Research Article www.ijcps.com

International Journal of Chemical and Pharmaceutical Sciences
2014, June., Vol. 5 (2)

ISSN: 0976-9390
IJCPS

Synthesis and biological activities of some chalcone derivatives

[1] Mohammed Al-Mamary [*], [2] Sadik Ahmed Al-Mikhlafi and [3] Bushra Jaadan.

[1] Department of Organic Chemistry, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen

[2] Department of Chemistry, Faculty of Sciences, Taibah University, Al-Madinah Al-Monawarah, KSA.

[3] Department of Medicinal Chemistry, Faculty of Pharmacy, Sana'a University, Sana'a, Yeme

[*] **Corresponding Author:** E-Mail: almamarym@hotmail.com

ABSTRACT

A series of chalcone derivatives were synthesized in order to obtain multipotent antioxidant and antimicrobial agents. These compounds were characterized by IR, and 1H-NMR. All the compounds were screened for in vitro antioxidant activity using DPPH, total reducing power activity (FRAP) based on the ability of compounds to reduce the Fe^{3+}–TPTZ complex to the Fe^{2+} /ferrous, NO scavenging activity, and H_2O_2 scavenging activity methods. The present results showed that chalcone containing a hydroxyl group attached to one of the aromatic rings showed relatively high ability to reduce the danger of free radicals either directly (DPPH, NO scavenging activity, and FRAP) or indirectly (H_2O_2 scavenging activity). However, the highest activity was obtained when two hydroxyl groups attached to the two aromatic rings in chalcones. In addition, these compounds have been screened for their antibacterial and antifungal activities against strains of *St. aureus, B. subtillis, E. coli, P. auriginosea,* and *C. albicans*. The tested chalcone compounds at 100 µg showed different antimicrobial activities depending on the type of the tested microorganism and the type of substituents and their positions. However, chalcone derivatives containing hydroxyl groups revealed broad spectrum as antimicrobial agents.

Keywords: Antioxidant, DPPH, FRAP, Antimicrobial, Chalcone Derivatives.

1. INTRODUCTION

Chalcones are considered as precursors of an important group of natural products known as the polyphenolic compounds. Chalcone and its derivatives have attracted increasing attention due to numerous pharmacological applications. They have displayed a broad spectrum of pharmacological activities, among which antimalarial [1,2], anticancer [3,4], antiprotozoal (antileishmanial and antitrypanosomal [5], anti-inflammatory [6, 7], antibacterial [8 - 10], antifungal [9, 10], larvicidal [11], and antioxidant [12, 13] activities have been reported. They have also shown inhibition of the enzymes, especially mammalian alpha-amylase [14], cyclo-oxygenase (COX) [15] and monoamine oxidase (MAO) [16]. Chalcones basic structure includes two aromatic rings bound by an α,β-unsaturated carbonyl group, a unique template associated with very diverse application [17]. Some researchers attributed the biological activities of this class of compounds, such as, antimicrobial [18 – 21] and antioxidant activities [18 – 21] to the presence of a reactive α,β-unsaturated keto function in chalcones, which may be altered depending on the type and position of substituent on the aromatic rings. Hydroxyl and phenyl substitutents are associated with antioxidant properties. The present work will focus primarily on prominent members of the chalcone family with an 1,3-diphenyl-2-propenon core structure. Thus, multipotent antioxidant and antimicrobial agents are of great interest for health protetion from different complex diseases. The aim of the present work is to find some antioxidants and antimicrobial from chalcone derivatives having the ability to prevent biological and chemical substances from radical-induced oxidation damage by different mechanisms. In addition, the obtained compounds will be tested for their antibacterial and antifungal activities .

2. MATERIALS AND METHODS

2.1. Synthesis and Identification of Chalcones

2.1.1. Synthesis of chalcones

The chalcone derivatives were synthesized by Claisen–Schmidt condensation with some slight modifications [10]. Generally, they

were prepared according to the following three methods:

2.1.1. Procedure, A (Compound 1, 3, 4, 5, 7-9, 11-13, 15-17, 19-20)

In a round bottom flask, substituted acetophenone (0.01mol) and substituted aldehyde (0.01 mol) were mixed in 40 ml ethanol in ice-bath. Drop wise with continuous stirring 10 ml of NaOH solution (60%) were added in 30 minutes. Mixing continued for 2-3 hours at room temperature. The mixture became quite thick. It was kept overnight in a refrigerator. It was diluted with 40 ml ice-cold distilled water, the product was filtered, washed well with cold water, dried in air and recrystallized from rectified spirit or methanol.

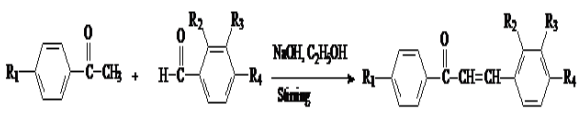
2.1.2. Procedure, B (Compound 2, 6, 10, 14, 18, 21, 23-24)

In a round bottom flask, substituted acetophenone (0.01mol) and substituted aldehyde (0.01mol) were mixed in 40 ml ethanol in ice-bath. Drop wise with continuous stirring 10 ml of NaOH solution (60%) were added in 30 minutes. The stirring of the mixture was continued for 2-3 days at room temperature. During this time, turbidity appeared in the mixture. It was diluted with 40 ml cold distilled water, and neutralized to litmus paper with 2N HCL. The product (ppt) was filtered, washed well with cold water, dried in air and recrystallized from rectified spirit or methanol.

2.1.3. Procedure, C (Compound 22)

In a round bottom flask, substituted acetophenone (0.01mol) and substituted aldehyde (0.01mol) were mixed in 40 ml ethanol in ice-bath. Drop wise with continuous stirring 10 ml of NaOH solution (60%) were added in 30 minutes (or 3 equivalents of NaOH dissolved in minimum amount of distilled water and added drop wise in 60 minutes). The mixture was refluxed overnight. The stirring of the mixture was continued for 2 days at room temperature (turbidity appeared in the mixture). It was diluted with 40 ml cold distilled water, and neutralized with 2N HCL. The product (ppt) was filtered, washed well with cold water, dried in air and recrystallized from rectified spirit or methanol.

General Scheme of Reaction:



2.2. Identification of Chalcones

The IR spectra were obtained using IR-FTIR-8300 (SHIMADZU) spectrophotometer in a disk of potassium bromide, at the range of 4000-400cm-1. The 1HNMR- spectra were recorded on spectrophotometer «Varian Mercury-VX-200 (300 MHz», using DMSO-d6 as solvent and TMS as the internal standard. The melting temperatures were determined in sealed of capillary on melting point apparatus SMP3 (ENGLAND).

All spectral data (IR and 1H-NMR) of the synthesized compounds are described as follows:

Compound 1: IR (см-1): 3030 (C-H aromatic), 1664 (C=O), 1598 (C=C); 1H-NMR (δ, ppm): 7.77 (d, 1Hα), 8.08 (d, 1Hβ),7.4-8 (m, 10H, Ar-H).

Compound 2: IR: 3083 (C-H aromatic), 1680 (C=O), 1580 (C=C), 3380 (-OH); 1H-NMR: 7.56 (d,1Hα), 7.99 (d, 1Hβ), 7.04-7.9 (m, 9H, Ar-H), 12.9 (s, 1H, -OH).

Compound 3: IR: 3030 (C-H aromatic), 1649 (C=O), 1598 (C=C); 1H-NMR: 7.38 (d, 1Hα), 8.1 (d, 1Hβ), 7.5-7.9 (m, 9H, Ar-H), 2.29 (s, 3H, -CH3).

Compound 4: IR: 3047 (C-H aromatic), 1649 (C=O), 1603 (C=C), 1128 (-OCH3); 1H-NMR: 7.19 (d,1Hα), 8.2 (d, 1Hβ), 7.29-7.9 (m, 9H, Ar-H), 3.79 (s, 3H, -OCH3).

Compound 5: IR: 3047 (C-H aromatic), 1664(C=O), 1608(C=C), 829 (Ar-Cl); 1H-NMR: 7.9 (d,1Hα), 8.2 (d, 1Hβ),7.39-8 (m, 9H, Ar-H).

Compound 6: IR: 3452 (-OH), 1690 (C=O), 1613 (C=C), 829 (Ar-Cl); 1H-NMR: 7.5 (d,1Hα), 8.19 (d, 1Hβ), 6.4-8 (m, 8H, Ar-H), 12.7 (s, 1H, -OH).

Compound 7: IR: 3052 (C-H aromatic), 1664(C=O), 1598(C=C), 829 (Ar-Cl); 1H-NMR: 7.9 (d,1Hα), 8.19 (d, 1Hβ), 7.3-8.1 (m, 8H, Ar-H), 2.3 (s, 3H, -CH3).

Compound 8: IR: 3034 (C-H aromatic), 1664 (C=O), 1590 (C=C), 1130 (-OCH3), 828 (Ar-Cl); 1H-NMR: 7 (d, 1Hα), 7.7 (d, 1Hβ), 6.9-8.1 (m, 8H, Ar-H), 3.79 (s, 3H, -OCH3).

Compound 9: IR: 3057 (C-H aromatic), 1664(C=O), 1590(C=C); 1H-NMR: 7.19 (d,1Hα), 8.2 (d, 1Hβ),7.2-8 (m, 9H, Ar-H), 2.3 (s, 3H, CH3).

Compound 10: IR: 3457 (-OH), 3011 (C-H aromatic), 1685 (C=O), 1591 (C=C); 1H-NMR: 7.2 (d,1Hα), 7.7 (d, 1Hβ), 7.1-8 (m, 8H, Ar-H), 12.8 (s, 1H, -OH) , 2.3 (s, 3H, CH3).

Compound 11: IR: 3027 (C-H aromatic), 1654(C=O), 1593(C=C); 1H-NMR: 7.8 (d,1Hα), 8.2 (d, 1Hβ), 7.1-8 (m, 8H, Ar-H), 2.3 (s, 3H, -CH3).

Compound 12: IR: 3011 (C-H aromatic), 1659 (C=O), 1588 (C=C), 1126 (-OCH3); 1H-NMR: 7.19 (d, 1Hα), 8.21 (d, 1Hβ), 6.9-8 (m, 8H, Ar-H), 3.8 (s, 3H, -OCH3), 2.96 (s, 3H, CH3).

Compound 13: IR: 3057 (C-H aromatic), 1659(C=O), 1588(C=C), 1168 (-OCH3); 1H-NMR: 7

(d, 1Hα), 8.2 (d, 1Hβ), 6.9-8.2 (m, 9H, Ar-H), 3.8 (s, 3H, -OCH3).

Compound 14: IR: 3380 (-OH), 3035 (C-H aromatic)1660 (C=O), 1590 (C=C), 1166 (-OCH3); 1H-NMR: 6.9 (d, 1Hα), 7.9 (d, 1Hβ), 6.7-8.1 (m, 8H, Ar-H), 12.8 (s, 1H, -OH), 3.8 (s, 3H, -OCH3).

Compound 15: IR: 3080 (C-H aromatic), 1649(C=O), 1588(C=C), 1170 (-OCH3); 1H-NMR: 7.7 (d,1Hα), 8.2 (d, 1Hβ), 6.9-8.2 (m, 8H, Ar-H), 2.29 (s, 3H, -CH3), 3.8 (s, 3H, OCH3).

Compound 16: IR: 3033 (C-H aromatic), 1659 (C=O), 1588 (C=C), 1168 (-OCH3); 1H-NMR: 7.7 (d,1Hα), 8.2 (d, 1Hβ), 6.9-8.2 (m, 8H, Ar-H), 3.8(s, 3H, -OCH3), 3.8 (s, 3H, OCH3).

Compound 17: IR: 3010 (C-H aromatic), 1654(C=O), 1562(C=C), 1340 (C-N); 1H-NMR: 6.7 (d, 1Hα), 8.21 (d, 1Hβ),7.3-7.8 (m, 9H, Ar-H), 2.9 (s, 6H, N-(CH3)2).

Compound 18: IR: 3380 (-OH), 3012 (C-H aromatic), 1664 (C=O), 1598 (C=C), 1372 (C-N); 1H-NMR: 6.8 (d, 1Hα), 8.7 (d, 1Hβ), 6.8-8.8 (m, 8H, Ar-H), 9.7 (s, 1H, -OH), 2.99 (s, 6H, N-(CH3)2).

Compound 19: IR: 3083 (C-H aromatic), 1649(C=O), 1603(C=C), 1360 (C-N); 1H-NMR: 6.7 (d, 1Hα), 8.21 (d, 1Hβ),7.3-8 (m, 8H, Ar-H), 2.98 (s, 6H, N-(CH3)2), 2.3 (s, 3H, -CH3).

Compound 20: IR: 3010 (C-H aromatic), 1650 (C=O), 1593 (C=C), 1161 (-OCH3), 1330 (C-N); 1H-NMR: 6.7 (d, 1Hα), 8.2 (d, 1Hβ),6.7-8.1 (m, 8H, Ar-H), 2.9 (s, 6H, N-(CH3)2), 3.79 (s, 3H, OCH3).

Compound 21: IR: 1639(C=O), 1557(C=C), 3206 (-OH), 3010 (C-H aromatic); 1H-NMR: 7.6 (d, 1Hα), 8.2 (d, 1Hβ), 6.7-8.2 (m, 9H, Ar-H), 10.3 (s, 1H, -OH).

Table - 1: Characterization of synthesized chalcone derivatives

Comp. No	R_1	R_2	R_3	R_4	Yield (%)	m.p. (°C)
1	H	H	H	H	75	56-57
2	OH	H	H	H	40	120-121
3	CH_3	H	H	H	71.5	70-72
4	OCH_3	H	H	H	72	108-110
5	H	Cl	H	H	77.5	53-54
6	OH	Cl	H	H	41.5	191-193
7	CH_3	Cl	H	H	72.5	50-51
8	OCH_3	Cl	H	H	62.5	124-126
9	H	H	CH_3	H	69	68-70
10	OH	H	CH_3	H	30	114-116
11	CH_3	H	CH_3	H	84	89-91
12	OCH_3	H	CH_3	H	80	74-75
13	H	H	H	OCH_3	80	77-78
14	OH	H	H	OCH_3	35	179-181
15	CH_3	H	H	OCH_3	87.5	99-100
16	OCH_3	H	H	OCH_3	84	102-103
17	H	H	H	$N(CH_3)_2$	80	111-113
18	OH	H	H	$N(CH_3)_2$	47	76-78
19	CH_3	H	H	$N(CH_3)_2$	70	124-125
20	OCH_3	H	H	$N(CH_3)_2$	88	129-131
21	H	OH	H	H	50	154-155
22	OH	OH	H	H	9	287-289
23	CH_3	OH	H	H	38.5	165-167
24	OCH_3	OH	H	H	45	151-153
25	H	H	Cl	H	80	78-80

Compound 22: IR: 1669 (C=O), 1590 (C=C), 3467 (-OH), 3005 (C-H aromatic); 1H-NMR: 7.8 (d, 1Hα), 7.5 (d, 1Hβ), 6.3-8.3 (m, 8H, Ar-H), 10.2 (s, 1H, -OH), 10.2 (s, 1H, -OH).

Compound 23: IR: 1644(C=O), 1577(C=C) , 3216 (-OH), 3030 (C-H aromatic); 1H-NMR: 6.8 (d, 1Hα), 7.8 (d, 1Hβ), 6.7-8.2 (m, 8H, Ar-H), 10.2 (s, 1H, -OH), 2.28 (s, 3H, -CH3).

Compound 24: IR: 3252 (-OH), 3000 (C-H aromatic), 1640 (C=O), 1603 (C=C), 1165 (-OCH3); 1H-NMR: 7.96 (d, 1Hα), 8.19 (d, 1Hβ), 6.7-8.2 (m, 8H, Ar-H), 10.19 (s, 1H, -OH), 3.69 (s, 3H, OCH3).

Compound 25: IR: 1654 (C=O), 1603 (C=C), 3052 (C-H aromatic), 823 (Ar-Cl); 1H-NMR: 7.9 (d,1Hα), 8.2 (d, 1Hβ),7.2-8 (m, 9H, Ar-H).

2.3. Biological Activities of Chalcone Derivatives:

2.3.1. Determination of antimicrobial activities

The anti-microbial activities of the recent synthesized chalcones were conducted against two gram positive bacteria: Bacillus subtilis and Staphylococcus aureus and two gram negative bacteria: Escherichia coli, and Pseudomonas aeruginosa, and against one fungi: Candida albican by using cup plate method. Ciproflaxacin and Fluconazole were employed as reference standards to compare the results. Each test compound was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1000 µg/ml. Ciprofloxacin and Fluconazole solutions were also prepared at a concentration of 1000 µg/ml. All chalcone compounds and references were tested at a concentration of 0.1ml (100 µg) level and DMSO was used as a control. The solution of each test compound and standard (100 µg) was added separately and kept undisturbed in cups and plates for at least 2 hours in a refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37^0C for 24 hrs. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader. All the tests were carried out in triplicate and the antibacterial activity was expressed as the mean zone of inhibition (mm) produced by the product solutions.

2.3.2. Determination of antioxidant activities

The antioxidant activities of chalcones solutions were measured in vitro using four complementary different methods, namely: the DPPH free radical scavenging assay [22], the ferric reducing ability power (FRAP) method [23], the nitric oxide (NO) scavenging activity [24], and the hydrogen peroxide scavenging activity [25]. All assays were carried out in triplicate using UV-VIS spectrophotometer (1061-Shimadzu, Japan) and the average value was obtained.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial Activities

The antimicrobial activity of the present synthesized chalcones was tested against two G-positive bacteria: *Staphylococcus aureus*, *Bacillus subtillis*, and two G-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa* and one fungi: *Candida albicans*. The results of the well diffusion assays are shown in table 2. These results revealed that all tested chalcones affected the growth of most microorganisms used in this experiment. In other words, if we exclude the compounds 4, 7, 12, and 20, which were not effective against *Staphylococcus aureus,* all other compounds were found to have antimicrobial activity against all tested microorganisms. This phenomenon could be due to the presence of a reactive α, β-unsaturated keto function in chalcones. Similar results were observed by other researchers when they investigated other chalcone derivatives [26 – 34]. However, this activity may be altered depending on the type and position of substituents on the aromatic rings [32]. In other words, the variation in the obtained antibacterial activities could be explained on the bases of the tested microorganisms and on the type and positions of substituents in chalcone. Therefore, the hydroxyl groups in the compounds 2 and 22 could be responsible for their broad spectrum as antimicrobial agents, because in most cases they were more effective against all tested microorganisms in comparison with used standard references (Table 2). These results are in agreement with those obtained by other researchers [33 - 35]. However, other chalcones containing hydroxyl group at *p*-position in ring A, such as, 6, 10, 14, and 18 have shown lower antibacterial activities, which could be attributed to the presence of other electron donating groups attached to ring B. Because, these electron donating groups can affect the availability of hydrogen in hydroxyl groups to create hydrogen bond with enzymes. In other words, the antimicrobial effectivness may decrease due to the abscence of hydrogen binding to the active site of enzymes [36, 37].

The present data indicate that *pseudomonas aeruginosa* is the most susceptible microorganism to the tested synthesized chalcones, because seventeen chalcones have shown similar or stronger antibacterial activity than the reference used in this work. In addition, the compound 10 seems to be the superior amongst chalcones in relation to the activity against P. aeruginosa as obserevd from its

Table - 2: Results of antimicrobial activities of tested chalcone compounds

Comp. No	Inhibition Zone (in mm) against different microorganisms				
	S. aureus	B. subtillis	E. coli	P. aeruginosa	C. Albicans
1	14	17	18	20	14
2	25	29	22	20	24
3	15	14	17	19	11
4	-	16	16	22	11
5	17	18	14	21	11
6	16	29	15	14	21
7	-	15	16	17	10
8	11	14	16	13	11
9	15	18	16	19	11
10	19	29	16	48	18
11	11	18	16	19	13
12	-	14	14	16	13
13	16	16	14	19	10
14	15	18	16	24	11
15	14	15	16	20	10
16	12	14	12	15	11
17	12	14	16	21	10
18	17	25	16	12	21
19	9	16	15	15	11
20	-	13	15	11	11
21	19	14	13	28	16
22	20	37	29	25	30
23	15	14	15	25	13
24	16	14	21	25	18
25	24	14	15	15	10
Ciproflixacin (100 µg)	18	19	20	19	-
Fluconazole (100 µg)	-	-	-	-	27

(-) indicates no zone of inhibition

inhibition zone (48 mm). This stduy has shown that, *Bacillus subtillis* is the second susceptible bacterial species to the investigated chalcones, because five compounds (2, 6, 10, 18, and 22) have shown greater antibacterial activity than the reference antibiotic (Table 2). Generally, the *S. aureus* appeared to be the most resistant bacterial species to most of the present compounds, since they had no or lower antibacterial activity in comparison with ciproflixacin. With the exception of the compound 22, which has shown stronger effect against the growth of *Candida albicans*, all other tested chalcones revealed weaker antifungal activity than the used reference standard (Fluconazole). However, chalcones containing hydroxyl groups (2, 6, 10, and 18) seem to be more potent as antifungal agent than other tested chalcones (Table 2).

3.2. Antioxidant activities

The results in table 3 expressed the antioxidant activity of chalcone and its different derivatives, which were tested using four complementary methods, namely: free radical scavenging activity using DPPH, FRAP, NO scavneging activity, and hydrogen peroxide scavenging activity methods. These four methods were used in order to find safe, and multipotent antioxidants. The first three methods are indicative to the ability of antioxidants to remove free radicals directly, while the fourth one is indicative to the ability of antioxidants to prevent

Table - 3: Results (Mean ± SD) of antioxidant activities of samples (50 µg) as measured by different methods: FRAP, H_2O_2 scavenging activity, NO scavenging activity, and DPPH radical scavenging activity

Comp. No	FRAP*	H_2O_2 (Inhibition, %)	NO (Inhibition, %)	DPPH (Inhibition, %)
1	32.62 ± 0.56	31.12 ± 0.43	44.57 ± 1.29	52.76 ± 0.94
2	54.73 ± 0.25	49.51 ± 2 .13	58.54 ± 3.21	85.93 ± 1.72
3	33.80 ± 0.29	31.45 ± 0.81	50.67 ± 0.69	57.91 ± 0.83
4	37.04 ± 0.68	40.01 ± 1.39	49.34 ± 1.78	52.42 ± 1.92
5	36.79 ± 0.73	29.42 ± 1.66	43.24 ± 0.76	53.86 ± 0.19
6	58.95 ± 1.22	42.54 ± 1.34	55.43 ± 1.72	88.02 ± 2.32
7	40.19 ± 0.69	36.56 ± 1 .12	43.19 ± 1.11	62.61 ± 1.60
8	41.86 ± 0.82	35.98 ± 0.59	51.27 ± 2.10	58.46 ± 0.35
9	42.59 ± 0.94	27.91 ± 0.71	48.98 ± 1.56	63.68 ± 0.54
10	63.57 ± 1.05	49.17 ± 1.13	63.67 ± 2.92	88.21 ± 2.24
11	40.18 ± 0.65	44.23 ± 1.10	57.02 ± 1.13	65.62 ± 1.13
12	39.89 ± 0.72	39.12 ± 0.21	53.32 ± 1.27	61.67 ± 1.02
13	40.85 ± 0.83	41.00 ± 1.24	49.49 ± 0.91	57.29 ± 1.18
14	66.83 ± 1.05	55.25 ± 1.32	59.33 ± 1.43	81.45 ± 2.95
15	38.37 ± 0.49	46.34 ± 1.65	53.38 ± 1.29	69.23 ± 0.81
16	28.83 ± 0.26	36.40 ± 1.42	55.26 ± 1.73	62.22 ± 0.41
17	28.77 ± 0.85	22.82 ± 0.78	50.37 ± 0.89	68.89 ± 1.24
18	66.83 ± 1.05	51.24 ± 0.07	61.49 ± 0.93	85.79 ± 3.10
19	28.83 ± 0.26	41.71 ± 1.23	51.29 ± 1.24	62.02 ± 0.84
20	30.88 ± 0.85	34.48 ± 1.89	49.34 ± 1.21	63.09 ± 0.71
21	63.28 ± 0.89	38.22 ± 0.82	65.19 ± 1.15	85.87 ± 1.92
22	103.44 ± 1.45	62.10 ± 0.99	74.23 ± 2.13	94.12 ± 1.21
23	67.77 ± 1.02	37.76 ± 0.77	67.61 ± 2.06	83.13 ± 2.10
24	54.42 ± 0.91	42.27 ± 1.23	59.25 ± 0.51	93.20 ± 2.05
25	19.10 ± 0.29	32.25 ± 0.69	51.23 ± 0.23	83.70 ± 0.96
Vit.C	-	54.45 ± 1.07	62.41 ± 0.89	91.18 ± 1.31

* (µg vit.C Eq. to 100µg Chalcone)

the formation of free radicals, and consequently reduce the danger of free radicals indirectly.

The present data (Table 3) showed that most of the tested chalcones have less ability to sacvenge free radicals than the positive control as measured by the DPPH method. However, the compound 22 revealed similar ability to scavenge free radicals as that observed for Vit.C. Generally, chalcones containing hydroxyl groups showed relatively high free radical scavenging ability, but the highest activity obtained from the compound 22 could be related to the presence of two hydroxyl groups attached to the two aromatic rings. Theses compounds (2, 6, 10, 14, 18, and 22) may be able to donate hydrogen atom ($H^{\bullet}$) and consequently neutralize the free radical from $DPPH^{\bullet}$, which is changed to DPPH-H, while new radicals from chalcones are formed. However, these new radicals are more stable due to the delocalization of the unpaired electron across the entire molecule. The present findings, are in agreement with those results obtained by other scientists [38] who studied some other chlacone derivatives containing hydroxyl groups attached to different positions.

With the exception of the compound 22, the ferric reducing ability power (FRAP) of other chalcone derivatives tested in the present study revealed to have low to moderate values and they were expressed as Vit.C equivalent (µg Vit.C/100 µg chalcone derivatives). However, the present work has shown that chalcone derivatives containing hydroxyl groups seem to have greater

ability to reduce Fe^{+3} to Fe^{+2} ions than other derivatives having no hydroxyl groups.

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. However, hydrogen peroxide can cross cell membranes rapidly, once inside the cell, it may react with Fe^{+2}, and possibly Cu^{+2}, ions to form hydroxyl radical and this may be the origin of its toxic effects. Therefore, it is important for cells to avoid the accumulation of H_2O_2. The present results (Table 3) showed that the recently tested chalcone derivatives have different activity to scavenge H_2O_2 from the reaction medium. Their activities ranged from low to moderate. However, the ability of chalcone derivatives containing hydroxyl groups to scavenge H_2O_2 from the reaction medium is greater than others and was comparable with that of the positive control (Vit.C).

The NO is generated from the terminal guanido nitrogen atom of L-arginine by various NADPH-dependent enzymes called NO synthases [38]. Oxygen reacts with the excess nitric oxide to generate nitrite (NO_2^-) and peroxynitrite anions (ONOO-), which act as free radicals [39 – 41]. The obtained data from the present study indicate that all chalcone derivatives have the ability to sacvenge NO from the reaction medium and in most cases chalcone derivatives containing hydroxyl groups seem to be more active than others. However, the compound 22 showed the highest activity and even greater than that of the positive control (Vit.C). In conclusion, the present data indicated that chalcone derivatives, especially those containing hydroxyl groups might be responsible for their biological activities, such as, antibacterial, antifungal and antioxidant activities. However, it was observed that chalcone containing two hydroxyl groups attached to two aromatic rings showed the highest biological activities.

4. REFERENCES

1. Awasthi SK, Mishra N, Kumar B, Sharma M, Bhattacharya A, Mishra LC and Bhasin VK.. **Med. Chem. Res.,** 2009; 18: 407.
2. Lim SS, Kim HS and Lee DU. **Bulletin of the Korean Chemical Society,** 2007; 28: 2495.
3. Achanta G, Modzelewska A, Feng L, Khan SR and Huang P. **Molecular Pharmacology,** 2006; 70: 426.
4. Romagnoli R, Baraldi PG, Carrion MD, Cara CL, Cruz-Lopez O and Preti D. Bioorgan. **Med. Chem.,** 2008; 16: 5367.
5. Lunardi F, Guzela M, Rodrigues AT, Corre R, Eger-Mangrich I, Steindel M, Grisard EC, Assreuy J, Calixto JB and Santos ARS. **Antimicrobial Agents and Chemotherapy,** 2003; 47: 1449.
6. Yadav HL, Gupta P, Pawar PS, Singour PK and Patil UK. **Med. Chem. Res.,** 2010; 19: 1.
7. Zhang XW, Zhao DH, Quan YC, Sun LP, Yin XM and Guan LP. **Med. Chem. Res.,** 2010; 19: 403.
8. Hamdi N, Fischmeister C, Puerta MC and Valerga P. **Med. Chem. Res.,** 2011; 20(4): 522.
9. Bag S, Ramar S and Degani MS. **Med. Chem. Res.,** 2009; 18: 309.
10. Lahtchev KL, Batovska DI, Parushev SP, Ubiyvovk VM and Sibirny AA. **Eur. J. Med. Chem.,** 2008; 43: 2220.
11. Begum NA, Roy N, Laskar RA and Roy K. **Med. Chem. Res.,** 2011; 20(2): 184.
12. Sivakumar PM, Prabhakar PK and Doble M. **Med. Chem. Res.,** 2011; 20(4): 482.
13. Vogel S, Ohmayer S, Brunner G and Heilmann **J. Bioorgan. Med. Chem.,** 2008; 16: 4286.
14. Najafian M, Ebrahim-Habibi A, Hezareh N, Yaghmaei P, Parivar K and Larijani B. **Molecular Biology Reports,** 2010; 10: 27.
15. Zarghi A, Zebardast T, Hakimion F, Shirazi FH, Rao PNP and Knaus EE. **Bioorgan. Med. Chem.,** 2006; 14: 7044.
16. Chimenti F, Fioravanti R, Bolasco A, Chimenti P, Secci D, Rossi F, Yanez M, Francisco OF, Ortuso F and Alcaro S. Med. Chem., 2009; 10, 1.
17. Moa MJG, Mandado M, Cordeiro M, Mosquera RA. **Chemical Physics Letters,** 2007; 446: 1.
18. Ahmed MR, Sastry VG, Bano N, Anwer S and Kumaraswamy G. **J. Chem. Pharm. Res,** 2011; 3(5): 710-717.
19. Kamble VM, Hatnapure GD, Keche AP, Birajdar S, Patil SG, Tale RH, Rodge AH, Turkar SS and Gour KJ. **J. Chem. Pharm. Res.,** 2011; 3(6): 639.
20. Aichaoui H, Guenadil F, Kapanda CN, Lambert DM, McCurdy CR and Poupaert JH. **Med. Chem. Res.,** 2009; 18: 467.
21. Anto RJ, Sukumaran K, Kuttan G, Rao MNA, Subbaraju V and Kuttan R. **Cancer letters,** 1995; 97: 33.
22. Benzie IFF and Strain JJ. **Analytical Biochemistry,** 1996; 239: 70.
23. Jayaprakasha GK, Jaganmohan RL and Sakariah KK. **Bioorgan. Med. Chem.,** 2004; 12: 5141.

24. Shirwaikar AN, Sirwaikar AR, Rajendrar K and Punitha ISR. Biol. Pharm. Bull., 2006; 29(9): 1906.
25. Brand-Williams W, Cuvelier ME and Berset C. **Lebensmittel Wissenschaft und Technologie,** 1995; 28: 25.
26. Chen ZH, Zheng CJ, Sun LP and Piao HR. Eur. **J. Med. Chem.,** 2010; 45: 5739.
27. Bandgar BP, Gawande SS, Bodade RG, Totre JV and Khobragade CN. Bioorgan. Med. Chem., 2010; 18: 1364.
28. Rao YK, Fang SH and Tzeng YM. **Bioorgan. Med. Chem.,** 2009; 17: 7909.
29. Rojas J, Paya M, Dominguez JN, Luisa M and Ndiza F. **Bioorgan. Med. Chem. Lett.,** 2002; 12: 1951.
30. Bandgar BP, Gawande SS, Bodade RG, Gawande NM and Khobragade CN. Bioorgan. Med. Chem., 2009; 17: 8168.
31. Bandgar BP and Gawande SS. Bioorgan. Med. Chem., 2010; 18: 2060.
32. Baviskar B, Sureshbhi PS, Baviskar B, Khadabadi SS and Shiradkar M. **Asian J. Research Chem,** 2008; 1: 67.
33. Naziabegum PS, Shahid FS and Salunke SD. **International Journal of Chemical and Pharmaceutical Sciences,** 2013; 4 (3): 97.
34. Pavan KP, Vivek C, Richard LJ, Jagatheesh K and Elangovan N. **International Journal of Chemical and Pharmaceutical Sciences,** 2012; 3(4): 67.
35. Ngaini Z, Haris-Fadzilla SM, Hussain H, Kamaruddin K. **World Journal of Chemistry,** 2009; 4(1): 9.
36. Beuchat LR, and Golden DA. **Food Technol.,** 1989; 43(1): 134.
37. Ceylan E and Fung DYC. **J. Rapid Meth. Auto. Micro.,** 2004; 12(1): 1.
38. Todorova IT, Batovska DI, Stamboliyska BA and Parushev SP. **J. Serb. Chem. Soc.,** 2011; 76(4): 491.
39. Nathan C and Xie QW. **Cell (Cambridge, Mass.),** 1994; 78: 915.
40. Knowles RG and Moncada S. **Biochem J.,** 1994; 298: 249.
41. Alderton WK, Chris E, Cooper CE and Knowles RG. **Biochem. J.,** 2001; 357: 593.