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Evaluation of Reducing Power Assay of Chalcone Semicarbazones

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ABSTRACT

In present study, a series of chalconesemicarbazones was synthesized, characterized and evaluated for their reducing power 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 reducing power assay an anti-oxidant study, 1-[1-{4-aminophenyl-3-(4-chlorophenyl)}allylidene]-4-o-tolylsemicarbazide (Compound 26) was the most active compound. It was found that Chloride substitution in aldehydic and amino substitution in acetophenic moiety of chalconesemicarbazones exhibited potent reducing power and unsubstituted compound showed less reducing potential.

Keywords: Chalcones, Anti-oxidant, Semicarbazones, Reducing power.

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, peroxyl 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 SECTION

Chalconesemicarbazones 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 Brucker 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 D_2O. 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.

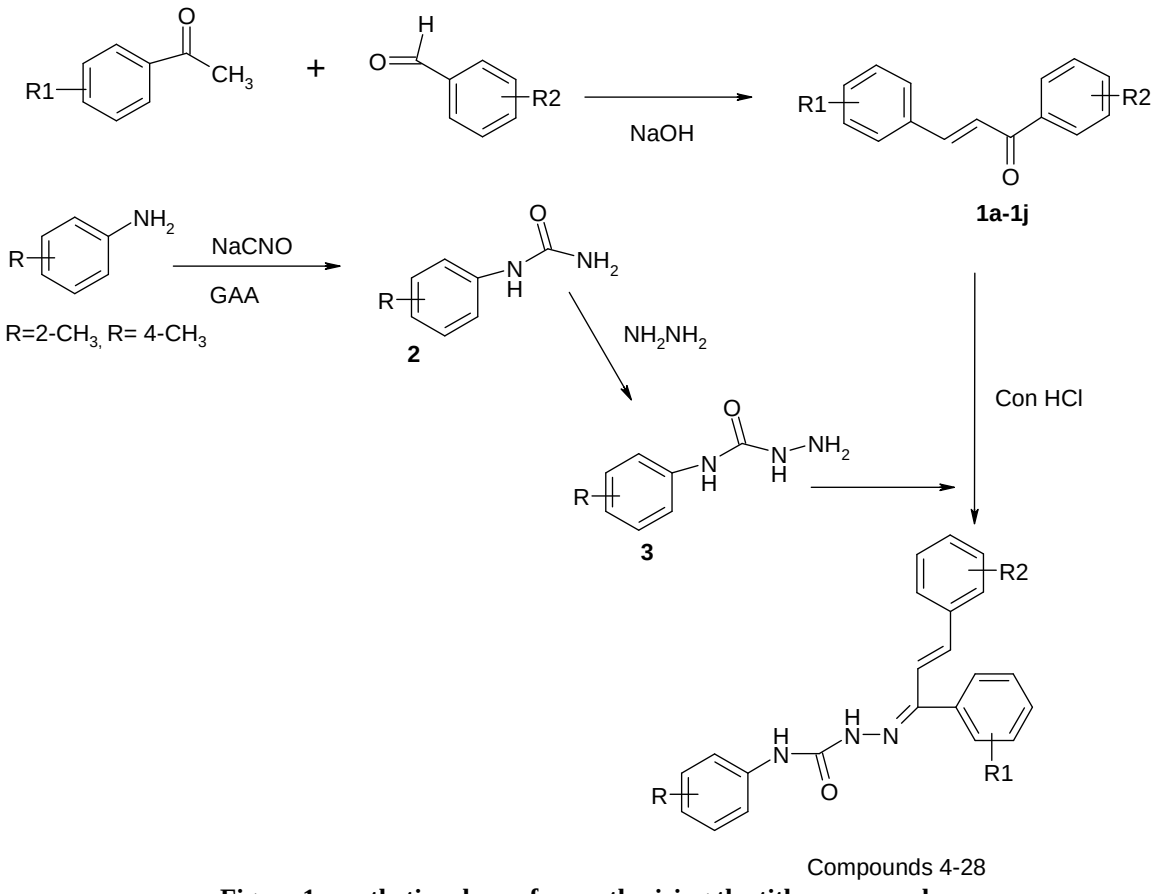


Figure 1: synthetic scheme for synthesizing the title compounds

Synthesis of substituted chalcone derivatives

Substituted benzaldehydes (0.012 mol) were added to a mixture of substituted acetophenones (0.01mol) 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.

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 methyl chalconesemicarbazone

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 Con. 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 (figure 2) and physicochemical properties of the synthesized title compounds are given in table 1.

Table 1: Physicochemical data of methyl semicarbazones

Comp no.	R	R_1	R_2	Yield (%)	Mol Wt.	Mol Formula	mp (°C)	Rf Value
4	2-CH_3	H	H	57	371	$C_{23}H_{21}N_3O_2$	150	0.78
14	4-CH_3	H	H	52	371	$C_{23}H_{21}N_3O_2$	206	0.53
24	2- CH_3	H	p-Cl	65	389.88	$C_{23}H_{20}ClN_3O$	115	0.49
25	2-CH_3	H	Cinnamaldehyde	73	381.47	$C_{25}H_{23}N_3O$	126	0.51
26	2-CH_3	p-NH_2	p-Cl	61	404.89	$C_{23}H_{21}ClN_4O$	192	0.73
27	4-CH_3	p-NH_2	H	63	370.45	$C_{23}H_{22}N_4O$	180	0.68
28	4-CH_3	p-NH_2	p-Cl	63	404.89	$C_{23}H_{21}ClN_4O$	173	0.72

1-[1-(2-hydroxyphenyl)-3-phenylallylidene]-4-(2-methylphenyl)semicarbazide (4)
1H-NMR (δ/ppm in $CDCl_3$): 2.12 (s, 3H, Ar-CH_3), 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, D_2O exchangeable), 9.42 (s, 1H, CONH, D_2O 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-phenylallylidene]-4-(4-methylphenyl)semicarbazide (14)
^{1}H-NMR (δ /ppm in $CDCl_3$): 2.15 (s, 3H, Ar-CH_3), 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, D_2O exchangeable), 9.38 (s, 1H, CONH, D_2O 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-(1,5-diphenylpenta-2,4-dienylidene)-4-o-tolylsemicarbazide (25)
1H-NMR (δ/ppm in $CDCl_3$): 7.11-7.64 (m, 15H, Ar-H), 7.69 (s, 1H, –CH=CH–), 7.72 (s, 1H, –CH=CH–), 7.88-8.12 (dd, 2H, –CH=CH–), 8.34 (s, 1H, ArNH), 9.42 (s, 1H, CONH); IR (KBr/cm^{-1}): 3450 (NH), 3300–3240 (CONH), 1670 (–CH=CH–),1590 (C-N), 1616, 1558 (aromatic), 754, 697 (monosubstituted benzene); MS, m/z 380; Elemental analysis calculated/found (%) C (78.71/78.56), H (6.08/5.98), N (11.02/10.92).

1-[1-{4-aminophenyl-3-(4-chlorophenyl)}allylidene]-4-o-tolylsemicarbazide (26)
1H-NMR (δ/ppm in $CDCl_3$): 6.52 (s, 2H, NH_2), 7.10-7.65 (m, 13H, Ar-H), 7.72 (s, 1H, –CH=CH–), 7.94 (s, 1H, –CH=CH–), 8.32 (s, 1H, ArNH), 9.46 (s, 1H, CONH); IR (KBr/cm^{-1}): 3452 (NH), 3300–3246 (CONH), 1678 (–CH=CH–),1597 (C-N), 1626, 1567 (aromatic), 872 (Cl), 755, 697 (monosubstituted benzene); MS, m/z 403; Elemental analysis calculated/found (%) C (68.23/67.96), H (5.23/5.17), N (13.84/13.75).

1-[1-(4-aminophenyl)-3-phenylallylidene]-4-p-tolylsemicarbazide (27)
1H-NMR (δ/ppm in $CDCl_3$): 6.41 (s, 2H, NH_2), 7.11-7.64 (m, 14H, Ar-H), 7.75 (s, 1H, –CH=CH–), 7.81 (s, 1H, –CH=CH–), 8.41 (s, 1H, ArNH), 9.64 (s, 1H, CONH); IR (KBr/cm^{-1}): 3459 (NH), 3309–3241 (CONH), 1674 (–CH=CH–),1593 (C-N), 1616, 1553 (aromatic), 754, 687 (monosubstituted benzene); MS, m/z 369; Elemental analysis calculated/found (%) C (74.57/74.46), H (5.99/5.78), N (15.12/15.02).

Reducing Power Assay
The reducing power of chalconesemicarbazones was determined by the method of Oyaizu [5]. Substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

$$\text{Potassium ferricyanide + Ferric chloride} \xrightarrow{\text{Antioxidant}} \text{Potassium ferrocyanide + ferrous chloride}$$

One ml of test sample solution (20μg/ml) was mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50^0C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a

freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Ascorbic acid (20μg/ml) was used as standard. A blank was prepared without adding standard or test compound. Increased absorbance of the reaction mixture indicates increase in reducing power [6].

The percent increase in reducing power was calculated using the following equation

$$\textbf{Increase in reducing power (\%)} = \frac{\text{A test - A blank}}{\text{A blank}} \times 100$$

where A test is absorbance of test solution; A blank is absorbance of blank.

RESULTS AND DISCUSSION

The antioxidant activity of the synthesized chalcone semicarbazones was evaluated using reducing power assay. The results of anti-oxidant screening were depicted in Table 2 and figure 3. Substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm [5,6].

Table 2: Antioxidant activity of chalcone semicarbazones by reducing power assay

Compounds	Absorbance (mean±S.D.; 700 nm)	% Increase in reducing power
Control	0.0952±0.0002	---------
Standard	0.187±0.0036[a]	96.43
Compound 4	0.1102±0.00026 [a,b]	15.76
Compound 14	0.1179±0.0002 [a,b]	23.84
Compound 24	0.1218±0.00148 [a,b]	27.94
Compound 25	0.1193±0.0004 [a,b]	25.31
Compound 26	0.1599±0.00032 [a,b]	67.96
Compound 27	0.1107±0.00071 [a,b]	16.28
Compound 28	0.1514±0.00078 [a,b]	59.03

[a,b] P<0.001 compared to control and standard respectively. One way ANOVA followed by Turkey test

As from the table it could be seen that most of the compounds showed significant antioxidant activity. The highest reducing activity observed in compound 26 is probably due to the presence of Amino group in the acetophenic and Chloride in the aldehydic moiety of chalcone. The order of activity regarding substitution on chalconyl group is Cl>NH_2> Cinnameldehyde> H [7-9].

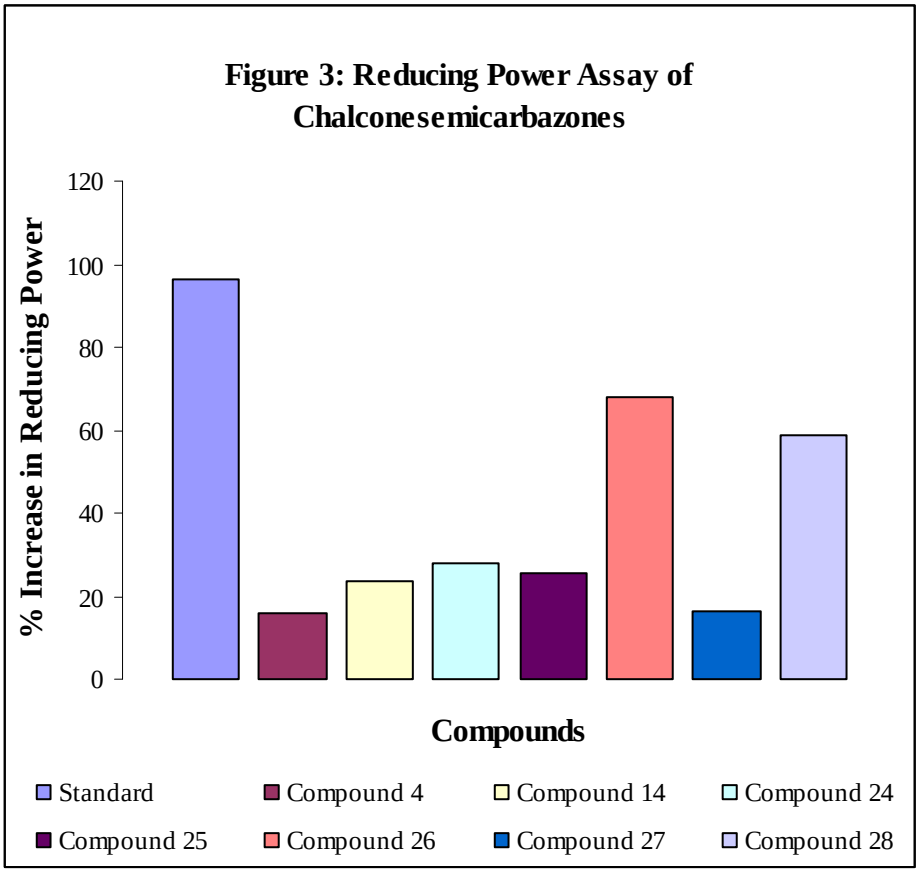
When the observed results compared, it observed that the 4 methyl substituted compounds showed more reducing power in comparison to the 2 methyl substituted compounds. The substitution with different substituent on the phenyl of the aldehydic and acetophenic group of chalcone moiety plays an important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

Compounds with the chlorine substitution in the aldehydic moiety and amino substitution in acetophenic moiety (compound 26, 28) exhibited good antioxidant activity [11]. Among the synthesized compounds, compound 26 and 28 showed comparable activity in comparision to the standard drug while the other compounds showed moderate reductive potential. The lengthening of carbon chain i.e. cinnameldehyde (compound 25) does not favor reducing effect. The

compounds with no substitution (compound 4, 14) or less substitution were showed very less reducing power in comparison to the substituted compounds due to lesser electronegativity [7-12].

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 lengthening of carbon chain may also disfavors the activity, may be due to the lesser electonegativity. The halide and ammonia substitution increases the activity of the compounds, may be due to increased electronegativity.

Figure 3: Reducing Power Assay of Chalconesemicarbazones



REFERENCES

[1] DH Setiadi; GA Chass; LL Torday; A Varro; JG Papp. *J. mol. Struc.*, **2003**, 620, 93-106.
[2] B Halliwell; JMC Gutteridge. Free radicals in biology and medicines, Oxford University Press, Oxford, **1985**.
[3] W Nickels. *European journal of biochemistry*, **2003**, 270, 2109-2119.
[4] S Dutta; S Padhye; KI Priyadarsini; C Newton. *Bioorganic & Medicinal Chemistry Letters*, **2005**, 15, 2738–2744.
[5] M Oyaizu. *Jpn. J. Nutr.*, **1986**, 44, 307-315.
[6] S Khanam; HN Shivaprasada; K Devi. *Ind. J. Pharm. Edu. Res.*, **2004**, 38(4), 180-183.
[7] S Venkateswarlu; MS Ramchandra; AV Krishnaraju; G Trimurtulu; GV Subbaraju. *Indian Journal of Chemistry*, **2005**, 45B, 252-257.
[8] D Singh; S Mohan; PC Sharma; J Sarvanan. *Acta Pharmaceutica Sciencia*, **2007**, 49, 29-38.
[9] DL Flynn; TR Belliotti; AM Boctor; DT Connor; CR Kostlan; DE Nies; DF Ortwine; JD Schrier; JC Sircar. *J. Med. Chem.*, **1991**, 34, 518-525.

[10] K Madhavi; K Bharathi; KVSRG Prasad. *Res. J. Pharm. Biol. Chem. Sci.*, **2010**, 1(4), 1073-1082.
[11] S Rajasekaran; GK Rao; S Pai PN; GS Sodhi. *J. Chem. Pharm. Res.*, **2010**, 2(1), 482-488.
[12] M Allegra; RJ Reiter; DX Tan; C Gentile; L Tesoriere; MA Livrea. *A. J. Pineal Res.*, **2003**, 34, 1–10.