20, 50, 100 and 200μg/mL) of chalcones C1-C7 were prepared in triplicate in methanol. A solution (45 mg/L) of DPPH was prepared in methanol. Then 2 mL of DPPH solution was mixed with 1 mL of each chalcone solution. The absorbance of DPPH radical solution was measured 30 min after addition of each chalcone sample at 517 nm. Each measurement was performed in triplicate at 25°C. The absorbance of DPPH at 517 nm was 1.073 at 25°C. Then the % antioxidant activities were plotted against the concentration of each the chalcone. The IC_{50} values were determined from the graph. Ascorbic acid was used as a positive control.

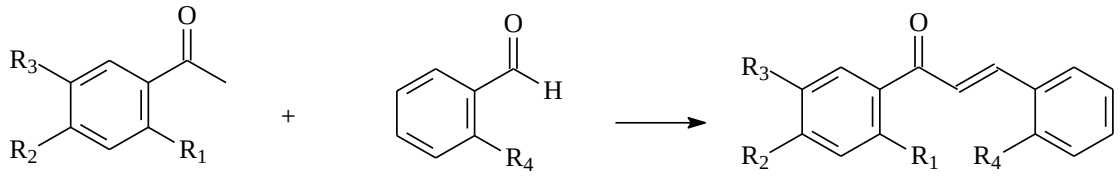
2.3 Antibacterial Activity Assay

Four bacterial strains were used as testing microbes for antibacterial activity assay of synthesized chalcones. All bacteria were obtained from the American Type Culture Collection (ATCC) as lyophilizates in ampoules. Gram (+) species included *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 6538). The Gram (-) species included *Escherichia coli* (ATCC 11775) and *Pseudomonas aeruginosa* (ATCC 10145). Cultures of these bacteria were grown on 25 ml nutrient agar (Oxoid) plates and incubated for 24 hours at 37°C.

Chalcones **C1**-**C6** were dissolved in dimethyl sulfoxide (DMSO) separately at the concentration of 1 mg/mL. The agar well diffusion techniques was employed. Overnight nutrient broth cultures of bacteria 10^5 (per 0.5 ml) were especially mixed with 20 ml of nutrient and the agar was cooled down to 50 °C in 9 mm plastic Petri dishes. Plates were allowed to stand for 30 min at room temperature then wells of 7 mm in diameter were made in the solidified agar medium with sterilized steel cork borer. Fifty μl of each chalcone sample was slowly loaded into the well by micropipettes with sterile tips. The loaded plates were later incubated for 24 hours at 37 °C. Each experiment was repeated at least twice and the diameter of inhibition zone surrounding the agar well for each plate was averaged and expressed in mm. The mean of the three readings per zone was measured and the standard deviation of all replicates was determined. Appropriate treatments, including wells loaded only with sterilized deionized water or DMSO, were considered as negative controls. For comparative purposes, standard antibacterial disks served as positive controls.

3. Results and Discussion

The Chalcones **C**1-**C7** were synthesized by reacting equimolar of substituted acetophenone with substituted aromatic aldehydes in KOH solution. The progress of the reaction was monitored by TLC analysis. Most of the synthesized chalcones were obtained as yellow-orange to dark brown solids with percentage yield ranging between 30-91%, as shown in scheme 1.



substituted acetophenone substituted aldehyde Chalcones **C1-C7**

C1, R_1 = OH, R_2 = OH, R_3 = H, R_4 = H, 29.7%
C2, R_1 = OH, R_2 = H, R_3 = Cl, R_4 = H, 41.9%
C3, R_1 = OH, R_2 = H, R_3 = Br, R_4 = H, 30.2%
C4, R_1 = OH, R_2 = OCH_3, R_3 = H, R_4 = H, 45.4%
C5, R_1 = H, R_2 = H, R_3 = H, R_4 = OH, 90.6%
C6, R_1 = H, R_2 = OCH_3, R_3 = H, R_4= OH, 44.7%
C7, R_1 = H, R_2 = Cl, R_3 = H, R_4 = OH, 87.1%

Scheme 1. The structures and % yields of chalcones **C1-C7**

3.1 IR and NMR results

The IR spectrums of all the synthesized chalcones displayed bands for OH group at about 3583-3590 cm^{-1} and carbonyl group at about 1588-1700 cm^{-1} and for α-β unsaturated ketones at 1450-1500 cm^{-1}. In the ^1H NMR the signals for chalcone vinyl protons appeared as two doublet with coupling constant (*J*) of 15-16 Hz. In ^{13}C NMR, the carbonyl carbon signal appeared in the range of 190-202 ppm and that for alkene carbons are at 120 and 147 ppm.

3.2 Antioxidant activity results

We followed the method reported by Sandip et al [14] to measure the antioxidant activity of C1-C7, where aliquot of different concentrations (25-200 µg/mL) of the chalcone is added to 2 mL of a (45 mg/L) methanolic solution of DPPH. The absorbance of the mixture was measured at 517 nm after 30 min of the addition of the chalcone. The % antioxidant activity was calculated using equation (1) below.

$$\% \text{ antioxidant activity} = (A_{br} - A_{ar}/A_{br}) \times 100 \qquad (1)$$

Where A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place. The IC_{50} of each chalcone is then determined from the curve of absorbance *versus* concentrations, as shown in Figure 2. The % antioxidant activity and IC_{50} data are reported in Table 1. Ascorbic acid was used as a reference. (Figure 2, Table 1, supporting materials)

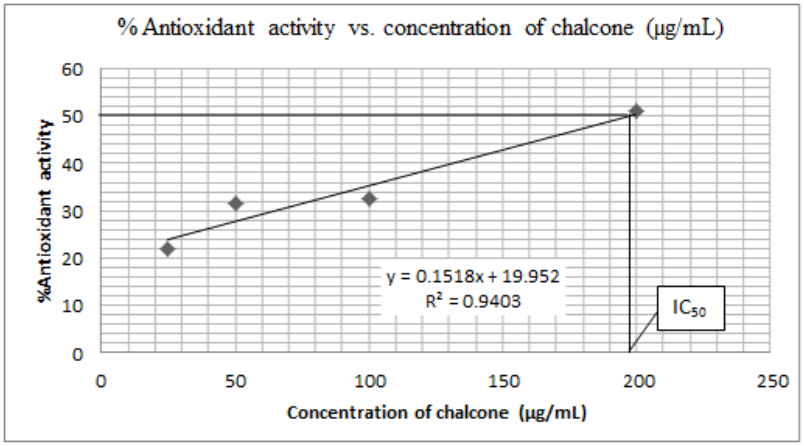
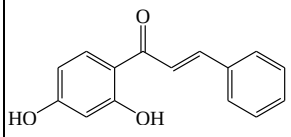
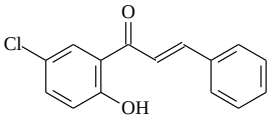
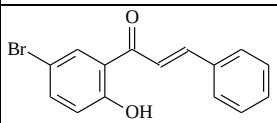
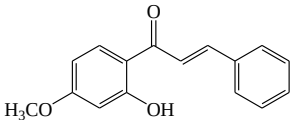
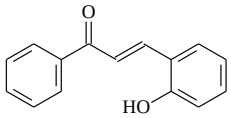
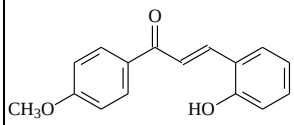
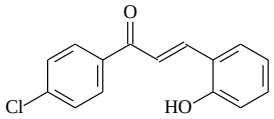


Figure 2. Determination of the IC_{50} value from the curve of % antioxidant activity *vs.* concentration of chalcone in µg/mL

Table 1. % Antioxidant activity and IC_{50} values for chalcones C1-C7.

Chalcone	Chalcone structure	% antioxidant activity (mean ± S*)				IC_{50} µg/mL	IC_{50}mM
		25µg/mL	50µg/mL	100µg/mL	200µg/mL		
C1		36.56±1.4	40.73±1.2	50.67±2.3	60.24±1.1	87.5	364
C2		40.66±1.0	41.32±2.0	43.85±1.0	51.17±0.6	187.1	723
C3		36.53±2.5	44.02±0.8	46.69±0.2	58.68±1.2	124.0	409
C4		37.65±1.2	42.22±0.9	43.58±0.6	56.97±0.8	140.4	552
C5		21.99±2.2	31.44±1.1	32.37±1.3	50.95±0.3	198.0	883
C6		29.02±2.2	31.75±2.3	34.08±1.2	48.65±1.3	221.0	868
C7		29.42±1.0	34.05±1.7	36.22±1.6	53.87±2.3	179.3	693

S is standard deviation ***

Chalcone **C1** showed the highest antioxidant activity among all the synthesized chalcones with IC_{50} value of 364 mM. The antioxidant activity of chalcones **C5** and **C7** was previously studied using DPPH method by Todorova et.al with IC_{50} values of 437.4 mM and 465.6 mM, respectively [15].

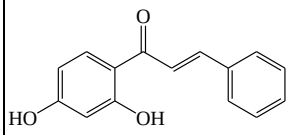
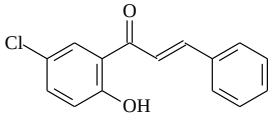
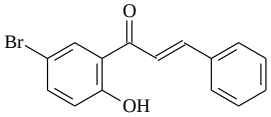
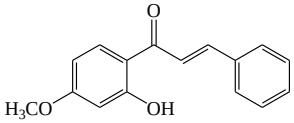
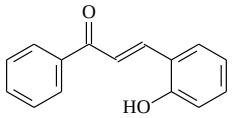
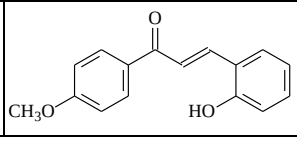
3.3 Antibacterial activity results

Results of the *in vitro* evaluation of six synthesized Chalcones compounds tested against two Gram-positive and two Gram-negative bacteria and presented as mean inhibition zone (mm) are reported in Table 2. Six chalcones were tested for their antimicrobial activity. Overall, among the six tested Chalcones, only **C3** did not show positive biological activities against the tested bacterial stains. Moreover, two of the tested Chalcones **C4** and **C5** showed positive effects against Gram (-) and Gram (+) bacteria, while the other Chalcones showed affirmative effects Gram (+) bacteria. Gram (+) bacteria appears to be more susceptible to the tested Chalcones than Gram (-) bacteria.

The highest antibacterial activity among all the six Chalcones was observed for **C5** and **C6** against the Gram (+) bacteria *B. subtilis* with 18.33 ± 1.00 and 17.33 ± 1.00 mm inhibition zones, respectively. Chalcone **C4** also revealed appreciable bioactivity against *E. coli* with 18.00 ± 1.53 mm inhibition zone. The inhibitory effect of the remaining Chalcones varied from as low as 12.00 ± 1.00 mm to as high as 15.00 ± 1.00 mm inhibition zone. Overall, chalcones **C4**, **C5** and **C6** have wider spectrum activity when compared to other chalcones.

Balaji et al. have studied the antibacterial of chalcone **C2** on various bacteria strains including *E. coli, S. aureus and B. subtilis*. The results revealed that **C2** has low bioactivity against *S. aureus* with 7 mm inhibition zone, moderate bioactivity against *B. subtilis* with 11 mm inhibition zone, and high antibacterial activity against *E.Coli* with 21mm inhibition zone [7]. Moreover, S. K. Pardeshi et al have studied the antimictobial of **C2** against *E. coli, S. aureus and B. subtilis* and obtained low bioactivity with 7 mm, 1mm and 7 mm inhibition zone, respectively [16].

Table 2. Antibacterial activity of six chalcones (C1-C6) against two Gram-negative and two Gram-positive bacteria (Mean ± SD (standard deviation))

Chalcone	Chalcone structure	Mean inhibition Zone Diameter (mm)			
		Gram (-) bacteria		*Gram (+) bacteria*	
		E.Coli	*P.aeruginosa*	*S.aureus*	*B.subtilis*
C1		-	-	-	12.33 ± 1.00
C2		-	-	-	15.00 ± 1.00
C3		-	-	-	-
C4		18.00 ±1.53			12.00 ±1.00
C5			14.00 ±1.00	14.00 ± 1.53	18.33 ± 1.00
C6					17.33 ± 1.00

4. Conclusion

Substituted chalcones were prepared *via* Aldol condensation under thermal conditions. The antioxidant activity of the prepared chalcones was investigated using DPPH method. Chalcone **C1** showed the highest antioxidant activity with IC_{50} of 0.364 μmol/mL and **C5** has the lowest antioxidant activity with IC_{50} of 0.886 μmol/mL. The antimicrobial activity of **C1**-**C6** was investigated against two Gram (+) and two Gram (-) bacteria. All tested samples show positive biological activity except **C3** which show negative results against the tested bacterial stains. The results revealed that Gram-positive bacteria are more susceptible to tested chalcones than Gram (-) bacteria. Chalcones **C5** and **C6** revealed the highest antibacterial activity against *B. subtilis* with inhibition zone of 18.33 ± 0.58 and 17.33 ± 0.58 mm, respectively. **C4** also exhibited appreciable bioactivity effect against *E. coli* with 17.67 ± 1.53 mm inhibition zone. Chalcones **C4**, **C5** and **C7** have potential antibacterial activity.

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