Evaluation of Antioxidant Activity of Coumarin Derivatives Conjugated with Benzophenone

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Abstract - A series of potential biologically active substituted methanone cumarins 5a-j were obtained via a multistep synthesis sequence beginning with the substituted Hydroxy benzophenone 1a–j. All the newly synthesized analogues were characterized by IR, ^{1}H NMR and mass spectral data. The newly synthesized compounds were screened for antioxidant activity and were compared with standard drug ascorbic acid.

Key Words: Methanone, Coumarin, Benzophenone, Antioxidant.

I. INTRODUCTION

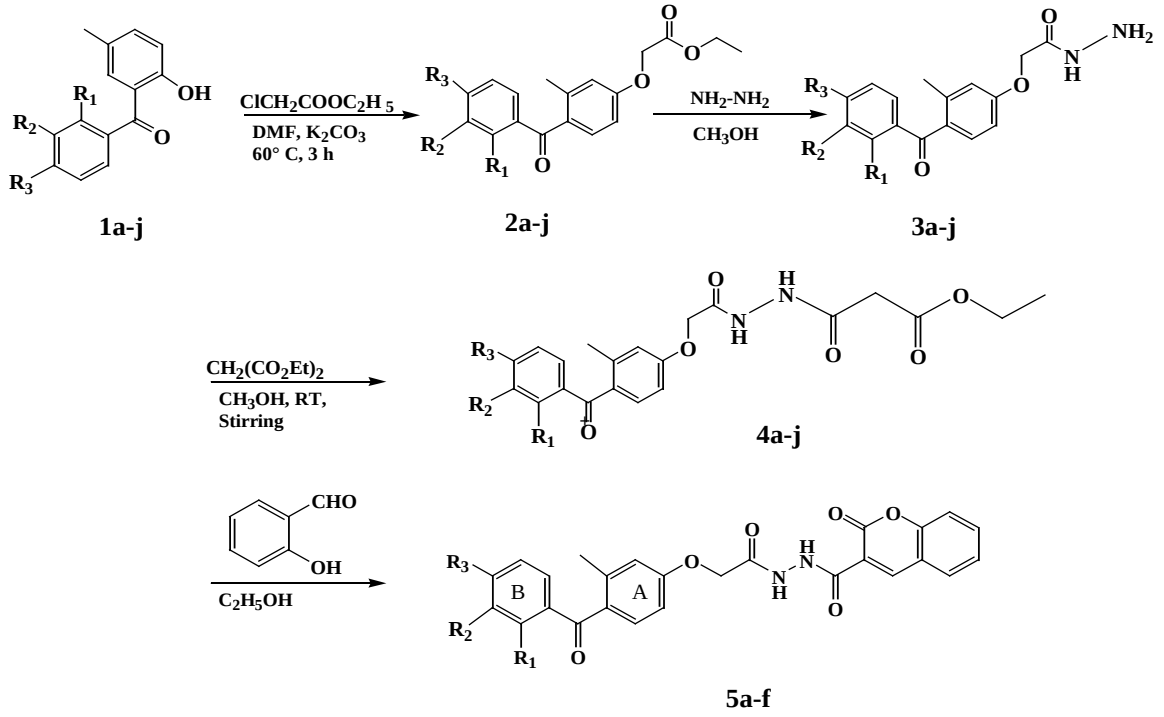
Coumarins are benzopyrone analogues widely distributed in nature.[1] The fused heterocyclic framework of coumarins has served as the prototype scaffold for the synthesis of a wide variety of analogues in order to investigate their biological activity. In the literature, coumarins are reported to possess a remarkable range of biological properties including antioxidant,[2] anticancer,[3] vasorelaxant,[4] antiviral[5] and anti-inflammatory activities.[6, 7] Moreover, several synthetic coumarin derivatives have important pharmacological potential as they proved to be efficient inhibitors of a variety of enzymes such as the human 5-lipoxygenase,[8] aromatase,[9] horseradish peroxidase,[10] hAChE/BACE1[11] and 17β-hydroxy steroid dehydrogenase type 3.[12]The literature survey reveals that benzophenone and its derivatives are an emerging class of molecules with multiple pharmacokinetic properties. New molecules with benzophenone moiety emerging day by day with potent biological activity in recent times.[13-16]Antioxidants are molecules that are capable of inhibiting the oxidation of other molecules and preventing cell death after the release of free radicals. The search for new molecules with antioxidant properties is an excep-tionally active area of research, as such molecules have the potential to reduce the risk of many chronic diseases, including atherosclerosis, stroke, diabetes, Alzheimer's disease, and some forms of cancer. Furthermore, oxidative stress is responsible for DNA, protein and membranedamage.[17] Different types of antioxidants (vitamins C and E, glutathione, lipoic acid, butylated phenols, etc.) are widely used in the chemical and pharmaceutical industries to interrupt radical-chain oxidation processes. Hence, there is great scientific interest in the discovery of efficient synthetic and/or natural antioxidants.[18, 19] The literature investigation reveals that no endeavor was proposed toward the evolution of antioxidant activity of benzophenone-N-ethyl piperidine ether analogues to verify the importance of title compounds on the pharmacological activity. Based on this information and in our search for new molecules with antimicrobial activity, it was considered valuable to synthesize benzophenone-N-ethyl piperidine ether analogues (**5a–j**) as antimicrobial agents as explained below, for a rational study of the structure–activity relationships.

II. RESULT AND DISCUSSION

A. Chemistry

The synthesis of the title compounds **5a-j** is as outlined in Scheme 1. A series of N-[2-(2-aroyl-4-methylphenoxy)-acetyl]-hy- drazide methanone coumarins **5a-j** were obtained starting from hydroxyl benzophenones **1a-j**. Compounds **1a-j** on reaction with ethyl chloroacetate afford ethyl 2-aroyl-4-methylphenoxy acetates **2a-j,** [20] which on treatment with hydrazine hydrate in the presence of ethanol yields 2-aroyl-4-methylphenoxy acetohy- drazides **3a-j.** Condensation of **3a-j** with diethyl malonate in the presence of methanol at room temperature affords {N-[2-(2- aroyl-4-methyl-phenoxy)-acetyl]-hydrazinocarbonyl}-acetic acid ethyl ester **4a-j**. Finally the title compounds **5a-j** were achieved by intramolecular cyclization of **4a-j** with o-hydroxy benzaldehyde in the presence of alcohol. The structures of the compounds were confirmed by IR, NMR and mass spectroscopy. In IR spectra the disappearance of O-C stretching band of ester group and appearance of amide C=O and ring

C=O stretching bands were observed. Besides, the compounds were confirmed by disappearance of $COCH_2$, CH_2 and CH_3 protons and enhancement in the number of aromatic protons in 1H NMR spectra and also by mass spectra and CHN analysis.



a: R_1, R_2=H, R_3=OCH_3; b: R_1, R_3=H, R_2=Cl
c: R_2, R_3 =H, R_1=Br; d: R_1, R_2, R_3=H
e: R_2, R_3 =H, R_1=Cl ; f: R_1, R_2 =H, R_3=Cl
g: R_1, R_3=H, R_2=Br; h: R_1, R_2 =H, R_3=Br
i: R_1, R_2 =H, R_3=F ; j: R_1, R_2 =H, R_3=CH_3

Scheme 1. Synthesis of coumarin conjugated benzophenone analogs.

B. Antioxidant activity

Free radicals play a very important role in the pathogenesis of various human diseases and aging. In food products free radicals also cause damage, resulting in diminish taste and shelf life. Antioxidants are therefore protecting against free radicals and save health. The aim of our study was to explore the most potent antioxidant and examine the factors that give a picture and establish the antioxidant activity with frequent comparison to various thiadiazole and oxadiazole analogues.

All the synthesized compounds were screened for their in vitro antioxidant activity by various methods such as 1,1-diphenylpicrylhydrazyl (DPPH), nitric oxide (NO), hydrogen peroxide (H_2O_2) and LPO assay, methods which were summarized in Tables **1-4** respectively.

The values of IC_{50}, the effective concentration at which 50% of the radicals were scavenged, were tested to evaluate the antioxidant activities. Generally, a lower IC_{50} value demonstrated greater antioxidant activity and IC_{50} values of less than 10 mg/mL usually indicated potent activities in antioxidant properties.

The investigation of antioxidant screening revealed that some of the tested compounds showed moderate to good antioxidant activity. DPPH radical scavenging activity evaluation is a rapid and convenient technique for screening the antioxidant activities of the antioxidants.

Compounds **5a** and **6j** have shown the best antioxidant activity with IC 50 values of 15.02 and 15.14 µg/mL respectively**,** compounds **5d, 5f** and **5e** showed moderate activity while **5i, 5c, 5g** and **5h** showed mild radical scavenging activity as compared to the standard, ascorbic acid by DPPH. Further nitric oxide activity methods have little different scavenging activity. Mainly, compounds **5a** and **5j** have shown the best antioxidant activity with IC_{50} values of 13.85 and14.34 µg/mL respectively**,** while **5d, 5g, 5i and 5f** showed moderate activity. In hydrogen peroxide IC_{50} values of compounds **5a** is 15.86 and **6j** is 16.02 and they have shown the best antioxidant activity. Compounds **5b, 5d and 5e** showed moderate activity as compared to the standard.

Compounds **5a and 5j** have shown the best antioxidant activity with IC 50 values of 16.21 and 17.12 respectively, where as **5d** showed good activity with IC 50 values 18.49, compounds **5h, 5g, 5e** and **5b** showed moderate activity as compared to the standard, ascorbic acid by LPO. In general, compounds **5a** and **5j** with methoxy and methyl group at para position respectively, in ring B have shown highest activity compared to other analogues for all the four methods. Further, Tables **1-4** indicate that remaining compounds showed moderate to mild radical scavenging activity in DPPH, NO, H_2O_2 and LPO methods.

III. EXPERIMENTAL SECTION

1. Material and methods:

Chemicals were procured from Sigma Aldrich Chemical Co. TLC was performed on aluminum-backed silica plates and visualized by UV-light. Melting points were determined on a Thomas Hoover capillary melting point apparatus with a digital thermometer. IR spectra were recorded in Nujol on FT-IR Shimadzu 8300 spectrophotometer, 1H NMR spectra were recorded on a Bruker 400 MHz NMR spectrophotometer in $CDCl_3$ and chemical shift were recorded in parts per million down field from tetramethylsilane. Mass spectra were obtained with a VG70-70H spectrophotometer and important fragments are given with the relative intensities in the brackets. Elemental analysis results are within 0.5% of the calculated value.

2. Chemistry

General procedure for synthesis of ethyl (2-benzoyl-4-methylphenoxy) acetates (2a-j)

A mixture of **1a-j** (0.028 mol) and ethyl chloroacetate (0.028 mol) in dry acetone (70 ml) and anhydrous potassium carbonate (0.056 mol) was refluxed for 7-8 h then cooled and the solvent removed under reduced pressure. The residual mass was triturated with ice water to remove potassium carbonate and extracted with ether (3 × 60 ml) and the ether layer was washed with 10% sodium hydroxide solution (3 × 40 ml) followed by distilled water (3 × 40 ml) and then dried over anhydrous sodium sulfate and evaporated to dryness to get crude solid, which on recrystallization with alcohol gave pure compounds **2a-j** [20].

Ethyl [2-(4-methoxybenzoyl)-4-methylphenoxy] acetate **2a**. Yield 88%; M.p. 58e60 ○C; IR (Nujol): 1660 (C=O), 1730 cm^{-1} (ester, C=O); 1H NMR ($CDCl_3$): 1.2 (t, J = 7 Hz, 3H, CH3 of ester), 2.25 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 4.2 (q, J = 6 Hz, 2H, CH_2 of ester), 4.42 (s, 2H, OCH_2), 7.0 (d, J = 8.3 Hz, 2H, Ar-H), 7.15-7.41 (m, 3H, Ar-H), 7.49 (d, J = 8.5 Hz, 2H, Ar-H); EI-MS: m/z 328 (M^+, 59). Anal. Calcd. for $C_{19}H_{20}O_5$ (328): C, 69.51; H, 6.09. Found: C, 69.49; H, 6.05%.

General procedure for synthesis of 2-(2-benzoyl-4-methylphenoxy) acetohydrazides (3a-j)

Compounds **2a-j** (0.027 mol) were dissolved in alcohol (20 ml) and then 80% hydrazine hydrate (0.027 mol) was added in drops and stirred for 1-2 h at room temperature. A white solid was separated, which was filtered, washed with distilled water (3 × 15 ml) and recrystallized with alcohol. A white solid of **3a-j** was obtained [21].

2-[2-(4-Methoxybenzoyl)-4-methylphenoxy] acetohydrazide **3a**. Yield 70%; M.p.175e177 ^oC; IR (Nujol): 1610 (C=O), 1645 (amide, C=O), 3100-3205 cm^{-1} (NH-NH_2); 1H NMR (CD_3COCD_3): 2.2 (s, 3H, CH_3), 3.5 (bs, 2H, NH_2), 3.9 (s, 3H, OCH_3), 4.55 (s, 2H, OCH_2), 7.0 (d, J = 8.3 Hz, 2H, AreH), 7.2-7.45 (m, 3H, Ar-H), 7.7 (d, J = 8.5 Hz, 2H, Ar-H), 9.4 (bs, 1H, CONH); EI-MS: m/z 314 (M^+, 42). Anal. Calcd. for $C_{17}H_{18}N_2O_4$ (314): C, 64.96; H, 5.73; N, 8.91. Found: C, 64.94; H, 5.70; N, 8.89%.

General procedure for synthesis of N-[2-(2-benzoyl-4-methyl- phenoxy)-acetyl hydrazinocarbonyl]-ethyl acetates (4a-j)

A mixture of **3a-j** (2.2 mmol) and diethyl malonate (2.4 mmol) was refluxed for 4-5 h in methanol (20 ml), cooled and poured into ice-cold water. The solid separated was filtered, dried and recrystallized from alcohol to achieve compounds **4a-j [**22].

*N-{2-[2-(4-methoxy-benzoyl)-4-methyl-phenoxy]-acetyl}hydrazinocarbonyl-ethyl acetate **4a***. Yield 70%; M.p. 230-232 ○C; IR(KBr): 1640 (C=O), 1660 (amide, C=O), 1730 (ester, C=O), 3200-3300 cm^{-1} (NH-NH); 1H NMR ($CDCl_3$): 1.25 (t, J = 7 Hz, 3H, CH_3 of ester), 2.3 (s, 3H, CH_3), 3.14 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 4.18 (q, J = 6 Hz, 2H, CH_2 of ester), 4.7 (s, 2H, OCH2), 6.8-7.7 (m, 7H, Ar-H),m 9.2 (bs, 2H, NH); 13C NMR ($CDCl_3$): 13.6, 20.9, 39.3, 56.0, 59.2, 78.0, 113.7, 113.8, 123.3, 129.7, 130.1, 131.1, 131.8, 133.9, 160.6, 165.7, 170.3, 171.0, 187.0. EI-MS: m/z 428 (M^+, 48). Anal. Calcd. for $C_{22}H_{24}N_2O_7$ (428): C, 61.68; H, 5.6; N, 6.54. Found: C, 61.65; H, 5.4; N, 6.57%.

General procedure for synthesis of N-[2-(2-benzoyl-4-methylphenoxy)-acetyl]-hydrazide methanone coumarins (5a-j)

To a solution of *o*-hydroxy benzaldehyde (2 mmol) in alcohol (20 ml), compounds **4a-j** (1.16 mmol) were added and the mixture was refluxed for 4-5 h in the presence of catalytic amount of acetic acid. The mixture was cooled and poured into ice-cold water, the solid separated was filtered, dried and recrystallized from alcohol to obtain compounds **5a-j.** Comound **5a** taken as a representive example to explain physical and charachterization data**[**22].

N-{2-[2-(4-methoxybenzoyl)-4-methylphenoxy]-acetyl}-hydrazidemethanone coumarin **5a**. Yield 69%; M.p. 200-202 ^{o}C; IR (KBr): 1640 (C=O), 1660 (amide, C=O), 1733 (ring C=O), 3250-3340 cm^{-1} (NH-NH); 1H NMR (CDCl3): □2.3 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 4.7 (s, 2H, OCH_2), 6.9-7.65 (m, 11H, Ar-H), 8.72 (s, 1H, =CH), 9.1 (bs, 2H, NH); 13C NMR (CDCl3): □ 20.9, 59.01, 78.02, 113.71, 113.82, 121.32, 123.31, 124.4, 125.2, 128.1, 129.71, 130.11, 131.1, 131.8, 133.91, 150.8, 151.4, 160.62, 162.0, 165.71, 165.9, 170.3, 187.01. EI-MS: m/z 486 (M^+, 48). Anal. Calcd. for $C_{27}H_{22}N_2O_7$ (486): C, 66.66; H, 4.52; N, 5.76. Found: C, 66.64; H, 4.55; N, 5.96%.

3. Biology:

DPPH radical scavenging activity

The hydrogen atom or electron donation ability of the compounds was measured [23] from the bleaching of the purple colored methanol solution of 1,1-diphenyl-1-picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 mL of various concentrations of the test compounds (25, 50, 75, 100 and 250 mg/mL) in methanol was added to 4 mL of 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. The percent of inhibition (I %) of free radical production from DPPH was calculated by the following equation (1)

% of scavenging = [(A control - A sample)/A blank] ×100 …….(1)

Where A control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. Tests were carried at in triplicate.

Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activity was measured by slightly modified methods of Green et al. and

Marcocci et al., [24] Nitric oxide radicals (NO) were generated from sodium nitroprusside. 1 mL of sodium nitroprusside (10 mM) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50, 75 and 100 mg/mL) of the test compounds and incubated for 150 min at 25 OC and 1 mL of the reaction mixture was treated with 1 mL of Griess reagent (1% sulfanilamide, 2% H3PO4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromatophore was measured at 546 nm. Nitric oxide scavenging activity was calculated using Eq. (1).

Hydrogen peroxide (H_2O_2) scavenging activity

The H_2O_2 scavenging ability of the test compound was determined according to the method of Ruch et al., [25] A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 25, 50, 75 and 100 mg/mL concentrations of the test compounds in 3.4 mL phosphate buffer were added to H_2O_2 solution (0.6 mL, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. The percent of scavenging of H_2O_2 was calculated using Eq. (1).

Lipid peroxidation assay

LPO inhibitory activity of compounds **5a-j** was measured according to Kulkarni et al. [26] Egg lecithin (3 mg/mL in phosphate buffer, pH 7.4) was sonicated (Hielscher GmbH UP 50H

ultra-challprocessor sonicator) for 30 min to obtain small membrane liposome vesicles. Different concentrations of the compounds were added to 0.5mL of liposome mixture. LPO was induced by adding 10mL of 400mM FeCl3 and 10mL of 200mL ascorbic acid. After 60 min of reaction at 37°C, the reaction was stopped by the addition of 1mL of 0.25N HCl containing 15% thiazolidine-4-carboxylic acid (TCA) and 0.375% 2,4,6-tribromoanisole (TBA) and incubation in a boiling water bath for 15 min. After centrifugation at 10,000 rpm, absorbance of the supernatant was measured at 532 nm. The scavenging effect was calculated using the equation as described for DPPH.

IV. CONCLUSION

From the results of the present study, it is concluded that, a series of novel biologically active substituted coumarin analogs **5a-j** were synthesized and screened for antioxidant activity. Compounds **5a** and **5j** with methoxy and methyl group at para position respectively, in ring B have shown highest radical scavenging activity as compared to the standard, ascorbic acid by DPPH, NO, H_2O_2 and LPO methods

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Tables

Table 1 In-vitro antioxidant activity of compounds 5a-j in DPPH method

Compounds	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
5a	73.65±	76.	80.	82.86	15.02
5b	51.53 ± 0.81	54.63 ± 1.32	57.76 ± 0.72	62.71 ± 1.01	21.44 ± 0.36
5c	63.75 ± 1.05	68.84 ±1.40	72.84± 0.84	76.82 ± 1.64	19.53 ± 1.08
5d	67. 86	72.89	74.88	78.71	16.02
5e	61.83 ± 1.38	65.82 ± 1.14	70.81± 1.50	74.62 ± 0.88	17.72 ± 1.08
5f	70.72±	73.81	75.	80.84±	16.22
5g	60.61 ± 1.08	63.73 ± 1.29	67.82 ± 1.58	70.53 ± 0.87	20.34 ± 0.55
5h	51.78	57.82±	60.84±	64.98	22.67
5i	67. 88	71.89	73.89±	78.92	18.32
5j	70. 83 ± 0.12	74.76 ± 0.37	76.89 ± 0.40	80.55± 0.61	15.14 ± 0.53
Ascorbic acid	80.75± 0.07	81.76± 0.32	83.85± 0.37	85.76± 0.44	14.32± 0.40
Blank	-	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ± SD.

Table 2 The in vitro antioxidant activity of compounds 5a-j in nitric oxide (NO) method.

Compounds	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
5a	74.96 ± 0.21	80.81 ± 0.40	80.88 ± 0.52	82.93± 0.71	13.85 ± 0.70
5b	66.75 ± 0.92	68.91 ± 1.38	72.65 ± 0.97	77.87 ± 1.25	19.12 ± 1.08
5c	67.48 ± 0.93	70.44 ± 1.39	74.52 ± 0.98	78.25 ± 1.26	19.58 ± 1.09
5d	70.75 ± 0.20	75.93 ± 0.37	79.85± 0.58	81.96 ± 0.70	15.32 ±0.80
5e	63.82 ± 1.34	70.79 ± 1.26	75.84 ±1.07	78.47± 0.79	18.98± 1.14
5f	58.99± 1.30	64.88± 1.56	68.96± 0.70	71.96± 1.04	16.94 ± 0.82
5g	68.31 ± 0.80	71.24 ± 1.01	74.87± 1.32	78.73 ± 1.20	15.37 ± 0.88
5h	61.85 ± 1.41	65.89 ± 1.58	70.26 ± 0.78	72.93 ± 1.11	20.31 ± 0.98
5i	68.66 ±0.10	73.93± 0.26	78.87 ±0.44	80.93 ± 0.61	15.68 ± 0.83
5j	72.91	76.86	80.21	81.86±	14.34±
	± 0.20	± 0.30	± 0.50	0.66	0.50
Ascorbic acid	82.87± 0.14	83.85± 0.31	86.86± 0.46	88. 86±0.74	13.32± 0.50
Blank	-	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ± SD.

Table 3: The in vitro antioxidant activity of compounds **5a-j** in hydrogen peroxide (H_2O_2) method.

Compounds	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
5a	61.88± 1.01	63.98± 1.20	66.82± 0.50	70.17± 0.60	15.86± 0.48
5b	61.55 ± 0.20	64.57 ± 0.57	66.92 ± 0.60	69.71 ± 0.70	18.12 ± 0.21
5c	52.87± 1.18	57.73 ± 0.98	61.87 ± 1.48	65.82 ± 0.86	22.11± 0.68
5d	59.79 ±1.27	62.13 ± 1.12	65.79± 1.01	70.92 ± 1.50	17.82± 0.70
5e	59.21 ± 0.81	60.92 ± 1.51	65.73 ± 1.01	68.85 ± 1.31	19.12 ± 1.17
5f	55.86 ± 1. 04	60.12± 1.11	62.85± 1.40	66.55 ± 1.47	20.04 ± 1.01
5g	59.81 ± 1.07	62.82 ± 1.28	66.91 ± 1.57	69.89 ± 0.86	20.21 ± 0.54
5h	53.92± 1.19	58.73 ± 0.89	62.88 ± 1.49	66.63 ± 0.87	22.82± 0.69
5i	50.79 ± 0.80	53.92 ± 1.31	56.81 ± 0.71	61.94 ± 1.01	21.16 ± 0.35
5j	64.84 ± 0.20	67.12 ± 0.40	70.84 ± 0.51	75.77 ± 0.74	16.02 ± 0.90
Ascorbic acid	75.29± 0.15	77.12± 0.27	81.11± 0.58	85.03± 0.64	15.13± 0.21
Blank	-	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ± SD.

Table 4: The in vitro antioxidant activity of compounds **5a-j** in lipid peroxidation method.

Compounds	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
5a	70.56 ± 0.85	72.86 ± 1.04	75.86± 1.38	80.81 ± 1.24	16.21 ± 0.91
5b	60.81± 1.52	61.93 ± 1.21	67.89 ± 1.31	71.85± 0.71	20.29 ± 0.94
5c	62.48± 1.53	63.21 ± 1.22	69.89 ± 1.32	74.77± 0.72	21.24 ± 0.93

5d	59.84± 1.27	62. 83± 1.14	66.64± 1.04	69.97± 1.52	18.49± 0.71
5e	50.91 ± 0.86	53.83± 1.36	56.93± 0.75	61.89 ± 1.02	20.12 ± 0.40
5f	52.81 ± 0.87	55.71 ± 1.37	58.72± 0.76	63.63 ± 1.03	22.72 ± 0.40
5g	55.71± 1.09	61.72± 1.18	66.74± 0.85	70.45± 0.92	19.64± 0.73
5h	54.89± 1.08	60.82± 1.17	65.83± 0.84	69.87± 0.91	19.22± 0.71
5i	52.81 ± 0.87	55.71 ± 1.37	58.72± 0.76	63.63 ± 1.03	22.74 ± 0.40
5j	61.75± 1.14	66.34± 1.52	71.97± 1.36	76.55± 0.68	17.12± 1.12
Ascorbic acid	77.91± 0.18	78.87± 0.28	82.92± 0.62	86.92± 0.68	15.15± 0.26
Blank	-	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ±

SD