

Antioxidant Activity of Synthesized Methyl Semicarbazone Derivatives by DPPH Scavenging Assay

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Abstract: In the present study, a series of chalcone-semicarbazones was synthesized and evaluated for antioxidant activity by DPPH free radical scavenging assay. Most of the compounds were found to be potent antioxidant. Free radicals play an important role in various pathological and xenotoxic effects so antioxidant may have protective role in these pathological conditions. Based on the results of an anti-oxidant study, Compound 23 was the most active compound. The highest scavenger activity observed in compound 23 is probably due to the presence of hydroxyl group in the acetophenic moiety and methoxy group in aldehydic moiety of the chalcone. It was found that methoxy and hydroxyl substituted chalcone-semicarbazones were potent free radical scavenger and unsubstituted compound showed very less activity.

Key words: Chalcones • Antioxidant • Semicarbazones • DPPH scavenging

INTRODUCTION

Free radicals are an atom or molecule that bears an unpaired electron and is extremely reactive, capable of engaging in rapid change reaction that destabilize other molecules and generate many more free radicals. In plants and animals these free radicals are deactivated by antioxidants. These antioxidants act as an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. The body is constantly exposed to the negative and sometimes lethal effects of oxidants during normal physiological processes. The harmful free radicals such as hydroxyl, peroxy and the superoxide anion are constantly being produced as a result of metabolic reactions in living systems. On a daily basis, up to 5% of inhaled oxygen may be converted to reactive oxygen species (ROS). These ROS have the ability to bind to cellular structures and have been implicated in number of pathological processes such as aging, inflammation, re-oxygenation of ischemic tissues, atherosclerosis, cancer and even Parkinson's disease in men [1]. Two processes, which produce free radicals *in vivo*, have been identified and named the Fenton reaction and the Haber-weiss reaction [2].

Antioxidants play an important role in animal health. Conventional antioxidants have been shown to

improve animal performance during conditions characterized by increased tissue oxidant levels such as stress, injury and infections [3]. The semicarbazone is an electron withdrawing group and exhibited antioxidant activity. Favorable substitution may increase their free radical scavenging effect [4].

Experimental Design: Chalcone-semicarbazones were synthesized according to synthetic scheme as shown in Figure 1. Melting points were measured in open capillary tubes on a Buchi 530 melting point apparatus and were uncorrected. Infrared (IR) and proton nuclear magnetic resonance (¹H NMR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Bruker Advance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. All exchangeable protons were confirmed by addition of D₂O. Mass spectra were measured with a Shimadzu GC-MS-QP5000 spectrophotometer. Only molecular ions (M⁺) and base peaks are given. Elemental analysis (C, H and N) were undertaken with a Perkin-Elmer model 240C analyzer and all analyses were consistent with theoretical values (within 0.4%) unless indicated. The homogeneity of the compounds was monitored by ascending thin-layer chromatography (TLC) on silica gel G (Merck) coated aluminum plates, visualized by iodine vapor.

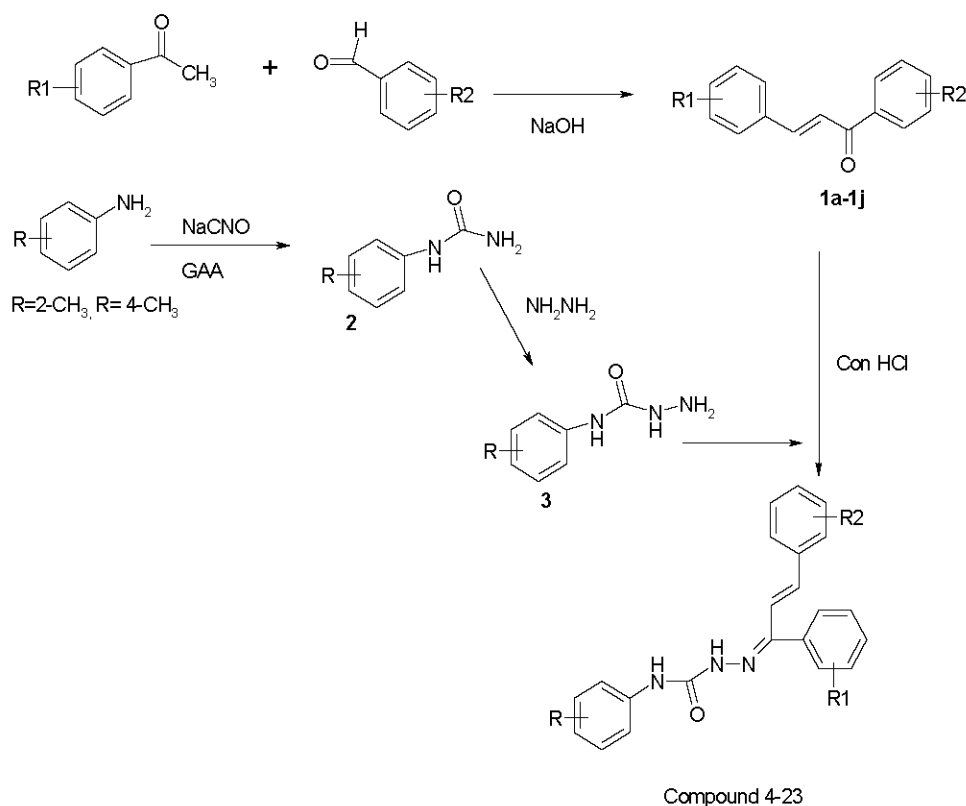


Fig. 1: Synthetic scheme for synthesizing the title compounds

Table 1: Physicochemical properties of chalcone derivatives

Compound No.	R ₁	R ₂	Molecular formula	Melting point (mp) (°C)	Yield (%)	Rf value
1a	H	H	C ₁₅ H ₁₂ O ₂	89	85	0.80
1b	H	4'-OH	C ₁₅ H ₁₂ O ₃	164	85	0.83
1c	H	4'-OCH ₃	C ₁₆ H ₁₄ O ₃	135	85	0.82
1d	H	4'-N(CH ₃) ₂	C ₁₇ H ₁₇ NO ₂	155	85	0.78
1e	4'-OH	6'-OH	C ₁₅ H ₁₂ O ₄	216	90	0.85
1f	4'-OH	4'-N(CH ₃) ₂	C ₁₇ H ₁₇ NO ₃	174	90	0.81
1g	H	6'-OH	C ₁₅ H ₁₂ O ₃	166	85	0.86
1h	5'-OH	6'-OH	C ₁₅ H ₁₂ O ₄	218	85	0.84
1i	5'-OH	4'-OH	C ₁₅ H ₁₂ O ₄	208	85	0.87
1j	5'-OH	4'-OCH ₃	C ₁₆ H ₁₄ O ₄	152	85	0.79

Synthesis of Substituted Chalcone Derivatives:

Substituted benzaldehydes (0.012 mol) were added to a mixture of substituted acetophenones (0.01 mol) in 25 ml of ethanol in a 200 ml beaker. The content of the beaker was mixed well and to that 10 ml of 10% potassium hydroxide solution was added and stirred vigorously at 25°C until the mixture was so thick that stirring was no longer effective (3-4 h).

After the completion of the stirring, the reaction mixture was kept in a refrigerator overnight.

The reaction mixture was then diluted with ice-cold water (50 ml), acidified with 10% aqueous hydrochloric acid to precipitate the chalcones. The product was filtered with suction on a Buchner funnel, washed with cold water until the washings were neutral to litmus and then washed with 10 ml of ice-cold rectified spirit. The dried product was recrystallized from chloroform. The physicochemical properties of the synthesized chalcone derivatives are given in table 1.

Table 2: Physicochemical data of methyl semicarbazones

Compound No.	R	R ₁	R ₂	Yield (%)	Mol. Wt.	Mol. Formula	mp (°C)	R _f Value
4	2-CH ₃	H	H	57	371	C ₂₃ H ₂₁ N ₃ O ₂	150	0.78
5	2-CH ₃	H	4''-OH	66	387	C ₂₃ H ₂₁ N ₃ O ₃	145	0.71
6	2-CH ₃	H	4''-OCH ₃	65	401	C ₂₄ H ₂₃ N ₃ O ₃	135	0.65
7	2-CH ₃	H	4''-N(CH ₃) ₂	58	414	C ₂₅ H ₂₆ N ₄ O ₂	148	0.57
8	2-CH ₃	4-OH	6''-OH	57	403	C ₂₃ H ₂₁ N ₃ O ₄	142	0.60
9	2-CH ₃	4-OH	4''-N(CH ₃) ₂	50	430	C ₂₅ H ₂₆ N ₄ O ₃	160	0.67
10	2-CH ₃	H	6''-OH	63	387	C ₂₃ H ₂₁ N ₃ O ₃	140	0.55
11	2-CH ₃	5-OH	6''-OH	61	403	C ₂₃ H ₂₁ N ₃ O ₄	135	0.63
12	2-CH ₃	5-OH	4''-OH	56	403	C ₂₃ H ₂₁ N ₃ O ₄	120	0.69
13	2-CH ₃	5-OH	4''-OCH ₃	57	417	C ₂₄ H ₂₃ N ₃ O ₄	126	0.51
14	4-CH ₃	H	H	52	371	C ₂₃ H ₂₁ N ₃ O ₂	206	0.53
15	4-CH ₃	H	4''-OH	65	387	C ₂₃ H ₂₁ N ₃ O ₃	188	0.63
16	4-CH ₃	H	4''-OCH ₃	63	401	C ₂₄ H ₂₃ N ₃ O ₃	204	0.70
17	4-CH ₃	H	4''-N(CH ₃) ₂	64	414	C ₂₅ H ₂₆ N ₄ O ₂	195	0.62
18	4-CH ₃	4-OH	6''-OH	55	403	C ₂₃ H ₂₁ N ₃ O ₄	178	0.58
19	4-CH ₃	4-OH	4''-N(CH ₃) ₂	56	430	C ₂₅ H ₂₆ N ₄ O ₃	185	0.66
20	4-CH ₃	H	6''-OH	54	387	C ₂₃ H ₂₁ N ₃ O ₃	180	0.69
21	4-CH ₃	5-OH	6''-OH	67	403	C ₂₃ H ₂₁ N ₃ O ₄	183	0.54
22	4-CH ₃	5-OH	4''-OH	50	403	C ₂₃ H ₂₁ N ₃ O ₄	165	0.59
23	4-CH ₃	5-OH	4''-OCH ₃	56	417	C ₂₄ H ₂₃ N ₃ O ₄	172	0.77

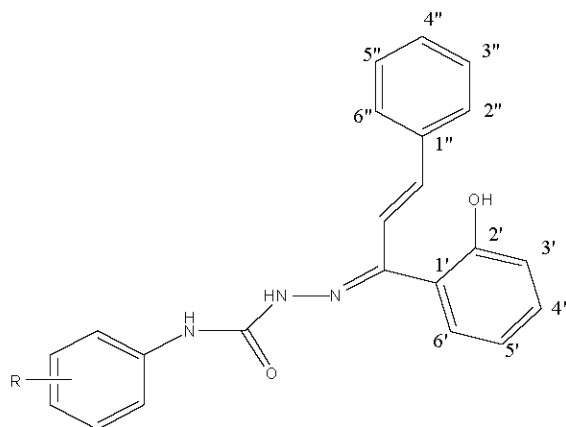


Fig. 2: Structure of synthesized title compounds

Synthesis of Methyl Phenyl Urea (2): Substituted aniline (0.1mol) was dissolved in 20 ml of glacial acetic acid and 10 ml of water. To this, 0.1 mol of sodium cyanate (6.5 g) in 80 ml of warm water was added with continuous stirring. The reaction mixture was allowed to stand for 30 min and then cooled in ice. The crude solid, thus obtained was filtered, dried and recrystallized with boiling water to yield methyl phenyl urea.

Synthesis of Substituted Phenyl Semicarbazide (3): Equimolar quantities (0.05mol) of above phenyl urea (2) and hydrazine hydrate (2.5 ml) in ethanol were refluxed for 27 h with continuous stirring. The two-third volume of

ethanol was distilled by vacuum distillation unit and then poured into ice. The resultant crude solid was filtered, washed with water and dried. The obtained solid was recrystallized with 50 ml of 90% alcohol.

General Method for the Synthesis of Substituted Phenyl Chalcone-Semicarbazone: To a solution of above (3) (0.005 mol) in 25 ml of ethanol added an equimolar quantity of the appropriate chalcone derivative previously dissolved in ethanol. Then few drops of Conc. hydrochloric acid was added and continuously stirred for 4-5 hrs.

The reaction mixture was poured into ice and precipitate, so obtained was filtered, washed with sodium acetate (0.005mol, 0.41 g) in 2ml water. The crude solid was dried and recrystallized with hot ethanol. The structures (Fig. 2) and physicochemical properties of the synthesized title compounds are given in table 2.

1-[1-(2-hydroxyphenyl)-3-phenylallylidene]-4-(2-methylphenyl) Semicarbazide (4): 1H-NMR (δ /ppm in CDCl₃): 2.12 (s, 3H, Ar-CH₃), 4.83 (s, 1H, 2-OH), 7.11-7.64 (m, J= 8.32 Hz, 12H, Ar-H) 7.7 (s, 1H, -CH=CH-), 7.9 (s, 1H, -CH=CH-), 8.34 (s, 1H, ArNH, D₂O exchangeable), 9.42 (s, 1H, CONH, D₂O exchangeable);

IR (KBr/cm⁻¹): 3450 (NH), 3480(-OH), 3300-3240 (CONH), 1670 (-CH=CH-), 1590 (C-N), 1616, 1558 (aromatic), 754, 697 (monosubstituted benzene);

MS, m/z 370; Elemental analysis calculated/found (%) C (74.37/74.26), H (5.70/5.48), N (11.31/11.12).

1-[1-(2-hydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(2-methylphenyl) Semicarbazide (5): ¹H-NMR (δ/ppm in CDCl₃): 2.18 (s, 3H, Ar-CH₃), 4.9 (s, 1H, 2-OH), 5.2 (s, 1H, 4-OH), 7.3-7.64 (m, J= 8.4 Hz, 11H, Ar-H) 7.8 (s, 1H, -CH=CH-), 8.0 (s, 1H, -CH=CH-), 8.44 (s, 1H, ArNH, D₂O exchangeable), 9.8 (s, 1H, CONH, D₂O exchangeable);

IR (KBr/cm⁻¹): 3455 (NH), 3475(-OH), 3310-3245 (CONH), 1675 (-CH=CH-), 1594 (C-N), 1615, 1556 (aromatic), 750, 695 (monosubstituted benzene);

MS, m/z 386; Elemental analysis, cal/fou (%) C (71.30/71.24), H (5.46/5.35), N (10.85/10.47).

1-[1-(2-hydroxyphenyl)-3-(4-methoxyphenyl)allylidene]-4-(2-methylphenyl) Semicarbazide (6): ¹H-NMR (δ /ppm in CDCl₃): 2.16 (s, 3H, Ar-CH₃), 4.7 (s, 1H, 2-OH), 3.88 (s, 3H, 4-OCH₃), 7.12-7.85 (m, J= 8.3 Hz, 11H, Ar-H), 7.98 (s, 1H, -CH=CH-), 8.35 (s, 1H, -CH=CH-), 8.87 (s, 1H, ArNH, D₂O exchangeable), 9.86 (s, 1H, CONH, D₂O exchangeable);

IR (KBr/cm⁻¹): 3458 (NH), 3478 (-OH), 3310-3243 (CONH), 1677 (-CH=CH-), 1587 (C-N), 1626, 1555 (aromatic), 758, 687 (monosubstituted benzene);

MS, m/z 400; Elemental analysis cal/fou (%) C (71.80/71.57), H (5.77/5.48), N (10.47/10.36).

1-[1-(2,4-dihydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(2-methylphenyl) Semicarbazide (9): ¹H-NMR (δ /ppm in CDCl₃): 2.48 (s, 3H, Ar-CH₃), 5.1 (s, 1H, 2-OH), 5.3 (s, 1H, 4-OH), 6.4 (s, 1H, 6-OH), 7.22-7.58 (m, J= 8.5 Hz, 10H, Ar-H) 7.88 (s, 1H, -CH=CH-), 8.4 (s, 1H, -CH=CH-), 8.77 (s, 1H, ArNH, D₂O exchangeable), 9.85 (s, 1H, CONH, D₂O exchangeable);

IR (KBr/cm⁻¹): 3453 (NH), 3482 (-OH), 3314-3242 (CONH), 1667 (-CH=CH-), 1594 (C-N), 1618, 1552 (aromatic), 758, 687 (monosubstituted benzene);

MS, m/z 402; Elemental analysis cal/fou (%) C (68.47/68.44), H (5.25/5.16), N (10.42/10.37).

1-[1-(2-hydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(2-methylphenyl) Semicarbazide (11): ¹H-NMR (δ /ppm in CDCl₃): 2.24 (s, 3H, Ar-CH₃), 5.1 (s, 1H, 2-OH), 5.3 (s, 1H, 2, 4-OH), 7.2-7.78 (m, J= 8.35 Hz, 11H, Ar-H), 7.8 (s, 1H, -CH=CH-), 8.2 (s, 1H, -CH=CH-), 8.78 (s, 1H, ArNH, D₂O exchangeable), 9.84 (s, 1H, CONH, D₂O exchangeable);

IR (KBr/cm⁻¹): 3462 (NH), 3488(-OH), 3300-3240 (CONH), 1666 (-CH=CH-), 1593 (C-N), 1618, 1554 (aromatic), 753, 694 (monosubstituted benzene);

MS, m/z 386; Elemental analysis cal/fou (%) C (71.30/71.17), H (5.46/5.37), N (10.85/10.66).

1-[1-(2,5-dihydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(2-methylphenyl) Semicarbazide (13): ¹H-NMR (δ /ppm in CDCl₃): 2.16 (s, 3H, Ar-CH₃), 5.4 (s, 1H, 2-OH) 5.2 (s, 1H, 4-OH), 5.6 (s, 3H, 5-OH) 7.22-7.88 (m, J= 8.6 Hz, 10H, Ar-H), 7.84 (s, 1H, -CH=CH-), 8.4 (s, 1H, -CH=CH-), 8.82 (s, 1H, ArNH, D₂O exchangeable), 9.96 (s, 1H, CONH, D₂O exchangeable);

IR (KBr/cm⁻¹): 3456 (NH), 3482(-OH), 3310-3245 (CONH), 1667 (-CH=CH-), 1593 (C-N), 1615, 1552 (aromatic), 755, 693 (monosubstituted benzene);

MS, m/z 402; Elemental analysis cal/fou (%) C (68.47/68.28), H (5.25/5.17), N (10.42/10.08).

1-[1-(2-hydroxyphenyl)-3-phenylallylidene]-4-(4-methylphenyl)semicarbazide (14): ¹H-NMR (δ /ppm in CDCl₃): 2.15 (s, 3H, Ar-CH₃), 4.82 (s, 1H, 2-OH), 7.22-7.64 (m, J= 8.3 Hz, 12H, Ar-H) 7.72 (s, 1H, -CH=CH-), 7.89 (s, 1H, -CH=CH-), 8.33 (s, 1H, ArNH, D₂O exchangeable), 9.38 (s, 1H, CONH, D₂O exchangeable);

IR (KBr/cm⁻¹): 3452 (NH), 3485(-OH), 3300-3243 (CONH), 1668 (-CH=CH-), 1591 (C-N), 1613, 1548 (aromatic), 753, 695 (monosubstituted benzene);

MS, m/z 370; Elemental analysis calculated/found (%) C (74.37/74.13), H (5.70/5.47), N (11.31/10.98).

1-[1-(2-hydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(4-methylphenyl) Semicarbazide (15): ¹H-NMR (δ /ppm in CDCl₃): 2.17 (s, 3H, Ar-CH₃), 4.91 (s, 1H, 2-OH), 5.3 (s, 1H, 4-OH), 7.3-7.68 (m, J= 8.32 Hz, 11H, Ar-H) 7.79 (s, 1H, -CH=CH-), 8.1 (s, 1H, -CH=CH-), 8.42 (s, 1H, ArNH, D₂O exchangeable), 9.85 (s, 1H, CONH, D₂O exchangeable);

IR (KBr/cm⁻¹): 3449 (NH), 3471(-OH), 3318-3245 (CONH), 1676 (-CH=CH-), 1593 (C-N), 1618, 1559 (aromatic), 751, 696 (monosubstituted benzene);

MS, m/z 386; Elemental analysis, cal/fou (%) C (71.30/71.25), H (5.46/5.33), N (10.85/10.58).

1-[1-(2-hydroxyphenyl)-3-(4-methoxyphenyl)allylidene]-4-(4-methylphenyl) Semicarbazide (16): ¹H-NMR (δ /ppm in CDCl₃): 2.19 (s, 3H, Ar-CH₃), 4.74 (s, 1H, 2-OH), 3.83 (s, 3H, 4-OCH₃), 7.12-7.85 (m, J= 8.3 Hz, 11H, Ar-H), 7.95 (s, 1H, -CH=CH-), 8.36 (s, 1H, -CH=CH-), 8.89 (s, 1H, ArNH, D₂O exchangeable), 9.86 (s, 1H, CONH, D₂O exchangeable);

IR (KBr/cm⁻¹): 3454 (NH), 3479 (-OH), 3310-3243 (CONH), 1672 (-CH=CH-), 1589 (C-N), 1624, 1556 (aromatic), 753, 687 (monosubstituted benzene);

MS, m/z 400; Elemental analysis cal/fou (%) C (71.80/71.68), H (5.77/5.67), N (10.47/10.33).

1 - [1 - (2 , 4 - d i h y d r o x y p h e n y l) - 3 - (2 - h y d r o x y p h e n y l) a l l y l i d e n e] - 4 - (4 - m e t h y l p h e n y l) S e m i c a r b a z i d e (18): ¹H-NMR (δ/ppm in CDCl₃): 2.38 (s, 3H, Ar-CH₃), 5.22 (s, 1H, 2-OH), 5.37 (s, 1H, 4-OH), 6.43 (s, 1H, 6-OH), 7.22-7.58 (m, J= 8.32 Hz, 10H, Ar-H) 7.89 (s, 1H, -CH=CH-), 8.421 (s, 1H, -CH=CH-), 8.77 (s, 1H, ArNH, D₂O exchangeable), 9.86 (s, 1H, CONH, D₂O exchangeable);

IR (KBr/cm⁻¹): 3456 (NH), 3482 (-OH), 3314-3242 (CONH), 1665 (-CH=CH-), 1598 (C-N), 1616, 1554 (aromatic), 752, 689 (monosubstituted benzene);

MS, m/z 402; Elemental analysis cal/fou (%) C (68.47/68.44), H (5.25/5.21), N (10.42/10.33).

1-[1-(2-hydroxyphenyl)-3-(2-hydroxyphenyl)allylidene]-4-(4-methylphenyl) Semicarbazide (20): ¹H-NMR (δ/ppm in CDCl₃): 2.25 (s, 3H, Ar-CH₃), 5.14 (s, 1H, 2-OH), 5.29 (s, 1H, 2, 4-OH), 7.2-7.77 (m, J= 8.3 Hz, 11H, Ar-H), 7.82 (s, 1H, -CH=CH-), 8.2 (s, 1H, -CH=CH-), 8.77 (s, 1H, ArNH, D₂O exchangeable), 9.87 (s, 1H, CONH, D₂O exchangeable);

IR (KBr/cm⁻¹): 3462 (NH), 3488(-OH), 3300-3240 (CONH), 1666 (-CH=CH-), 1593 (C-N), 1618, 1554 (aromatic), 753, 694 (monosubstituted benzene);

MS, m/z 386; Elemental analysis cal/fou (%) C (71.30/71.13), H (5.46/5.42), N (10.85/10.72).

1-[1-(2,5-dihydroxyphenyl)-3-(4-hydroxyphenyl)allylidene]-4-(4-methylphenyl) semicarbazide (22): ¹H-NMR (δ/ppm in CDCl₃): 2.17 (s, 3H, Ar-CH₃), 5.45 (s, 1H, 2-OH) 5.22 (s, 1H, 4-OH), 5.61 (s, 3H, 5-OH) 7.22-7.88 (m, J= 8.6 Hz, 10H, Ar-H), 7.85 (s, 1H, -CH=CH-), 8.4 (s, 1H, -CH=CH-), 8.82 (s, 1H, ArNH, D₂O exchangeable), 9.98 (s, 1H, CONH, D₂O exchangeable);

IR (KBr/cm⁻¹): 3458 (NH), 3483 (-OH), 3311-3246 (CONH), 1669 (-CH=CH-), 1595 (C-N), 1617, 1555 (aromatic), 756, 699 (monosubstituted benzene);

MS, m/z 402; Elemental analysis cal/fou (%) C (68.47/68.33), H (5.25/5.13), N (10.42/10.31).

DPPH Free Radical Scavenging Assay: The antioxidant activity of the synthesized semicarbazone derivatives was evaluated using the DPPH free radical scavenging assay [5-7]. 200 μL of test sample solution (100 μg/ml) was added to 4 mL of 100 μM methanolic DPPH. After vortexing, the mixture was incubated for 20 minutes at room temperature and the absorbance at 517 nm was measured. Ascorbic acid (100 μg/ml) was used as standard. A blank was

prepared without adding standard or test compound. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH scavenged (\%)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Where A control is the absorbance of the control reaction and A test is the absorbance in the presence of the test compounds. The antioxidant activity of the chalcone semicarbazones is expressed comparing with standard ascorbic acid.

RESULTS AND DISCUSSION

The antioxidant activity of the synthesized chalcone semicarbazones was evaluated using DPPH free radical scavenging assay. The results of anti-oxidant screening were depicted in Table 3 and Fig. 3. DPPH radical scavenging is considered a good *in vitro* model and is widely used to conveniently assess antioxidant efficacy[8]. In its radical form, DPPH has an absorbance at 517 nm which disappears when DPPH is reduced by an antioxidant compound or a radical species to become a stable diamagnetic molecule. As a result, the color changes from purple to yellow. This color change is taken as an indication of the hydrogen donating ability of the tested compounds. Antioxidants can react with DPPH and produce 1,1-diphenyl-2-picryl-hydrazine. The reducing abilities of the synthesized compounds were determined by their interaction with the free stable radical 1,1-diphenyl-2-picryl-hydrazine (DPPH) at 20 μg concentrations for 20 min.

As from the tables it could be seen that most of the compounds showed significant antioxidant activity. The highest scavenger activity observed in compound 23 is probably due to the presence of hydroxyl group in the acetophenic moiety and methoxy group in aldehydic moiety of chalcone. The order of activity regarding substitution on chalconyl group is OH>OCH₃>(CH₃)₂-N>H [9,10].

When the observed results compared, it observed that the 4 methyl substituted compounds showed more DPPH scavenging activity in comparison to the 2 methyl substituted compounds. The substitution with different substituent on the phenyl of the aldehydic and acetophenic group of chalcone moiety plays an important role in the scavenging of free radicals.

Table 3: Antioxidant activity of chalcone semicarbazones by DPPH scavenging assay

Group	Absorbance (mean±S.D.; 517 nm)	% DPPH scavenging
Control	0.853±0.001	-----
Standard	0.329±0.0015 ^a	61.43
Compound 4	0.76±0.02 ^{ab}	10.90
Compound 5	0.476±0.003 ^{ab}	44.197
Compound 6	0.366±0.006 ^a	57.09
Compound 7	0.611±0.0085 ^{ab}	28.37
Compound 8	0.349±0.0085 ^a	59.08
Compound 9	0.557±0.004 ^{ab}	34.7
Compound 10	0.457±0.0075 ^{ab}	46.42
Compound 11	0.326±0.0055 ^a	61.78
Compound 12	0.312±0.0025 ^a	63.42
Compound 13	0.281±0.003 ^a	67.06
Compound 14	0.72±0.005 ^{ab}	15.59
Compound 15	0.486±0.004 ^{ab}	43.02
Compound 16	0.424±0.003 ^{ab}	50.29
Compound 17	0.603±0.003 ^{ab}	29.31
Compound 18	0.304±0.003 ^a	64.36
Compound 19	0.542±0.002 ^{ab}	36.46
Compound 20	0.383±0.004 ^a	55.09
Compound 21	0.298±0.002 ^a	65.06
Compound 22	0.29±0.0046 ^a	66.002
Compound 23	0.253±0.003 ^{ab}	70.34

^{ab} P<0.001 compared to control and standard respectively. One way ANOVA followed by Turkey test

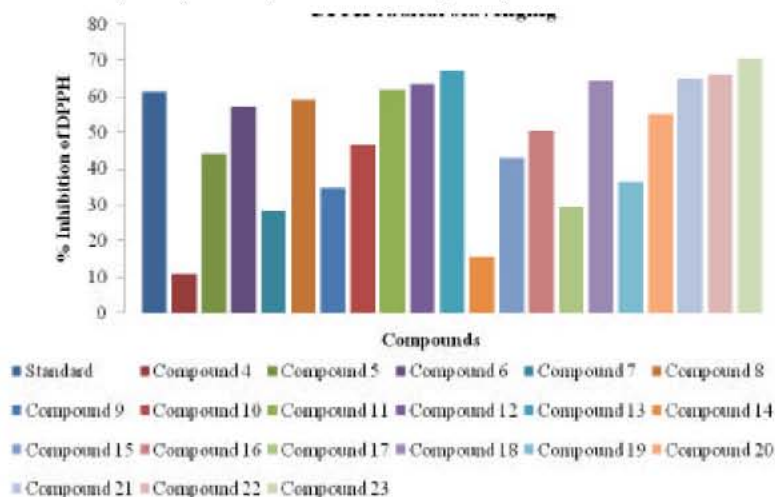


Fig. 3: Antioxidant activity of Chalcone semicarbazones by DPPH radical scavenging

When the phenyl group of aldehydic and acetophenic moiety of chalcone is substituted with -OH group (Compound 8,11, 12,18, 21,22) the compounds exhibited better activity in comparison to substitution with the other groups like p-dimethyl amino groups (compound 7, 17, 19) which may be due to more reducing potential. Hydroxyl substitution on both moieties of chalcone has more scavenging effect than substitution on any one moiety[9,10]. It was found that 5 hydroxyl substitution is more favorable than 4 or 6 hydroxyl

substitution in the aldehydic moiety for antioxidant activity.

Methoxy substitution in the aldehydic moiety of chalcone also favors antioxidant activity [9,10]. Highest DPPH free radical scavenging activity is shown when 5 hydroxyl substitution in acetophenic moiety and 4 methoxy substitution in aldehydic moiety is done.

Among the synthesized compounds, compound 6, 8, 11, 12, 13, 18, 20, 21, 22 and 23 showed the better or comparable activity in comparison to the standard drug.

Bulkier substitution (compound 7, 9, 17, 19) is less favourable for antioxidant activity. The compounds with no substitution (compound 4, 14) or less substitution were showed very less scavenging effect in comparison to the substituted compounds due to lesser electronegativity [10,11].

In summary, most of the synthesized compounds were potential lead for antioxidant activity. On the bases of observed results, it may be concluded that the substitution favors the activity, but the bulkier substitution disfavors the scavenging activity. The methoxy and hydroxyl substitution increases the DPPH free radical scavenging activity of the compounds.

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