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Original article

Synthesis and antioxidant properties of some novel 5H-dibenz[b,f]azepine derivatives in different in vitro model systems

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ABSTRACT

A series of 5H-dibenz[b,f]azepine containing different aminophenols and substituted aminophenols were synthesized. 3-chloro-1-(5H-dibenz[b,f]azepine-5yl)propan-1-one (**2**) was obtained by N-acylation of 5H-dibenz[b,f]azepine (**1**) with 3-chloro propionyl chloride. Further base condensation with different aminophenols and substituted aminophenols to produce series of 5H-dibenz[b,f]azepine containing aminophenol and substituted aminophenol (**2a–e**). The structures of newly synthesized compounds were characterized by spectral and elemental analysis. Their antioxidant properties were evaluated by using several methods: scavenging effects on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, inhibition of lipid peroxidation using β-carotene linoleate system, inhibition of human low-density lipoprotein (LDL) oxidation and reducing power. Butylated Hydroxy Anisole (**BHA**) and Ascorbic acid (**AA**) were used as the reference antioxidant compounds and also the comparative study with the synthesized compounds was done. Under our experimental conditions, Compound (**2**) showed negligible activity over all the antioxidant assays but 5H-dibenz[b,f]azepine containing different aminophenols and substituted aminophenols (**2a–e**) showed good antioxidant activities over all the methods and compounds containing substituted aminophenols **2e** and **2d** showed predominant antioxidant activities among the synthesized analogues.

1. Introduction

Antioxidants are chemical compounds that can quench reactive radical intermediates formed during the oxidative reactions. The primary antioxidants comprise essentially sterically hindered phenols and secondary aromatic amines [1,3]. These antioxidants act usually both through chaintransfer and chain termination [1]. The first step of the reactive radicals termination by this type of antioxidants is hydrogen atom transfer from the antioxidant molecule to the reactive radical intermediate [1,2]. Small amounts of antioxidants are added into most synthetic polymers to prevent or retard oxidation and to increase the service lifetimes of the products [1,2,4,5]. Free radicals and active oxygen species have been related with cardiovascular and inflammatory diseases, and even with a role in cancer and ageing [6,7]. Efforts to counteract the damage caused by these species are gaining acceptance as a basis for novel therapeutic approaches and the field of preventive medicine is experiencing an upsurge of interest in medically useful antioxidants [8,9]. Food oxidation is one of the main causes of food deterioration, especially in food items with a high lipid fraction. Antioxidants have been added to food for years to prevent this process and are widely used today for better food preservation.

Phenolic derivatives are one of the groups of antioxidants that have been studied by many research groups. A great number of examples have been described in the literature, such as caffeic acid and its analogues, which are known to have antiviral, anti-inflammatory and antiatherosclerotic properties [10], resveratrol with known anticancer and heart protecting effects [11] and olive oil phenols, particularly hydroxyl tyrosol, which inhibits human low-density lipoprotein (LDL) oxidation (a critical step in atherosclerosis) [12] inhibits platelet aggregation [13] and exhibits anti-inflammatory [14] and anticancer properties [15]. Phenols have been utilized extensively for food preservation. Synthetic phenolic antioxidants, such as Butylated hydroxyl toluene (BHT), Butylated hydroxy anisole (BHA) or tertiary butylated hydroxyl quinine (TBHQ) posses good antioxidant capacity but have been questioned due to possible side effects for human health [16]. The main structural feature responsible for the antioxidative and free radical scavenging activity of phenolic derivatives is the phenolic OH-group. Phenols are able to donate the hydrogen atom of the phenolic OH to the free radicals, thus stopping the propagation chain during the oxidation process.

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The research on free radicals provides theoretical information for the medicinal development, and supplies some in vitro methods for quick-optimizing drugs, it attracts more scientific attention from bioorganic and medicinal chemists. In addition to the traditional O–H bond type antioxidant, tricyclic amines having N–H bond functions as the antioxidant have attracted much research attention because Ar_2NHs have always been the central structure in many currently used drugs [17].

In the literature some tricyclic amines and their chemical structure shows antioxidant neuroprotective activity in vitro [18]. Nowaday, the free radical scavenging mechanism of aromatic amines (Ar_2NHs) has been discussed from the view of chemical kinetics [19]. Basic molecule 5H-dibenz[b,f]azepine i.e., iminostilbene (**1**) is common basic fused tricyclic amine. It is used as an intermediate for the synthesis of the registered anticonvulsant drug oxcarbazepine [20], the structure of which has recently been reported [21]. Dibenz[b,f]azepine and its derivatives has been variously reported as having antiallergic activity, specifically antihistaminic activity, spansmolytic, serotonin antagonistic, anticonvulsive, antiemetic, antiepileptic, anti-inflammatory, sedative and fungicidal action [22]. From the literature, usually phenolic compounds were found to have antioxidant and radical scavenging activity, they also inhibit LDL oxidation [23,24].

On the other hand aminophenols are also showed dominant in vitro antioxidant activities over various assays [25].

3-chloro-1-(5H-dibenz[b,f]azepine-5yl)propan-1-one belonging to one of the analogues of 5H-dibez[b,f]azepine and their structure may justify the possibilities of coupling different aminophenols and substituted aminophenols, it has taken as a model compound in this study.

In order to establish some structure–activity relationship based on the position and presence of different aminophenols and substituted aminophenols on 5H-dibenz[b,f]azepine and to understand how the different functionalities affects the antioxidant activities the present study was taken. Herein, we reported the synthesis of 5H-dibenz[b,f]azepine containing several aminophenols and substituted aminophenols obtained by base condensation using potassium carbonate and evaluation of their antioxidant properties by various methods namely DPPH free radical scavenging activity, inhibition of lipid peroxidation in β-carotene linoleate system, inhibition of human low-density lipoprotein (LDL) oxidation and reducing power assay. These studies may reflect the possibility for therapeutic uses and as a source of synthetic antioxidants.

2. Chemistry

In the present work, the basic molecule 5H-dibenz[b,f]azepine (**1**) was obtained according to the literature method [20]. The model compound, 3-chloro-1-(5H-dibenz[b,f]azepine-5-yl)propan-1-one (**2**) was prepared from N-acylation of 5H-dibenz[b,f]azepine with 3-chloro propionyl chloride in the presence of triethyl amine as base. Further coupling of three different aminophenols (4-aminophenol, 2-aminophenol, 3-aminophenol) and two different substituted aminophenols (4-amino-2-methoxyphenol and 4-amino-2-nitrophenol) by base condensation to obtain series of aminophenol and substituted aminophenol analogues of 5H-dibenz[b,f]azepine (**2a**–**e**) in moderate to high yield. The reaction sequences are outlined in Schemes 1 and 2.

3. Results, discussion and conclusion

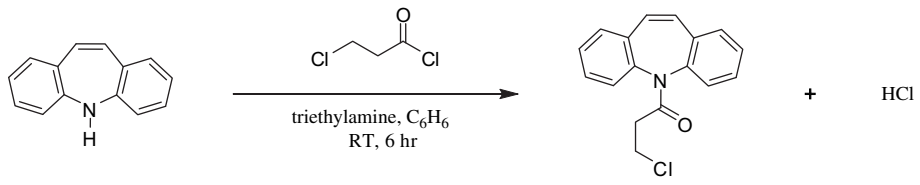
In this present work, a series of six new compounds were synthesized. Schemes 1 and 2 illustrate the way used for the preparation of target molecules. As a starting material 5H-debenz[b,f]azepine (**1**) was used to produce 3-chloro-1-(5H-dibenz[b,f]azepine-5yl)propan-1-one (**2**), a model compound. Further this model compound is used to synthesize series of three aminophenol and two substituted aminophenol derivatives (**2a**–**e**). Structural conformation was done using IR, ^{1}H NMR, mass spectra and elemental analysis. The IR spectrum of compound (**2**) showed characteristic absorption at 1675.3 cm^{-1} for carbonyl group and showed two $-CH_2$ stretching bands at 2971.0–3026.1 cm^{-1}. The ^{1}H NMR spectrum showed two characteristic peak of $-CH_2$ at 2.8 and 3.7 ppm, the absent of N–H peak was observed at 2.0 ppm conforming the N-acylation of 5H-dibenz[b,f]azepine (**1**). The IR spectrum of three aminophenol and two substituted aminophenol analogues of 3-chloro-1-(5H-dibenz[b,f]azepine-5yl)propan-1-one (**2a**–**e**) revealed the presence of N–H stretching band at 3300–3360 cm^{-1} in all the analogues and showed broad phenolic stretching at 3200–3500 cm^{-1}. The Ar–H and C═O absorption band was appeared at the expected regions. ^{1}H NMR spectra of all aminophenol and substituted aminophenol analogues showed N–H proton as singlet at 5.82–8.02 ppm. The signal due to phenolic OH in all the analogues appeared as singlet at about 9.4–10 ppm. In addition to phenolic OH, $-OCH_3$ protons present in the compounds **2e** resonated as a triplet at 3.8 ppm. Other aromatic protons were observed at expected regions. Mass spectra of all newly synthesized compounds showed M^+ peak, in agreement with their molecular formula.

4. Antioxidant evaluation

The antioxidant activities of 3-chloro-1-(5H-dibenz[b,f]azepine-5yl)propan-1-one (**2**) bearing different aminophenols like 4-aminophenol **2a**, 2-aminophenol **2b**, 3-aminophenol **2c** and substituted aminophenols like 4-amino-2-nitrophenol **2d** and 4-amino-2-methoxyphenol **2e** was evaluated by several in vitro methods in order to compare the results and to establish some structure–antioxidant–activity relationships for each method. The evaluation study was carried out at various concentrations and also comparative studies were done with the standard antioxidants.

DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical scavenging activity (RSA) evaluation is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical scavenging activity of specific compounds or extracts [30]. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades/disappears when an antioxidant is present in the medium. Thus, antioxidant molecule can quench DPPH free radical (i.e., by providing hydrogen atoms or by electron donating, conceivable) and convert them to a colorless/bleached product (i.e., 2,2-diphenyl-1-picrylhydrazine, or a substituted analogues hydrazine), resulting in a decrease in absorbance. Hence, more rapidly the absorbance decrease, the more potent the antioxidant activity of the compound.

Percentage activity of ethanolic solutions of 3-chloro-1-(5H-dibenz[b,f]azepine-5yl)propan-1-one (**2**) and 5H-dibenz[b,f]-azepine containing different aminophenols and substituted aminophenols (**2a**–**e**) were examined and compared (Fig. 1). Initially, compound (**2**) showed negligible DPPH activity, further coupling of different aminophenols and substituted aminophenols gives the significant enhancement in the activity. From Fig. 1 we can conclude that the coupling of different aminophenols and substituted aminophenols to 5H-dibenz[b,f]azepine (**2**) will increase the DPPH activity. All the compounds (**2a**–**e**) showed comparable or slight less activity to the standards (Ascorbic acid and BHA). The compound **2e** bearing a methoxy group (electron donating group) at para position showed dominate DPPH activity. The presence of Nitro group (electron withdrawing group) **2d** instead of a methoxy in the same positions exhibit slightly less to that of compound **2e**. IC_{50} for all the compounds were calculated.



(1) (2)

Scheme 1. Reaction protocol for the synthesis of 3-chloro-1-(5H-dibenz[b,f]azepine-5yl)propan-1-one (**2**).

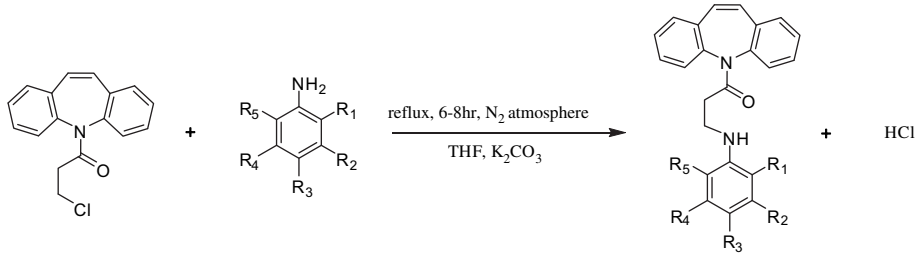
Table 1 reveals the 50% inhibitory concentration towards DPPH activity of newly synthesized compounds. Initially 3-chloro-1-(5H-dibenz[b,f]azepine-5yl)propan-1-one (**2**) showed negligible activity towards DPPH but further coupling of different aminophenols and substituted aminophenols enhance the DPPH activity by showing significant activity.

Coupling of 4-aminophenol showed good RSA values followed by 2-aminophenol and 3-aminophenol respectively. On the other hand coupling of substituted aminophenols containing nitro and methoxy group showed significant activity which is comparable but slightly less than the standards.

The antioxidant activity of carotenoid is based on its radical adducts with free radicals from linoleic acid. The linoleic acid free radical attacks the highly unsaturated β-carotene model. The presence of antioxidants can decrease the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system [31]. Accordingly, the absorbance decreases rapidly in the samples without antioxidant. Whereas in presence of antioxidant, they retain the colour for a longer time. The extent of β-carotene bleaching by newly synthesized compound (**2**) and (**2a**–**e**) at different concentrations (25 μM and 50 μM) is showed in Fig. 2. From Fig. 2, the extent of the bleaching of β-carotene in the presence of antioxidants (**2a**–**e**) is very less showing good activity. Whereas, in the presence of compound (**2**) the extent of bleaching of β-carotene was more showing less activity among the synthesized compounds.

The percentage (%) antioxidant activity of newly synthesized compounds under study was showed in Fig. 3. Among the compounds, substituted aminophenol analogues **2e** and **2d** showed excellent activity (91.5% and 88.36%) followed by aminophenol analogues **2a** (87.07%) at 25 μM and (91.3% and 89.95%) for **2e** and **2d** followed by **2a** (88.72%) at 50 μM concentration. The antioxidant activity of all compounds presents comparable or slightly less value than the standards, ascorbic acid and BHA. Ultimately, the coupling of different aminophenols and substituted aminophenols to 5H-dibenz[b,f]azepine (**2**) will enhance the lipid peroxidation inhibition.

Fig. 4 shows the reducing power of 3-chloro-1-(5H-dibenz[b,-f]azepine-5yl)propan-1-one (**2**) and 5H-dibenz[b,f]azepine analogues containing different aminophenols and substituted aminophenols (**2a**–**e**) examined as a function of their concentration. In this assay, the yellow colour of the test solution change to various shades of green and blue depending upon the reducing power of each compound. The presence of reducers (i.e., antioxidants) causes the reaction of the Fe^{3+}/ferricyanide complex to the ferrous form giving, after the addition of trichloroacetic acid and Ferric chloride, the Perl's Prussion blue that can be monitored at 700 nm. The reducing power of the standards BHA and ascorbic acid at various concentrations showed higher absorbance value to that of newly synthesized compounds. The reducing power of newly synthesized compound solutions in ethanol increases with increase in concentration. All the analogues (**2a**–**e**) showed higher



(2a-e)

Where,

Compound	R_1	R_2	R_3	R_4	R_5
2a	H	H	OH	H	H
2b	OH	H	H	H	H
2c	H	OH	H	H	H
2d	H	H	OH	NO_2	H
2e	H	H	OH	OCH_3	H

Scheme 2. Reaction sequence for the synthesis of 5H-dibenz[b,f]azepine analogues (**2a-e**).

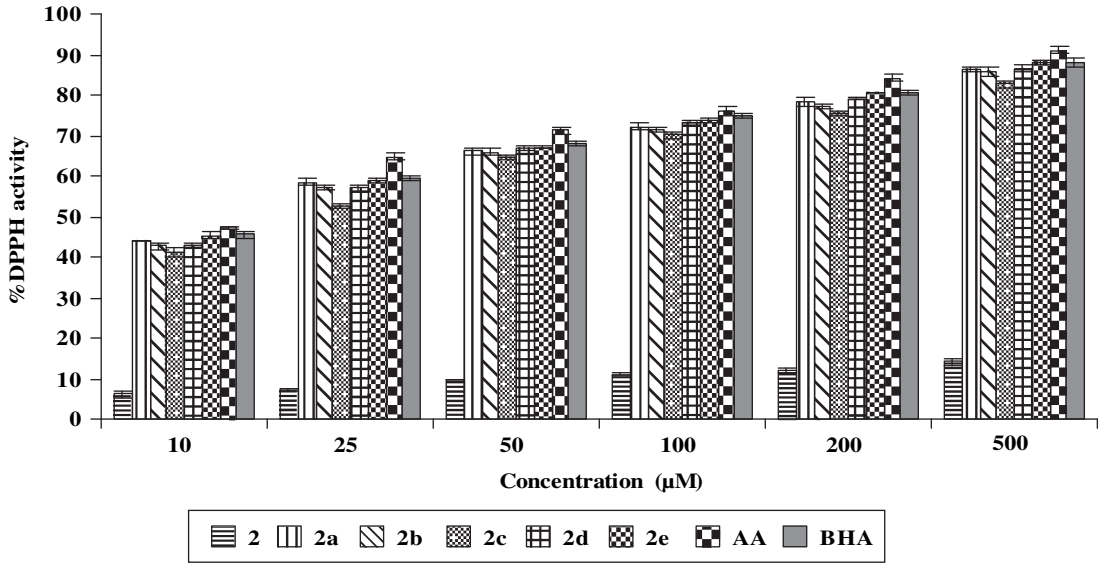


Fig. 1. Percentage (%) scavenging effect on DPPH by 5H-dibenz[b,f]azepine analogues. Each value represents means ± SD ($n = 3$).

absorbance which is comparable or slightly less than the standards. The presence of substituted aminophenols i.e., methoxy group (electron donating group) in para position to the N–H i.e., **2e** was much better than a nitro group (electron withdrawing group) in the same position i.e., **2d** giving **2e** higher reducing power. The absorbance values for compound (**2**) was very less showing low reducing power, while for compounds (**2a–e**) showed higher values possessing higher reducing power but lower than the standards. The coupling of 4-amino-2-methoxyphenol showed much better activity than the 4-amino-2-nitrophenol. Thus coupling of various aminophenols and substituted aminophenols to 5H-dibenz[b,f]azepine is the important features for the good results of reducing power of these compounds.

Oxidation modification is known to play an important role in the pathogenesis of atherosclerosis and coronary heart diseases [32] and the dietary antioxidants that protect LDL from oxidation may therefore reduce atherogenesis and coronary heart diseases [33]. In general, oxidation of LDL follows a radical chain reaction that generates conjugated diene hydroperoxides as its initial products. It has been reported that inhibition of human LDL oxidation may arise due to free radical scavenging and/or metal ion chelation [34]. Phenolic compounds were found to have antioxidant activity in the inhibition of LDL oxidation [24,35]. The antioxidant activity of 5H-dibenz[b,f]azepine containing aminophenols (**2a–e**) against human LDL oxidation with different concentrations (10 μM and 25 μM) is shown in Fig. 5.

The poly unsaturated fatty acid (PUFA) of human LDL were oxidized, and the malonoldehyde (MDA) formed have been estimated by using thiobarbituric acid (TBA) method. Initially the formation of conjugated dienes due to copper-induced LDL oxidation was unaffected by compound (**2**) showing less activity but coupling of aminophenols and substituted aminophenols i.e., compound (**2a–e**) effectively inhibited LDL oxidation showed good activity. The average induction time for copper mediated LDL oxidation was around 20 min without the addition of compounds. The compounds protected LDL from oxidation as measured by the prolongation of the induction time of the formation of conjugated dienes. Among the synthesized compounds, **2e** exhibited 56.13 and 70.41% protection at the 10 and 25 μM levels at the end of 2 h after the induction of oxidation. Whereas, it was 72.93 and 89.61% protection at the end of 6 h showing dominant inhibition over LDL oxidation and also comparable or slightly less protection with respect to the standards, ascorbic acid and BHA. Compound (**2**) exhibited 5.34 and 7.43% protection at the 10 and 25 μM levels at the end of 2 h after the induction of oxidation. Whereas, it showed 8.87 and 12.43% protection at the end of 6 h showing less activity. The results indicate a dose-dependent inhibition effect of compounds against LDL oxidation.

Table 1
50% Inhibition of DPPH radical by 5H-dibenz[b,f]azepine analogues. Where – corresponds to no significant 50% inhibition. Each value represents means ± SD ($n = 3$).

Compound name	IC_{50} (μM/mL)
Compound (**2**)	–
Compound **2a**	15.3 ± 0.22
Compound **2b**	16.1 ± 0.31
Compound **2c**	17.4 ± 0.11
Compound **2d**	14.8 ± 0.14
Compound **2e**	13.9 ± 0.18
AA	13.2 ± 0.10
BHA	14.1 ± 0.21

In conclusion, 5H-dibenz[b,f]azepine containing different aminophenols and substituted aminophenols were prepared by base condensation in moderate to high yields. The antioxidant properties of the new analogues obtained were evaluated by several methods. Initially 3-chloro-1-(5H-dibenz[b,f]azepine-5yl)propan-1-one (**2**) showed no significant activity over DPPH, reducing power, lipid peroxidation inhibition and human LDL oxidation inhibition. Coupling of aminophenols and substituted aminophenols to 5H-dibenz[b,f]azepine (**2a–e**) enhance the antioxidant activities which are comparable and slightly less than the standards, but the coupling of two substituted aminophenols containing nitro compound **2d** and methoxylated compound **2e** revealed high RSA, reducing power, lipid peroxidation inhibition and human LDL oxidation inhibition values. The coupling of different aminophenol and substituted aminophenols is the most important feature for the significant antioxidant activities of the 5H-dibenz[b,f]azepine analogues studied. As a result, our study provides evidence that the coupling of different aminophenols and substituted aminophenols to 5H-dibenz[b,f]azepine had significant influence for the antioxidant activities in different in vitro model system. These effects may be useful in the treatment of pathologies in which free radical oxidation plays a fundamental role.

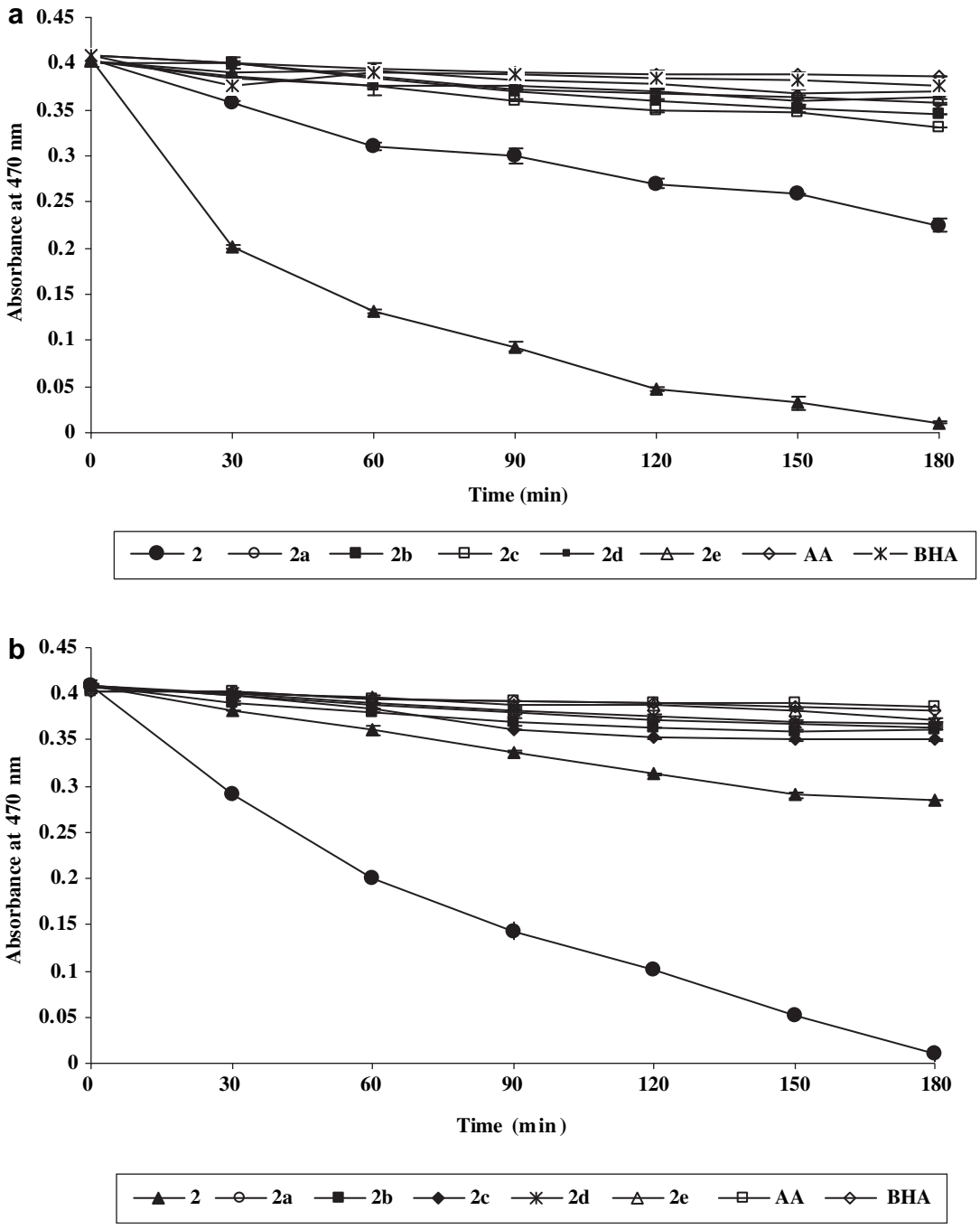


Fig. 2. Bleaching of β-carotene with respect to time in the presence of 5H-dibenz[b,f]azepine analogues at different concentrations ($a = 25$ μM and $b = 50$ μM). Each value represents the mean ± SD ($n = 3$).

5. Experimental protocols

All the reagents were used as purchased from commercial suppliers without further purification. Melting points were determined by using an open capillary method and are uncorrected. Thin layer chromatography (TLC) was performed with aluminium sheets -Silica gel 60 F_{254} purchased from Merck. The compounds were purified by using column chromatography with silica gel (60–120 mesh), using chloroform:methanol = 80:20 as eluent. IR: Shimadzu IR-435 spectrophotometer; ^{1}H NMR: Bruker 250 MHz spectrometer; mass spectra were obtained by Waters-Q-TOF ultima spectrometer. Micro analytical data were obtained by Elementar–Vario EL–III.

5.1. Chemistry

*5.1.1. General procedure for the synthesis of 3-chloro-1-(5H-dibenz-[b,f]azepine-5yl) propan-1-one (Compound **2**)*

To the well stirred solution of 5H-dibenz[b,f]azepine (2 mM) and triethyl amine (2.2 mM) in 50 mL benzene, 3-Chloro propionyl chloride (2.2 mM) in 25 mL benzene was added drop by drop for about 30 min. Then the reaction mixture is stirred at room temperature for about 6 h. Progress of the reaction is monitored by TLC using 9:1 Hexane:Ethyl acetate mixture as mobile phase. After the completion of reaction, the reaction mass was quenched in ice cold water and extracted in diethyl ether. The ether layer was

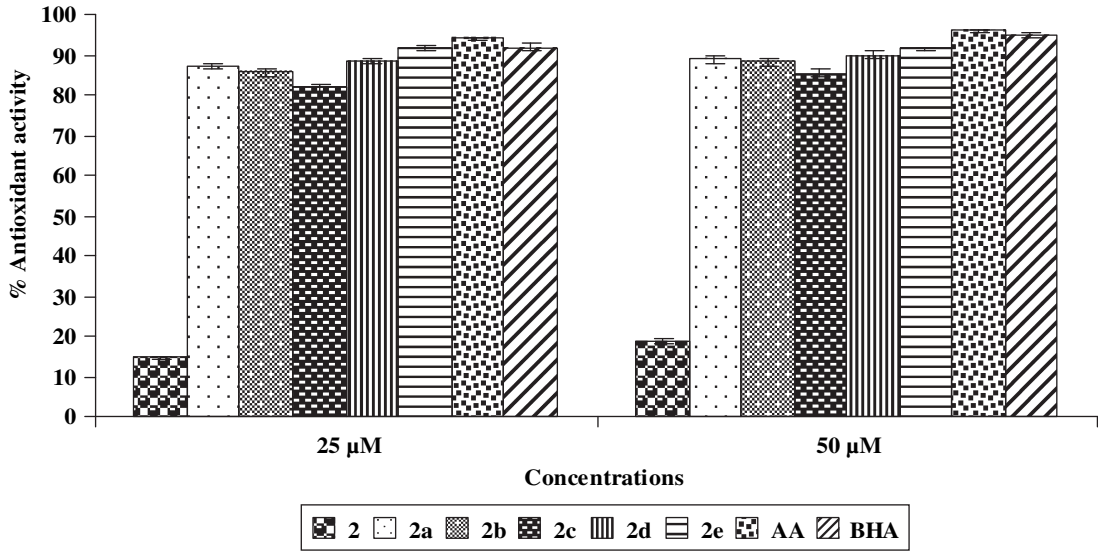


Fig. 3. % Antioxidant activity of 5H-dibenz[b,f]azepine analogues by β-carotene-linoleic acid model. Each value represents the mean ± SD ($n = 3$).

washed twice with 5% $NaHCO_3$ and once with distilled water. Finally the ether layer is dried with anhydrous Na_2SO_4. The light yellow solid product was obtained by desolventation through rotary evaporator at <50 °C.

Light yellow solid, Yield (85%), M.p. 109–110 °C. IR (KBr) ν_{max} (cm^{-1}): 3067.9 (Ar C–H), 1675.3 (C=O), 2971.0–3026.1 (CH_2); ^1H NMR (250 mHz) ($CDCl_3$) δ (ppm): δ 2.8 (d, 2H, CH_2–C=O), 3.7 (d, 2H, CH_2Cl), 7.5–7.6 (m, 8H, Ar–H), 6.9 (d, 2H, Ar–H of seven membered ring); Mass (*m/z* %): M^+ 284.56 (100.0%), 285.07 (32.0%), 284.08 (18.6%), 286.08 (6.0%), 285.08 (1.9%); Anal. Calcd. for $C_{17}H_{14}ClNO$: C, 71.96; H, 4.97; Cl, 12.49; N, 4.94; O, 5.64%; Found: C, 71.20; H, 4.88; Cl, 12.26; N, 4.79; O, 5.52%.

*5.1.2. Synthesis of 1-(5H-dibenz[b,f]azepin-5-yl)-3-(4-hydroxyphenylamino)propan-1-one (Compound **2a**)*

p-Aminophenol (1.2 mM) in tetrahydrofuron (THF, 25 mL) was completely solubalized with triethylamine under inert (N_2) atmosphere. To this, K_2CO_3 (600 mg) was added. Later the solution of 3-Chloro-1-(5H-dibenz[b,f]azepine-5-yl) propan-1-one (1 mM) THF (50 mL) was added drop by drop for 30 min. The reaction mixture was refluxed for 6–8 h. The progress of the reaction mixture was monitored by TLC. The reaction mixture was then desolventized in rotavapour and the compound is extracted in ethyl acetate. The ethyl acetate layer was washed with water and dried over anhydrous Na_2SO_4. The light brown solid was obtained by further desolventation in rotary evaporator at 50 °C.

o-Aminophenol, m-aminophenol, methoxy aminophenol, nitro aminophenol analogues of 3-chloro-1-(5H-dibenz[b,f]azepine-5yl)propan-1-one were obtained by following the same procedure. The products were separated and purified by column chromatography, using mixture of chloroform:methanol = 80:20.

Light brownish solid, Yield (85%), M.p. 127–129 °C. IR (KBr) ν_{max} (cm^{-1}): 3053–2829.5 (Ar C–H), 1670.9 (C=O), 3234.5 (N–H), 3222.4–3500.8 (phenolic-OH), 2971.0–3026.1 (CH_2); ^1H NMR

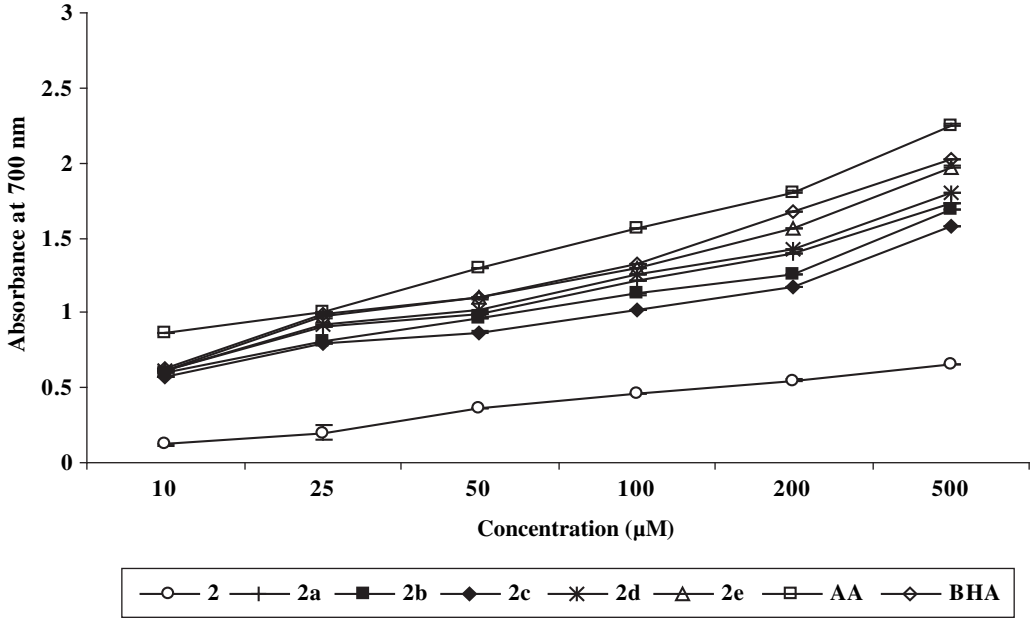


Fig. 4. Reducing power of 5H-dibenz[b,f]azepine analogues. Each value represents the mean ± SD ($n = 3$).

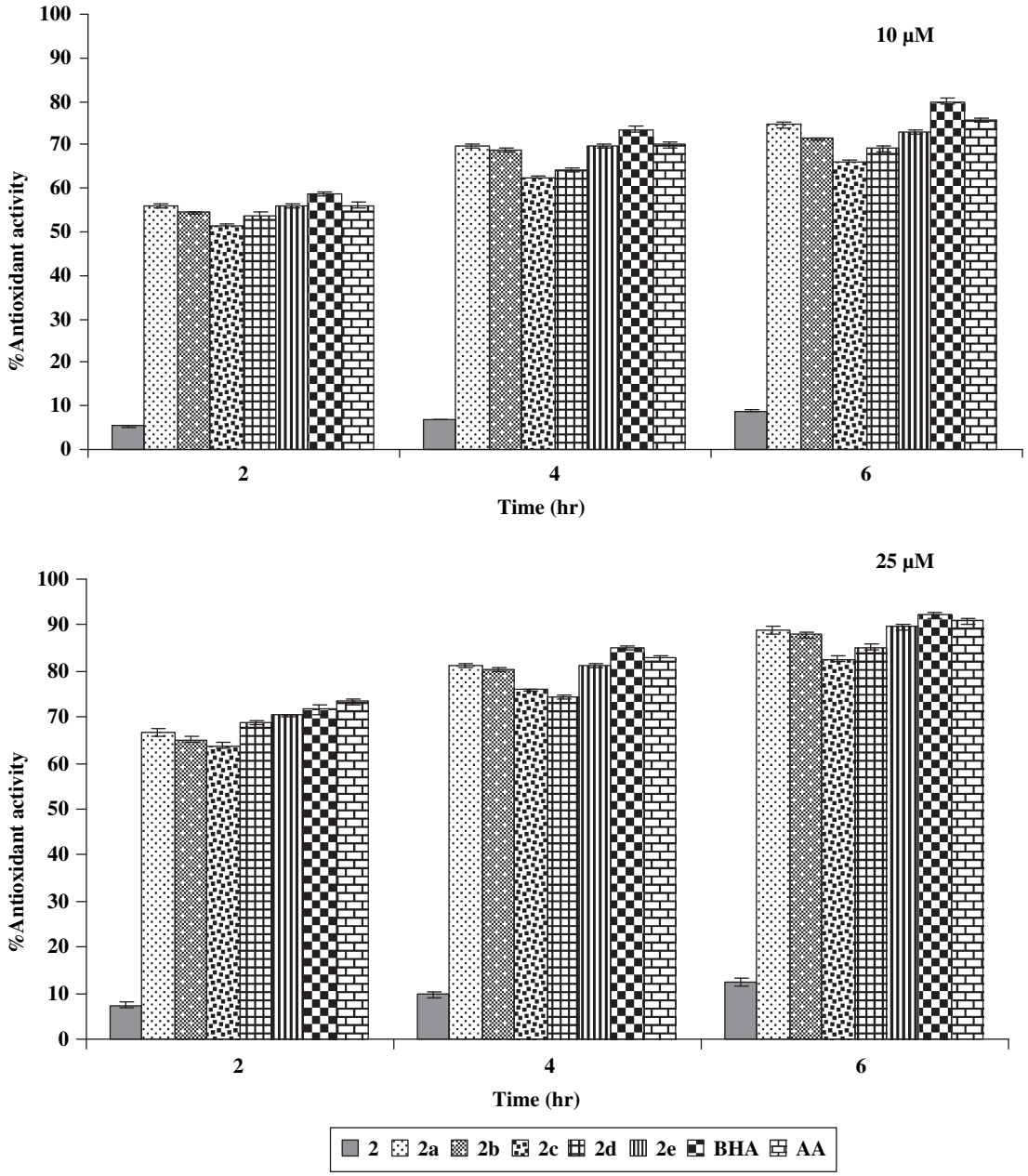


Fig. 5. Antioxidant activity (%) of 5H-dibenz[b,f]azepine analogues on human LDL oxidation in different concentrations (10, and 25 µM/mL of LDL). Values represent means ± SE ($n = 3$).

(250 mHz) ($CDCl_3$) δ (ppm): δ 7.19–7.18 (m, 8H, Ar–H), 6.9 (s, 2H, Seven membered Ar–H), 2.68 (*t*, 2H, CH_2, C═O), 3.55 (*t*, 2H, CH_2, CH_2N–H), 5.82 (s, 1H, N–H), 6.71–6.73 (m, 4H, Ar–H of aminophenol), 9.43 (s, 1H, OH); Mass (*m*/*z* %): M^+ 356.21 (100.0%), 357.14 (25.2%), 358.17 (3.4%); Anal. Calcd. for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86; O, 8.98%; Found: C, 77.50; H, 5.63; N, 7.89; O, 8.97%.

*5.1.3. 1-(5H-dibenz[b,f]azepin-5-yl)-3-(2-hydroxyphenylamino)-propan-1-one (Compound **2b**)*

Light brownish solid, Yield (81%), M.p. 126–128 °C. IR (KBr) ν_{max} (cm^{-1}): 3052–2803.5 (Ar C–H), 1659.5 (C═O), 3360.4 (N–H), 3213.1–3450.6 (phenolic-OH), 2971.0–3026.1 (CH_2); ^1H NMR (250 mHz) ($CDCl_3$) δ (ppm): δ 7.19–7.18 (m, 8H, Ar–H), 6.9 (s, 2H, Seven membered Ar–H), 2.68 (*t*, 2H, CH_2, C═O), 3.55 (*t*, 2H, CH_2, CH_2N–H), 8.02 (s, 1H, N–H), 6.71–6.9 (m, 4H, Ar–H of aminophenol), 10.0 (s, 1H, OH); Mass (*m*/*z* %): M^+ 356.19 (100.0%), 357.15 (25.2%), 358.17 (3.4%); Anal. Calcd. for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86; O, 8.98%; Found: C, 77.53; H, 5.64; N, 7.87; O, 8.99%.

*5.1.4. 1-(5H-dibenz[b,f]azepin-5-yl)-3-(3-hydroxyphenylamino)-propan-1-one (Compound **2c**)*

Brownish solid, Yield (80%), M.p. 124–126 °C. IR (KBr) ν_{max} (cm^{-1}): 3048.1–2834.3 (Ar C–H), 1662.4 (C═O), 3315.2 (N–H), 3210.3–350.5 (phenolic-OH), 2971.0–3026.1 (CH_2); ^1H NMR (250 mHz) ($CDCl_3$) δ (ppm): δ 7.19–7.18 (m, 8H, Ar–H), 6.9 (s, 2H, Seven membered Ar–H), 2.68 (*t*, 2H, CH_2, C═O), 3.55 (*t*, 2H, CH_2, CH_2N–H), 5.82 (s, 1H, N–H), 6.71–6.93 (m, 4H, Ar–H of aminophenol), 9.0 (s, 1H, OH); Mass (*m*/*z* %): M^+ 356.21 (100.0%), 357.17 (25.2%), 358.18 (3.4%); Anal. Calcd. for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86; O, 8.98%; Found: C, 77.52; H, 5.65; N, 7.86; O, 8.95%.

*5.1.5. 1-(5H-dibenz[b,f]azepin-5-yl)-3-(2-hydroxy-5-nitrophenylamino)propan-1-one (Compound **2d**)*

Light yellow solid, Yield (61%), M.p. 145–147 °C. IR (KBr) ν_{max} (cm^{-1}): 3047.5–2967.5 (Ar C–H), 1662.4 (C=O), 3313.4 (N–H), 3217.4–3510.2 (phenolic-OH), 2971.0–3026.1 (CH_2); ^1H NMR (250 mHz) ($CDCl_3$) δ (ppm): δ 7.19–7.18 (m, 8H, Ar–H), 6.9 (s, 2H, Seven membered Ar–H), 2.68 (*t*, 2H, CH_2, C=O), 3.55 (*t*, 2H, CH_2, CH_2N–H), 5.82 (s, 1H, N–H), 6.71–6.93 (m, 3H, Ar–H of aminophenol), 10.0 (s, 1H, OH); Mass (*m*/*z* %): M^+ 401.15 (100.0%), 402.16 (25.2%), 403.14 (4.1%), 402.13 (1.1%); Anal. Calcd. for $C_{23}H_{19}N_3O_4$: C, 68.82; H, 4.77; N, 10.47; O, 8.94%; Found: C,68.84; H, 4.75; N, 10.46; O, 8.95%.

*5.1.6. 1-(5H-dibenz[b,f]azepin-5-yl)-3-(3-hydroxy-4-methoxyphenylamino)propan-1-one(Compound **2e**)*

Brownish solid, Yield (71%), M.p. 134–137 °C. IR (KBr) ν_{max} (cm^{-1}): 3049.7–2834.4 (Ar C–H), 1662.1 (C=O), 3364.8 (N–H), 3196.4–3524.7 (phenolic-OH), 2971.0–3026.1 (CH_2); ^1H NMR (250 mHz) ($CDCl_3$) δ (ppm): δ 7.19–7.18 (m, 8H, Ar–H), 6.9 (s, 2H, Seven membered Ar–H), 2.68 (*t*, 2H, CH_2, C=O), 3.55 (*t*, 2H, CH_2, CH_2N–H), 5.82 (s, 1H, N–H), 6.0–6.6 (m, 3H, Ar–H of aminophenol), 9.4 (s, 1H, OH), 3.8 (*t*, 3H, OCH_3); Mass (*m*/*z* %): M^+ 386.36 (100.0%), 387.16 (26.3%), 388.16 (3.9%); Anal. Calcd. for $C_{24}H_{22}N_2O_3$: C, 74.59; H, 5.74; N, 7.25; O, 12.42%; Found: C, 74.55; H, 5.73; N, 7.27; O, 12.4%

5.2. Antioxidant activity

5.2.1. DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging effect was carried out according to the method first employed by Blois [26]. Compounds of different concentrations were prepared in distilled ethanol, 1 mL of each compound solutions having different concentrations (10, 25, 50, 100, 200 and 500 μM) were taken in different test tubes, 4 mL of 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound, and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV–visible spectrophotometer (Shimadzu 160A). The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:

$$\text{Radical scavenging activity}(\%) = [(A_0 - A_1/A_0) \times 100]$$

where A_0 is absorbance of the control (blank, without compound) and A_1 is absorbance of the compound. The radical scavenging activity of BHA and ascorbic acid was also measured and compared with that of the different synthesized compound. The compound concentration providing 50% inhibition (IC_{50}) was calculated from the graph of RSA percentage against compound concentrations.

5.2.2. Antioxidant activity by β-carotene-linoleic acid assay

Each compound at the final concentrations of 50 and 100 μM/mL were incorporated into β-carotene-linoleic acid model system independently and the activity was monitored spectrophotometrically at 470 nm [27].

5.2.2.1. Preparation of the suspension. The substrate suspension was prepared by addition of β-carotene (4 mg dissolved in 5 mL chloroform) into a covered round bottomed flask containing Tween-40 (600 mg) followed by the addition of linoleic acid (60 μL). The chloroform was removed completely under vaccum using rotary evaporator at 40 °C. The resulting solution was diluted with triple distilled water (30 mL) and the emulsion was mixed well and diluted with oxygenated water (120 mL). The aliquots (4 mL) was transferred to different stopper test tubes containing compound (50 and 100 μM/mL) in distilled ethanol. Control was prepared with distilled ethanol (1 mL) and emulsion (4 mL). BHA and ascorbic acid solution as internal standards of the same concentration were also analyzed for comparison. Zero adjustment was done using distilled water. As soon as the emulsion was added to each test tube, the zero time ($t = 0$) absorbance was measured at 470 nm using spectrophotometer (Shimadzu 160A) and subsequently absorbance was measured for every 30 min up to 3 h ($t = 180$) time interval. The tubes were placed in a water bath at 50 °C between the readings. Percentage antioxidant activities of each compound were evaluated in triplicates in terms of photo-oxidation of β-carotene using the following formula:

$$\%\ \text{Antioxidant activity} = 100\left[1 - \left(A_o - A_t/A_o^o - A_o^t\right)\right]$$

where,

A_o = Initial absorbance of the sample. ($t = 0$ min)
A_t = Absorbance of the sample after time'*t*'. ($t = 180$ min)
A_o^o = Initial absorbance of the control. ($t = 0$ min)
A_o^t = Absorbance of control after time'*t*'. ($t = 180$ min)

5.2.3. Reducing power assay (iron reducing activity)

The reducing power of synthesized compounds was determined according to the method of Oyaizu [28]. The compounds having 50 and 100 μM were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferric cyanide, and then incubated at 50 °C for 20 min. To this mixture 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 20 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride and the absorbance was measured at 700 nm using a spectrophotometer (Shimadzu 160A). Increases of absorbance of the reaction mixture indicate higher reducing power. Mean values from three independent samples were calculated for each compound and standard deviations were less than 5%.

5.2.4. Human LDL oxidation

Fresh blood was obtained from fasting adult human volunteers and plasma was immediately separated by centrifugation at 1500 rpm for 10 min at 4 °C. LDL (0.1 mg LDL protein/mL) was isolated from freshly separated plasma by preparative ultra centrifugation using a Beckman L8-55 ultra centrifuge. The LDL was prepared from the plasma according to the method of [29]. The isolated LDL was extensively dialyzed against phosphate buffered saline (PBS) pH 7.4 and sterilized by filtration (0.2 μm Millipore membrane system, USA) and stored at 4 °C under nitrogen. 1 mL of various concentrations (10, and 25 μM) of compounds were taken in test tubes, 40 μL of copper sulphate (2 mM) was added and the volume was made up to 1.5 mL with phosphate buffer (50 mM, pH 7.4). A tube without compound and with copper sulphate served as a negative control, and another tube without copper sulphate with compound served as a positive control. All of the tubes were incubated at 37 °C for 45 min. To the aliquots of 0.5 mL drawn at 2, 4 and 6 h intervals from each tube, 0.25 mL of thiobarbutaric acid (TBA, 1% in 50 mM NaOH) and 0.25 mL of trichloro acetic acid (TCA, 2.8%) were added. The tubes were incubated again at 95 °C for 45 min and cooled to room temperature and centrifuged at 2500 rpm for 15 min. A pink chromogen was extracted after the mixture was cooled to room temperature by further centrifugation at 2000 rpm for 10 min. Thiobarbituric acid reactive species in the pink chromogen were detected at 532 nm by a spectrophotometer against an appropriate blank. Data were expressed in terms of malondialdehyde (MDA) equivalent, estimated by comparison with

standard graph drawn for 1,1,3,3-tetramethoxy-propane (Which was used as standard) which give the amount of oxidation and the results were expressed as protection per unit of protein concentration (0.1 mg LDL protein/mL). Using the amount of MDA, the percentage protection was calculated using the formula:

$$\%\ \text{inhibition of LDL oxidation} = (\text{Oxidation in control} - \text{oxidation in experimental} / \text{oxidation in control}) \times 100$$

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Abbreviations

°*C:* centigrade
min: minute
h: hour
mL: milli Liter
μM: micro molar
mg/mL: milli gram per milli Liter
g/mL: gram per milli Liter
%: percentage
IC_{50}*:* 50 percent inhibition concentration
nm: nano meter
mM: milli molar
rpm: rotation per minute
RT: room temperature
DPPH: 2,2-diphenyl-1-picrylhydrazyl
TCA: trichloro acetic acid
TBA: thiobarbutaric acid
LDL: low-density lipoprotein
MDA: malondialdehyde
AA: ascorbic acid
BHA: butylated hydroxy anisole
BHT: butylated hydroxy toluene
TBHQ: tertiary butylated hydroxy toluene
RSA: radical scavenging activity
<: less than