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Research article

Synthesis, and in *vitro* antioxidant activity of novel 1, 3, 4- oxadiazole-2-thione

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Abstract

As a continuation of our efforts to discover and develop 1,3,4-oxadiazole-2-thione,a new series of 5-[4-4′-nitrophenyl)-1,3-thiazole-2yl]-1,3,4-oxadiazole-2-thione (E) were synthesized and derivatized in to mannich bases 5-[4-4′-nitro thiazole-2yl]-3-substituted-1,3,4-oxadiazole-2-thione [Ea-e]. Physical characterization of compounds which were established by performing melting point, R_f value, percentage yield and solubility. The derivative structures were identified using UV, FT-IR, ^{1}HNMR and MASS spectral analysis. The derivatives were then screened for antioxidant activity by DPPH, Nitric oxide and Hydrogen peroxide scavenging assays. When compared with other derivatives, compound 5c was found to have maximum *in* vitro antioxidant activity in all the three methods. This may be due to the presence of electron donating diethyl group present in compound 'Ec'. The electron donating nitro group may also have an influence in the antioxidant activity which is present in all derivatives.

Key words: 1,3,4-oxadiazole, Spectroscopy, Mannich bases, DPPH and H_2O_2 assay.

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1. Introduction

The living cells are disrupting by the chain reaction, that is, the attacked molecules lose its electrons and become a free radical which attacks the living cell. Natural and synthetic are the two basic types of antioxidants structures. Various alkyl substitutions containing phenolic group are in general natural antioxidants. Nitrogen compounds, phenolic compounds are categorized as synthetic antioxidants. The sterically hindered phenols and secondary aromatic amines form the primary antioxidants compounds. The hydrogen atom moves from the antioxidant molecule to radical intermediate is the first step of the radical termination [1].

Free radical scavenging is one of the best known mechanisms by which antioxidant inhibit lipid oxidation. The *in* vitro antioxidant activity can be performed by three methods, DPPH assay, Nitric oxide scavenging assay and Hydrogen peroxide radical scavenging assay.

Among the five membered aromatic compounds which are hetrocyclic1,3,4-Oxadiazoles are critical compounds. 1,3,4-oxadiazole is widely being exhibit diverse biological activities[2] like antibacterial [3], antitubercular [4], vasodilatory [5], antifungal [6], anti-inflammatory [7], anticonvulsant [8], cytotoxic [9], anaesthetic [10], analgesic [11], hypolipidimic [12], anticancer [13], antioxidant [14], and ulcerogenic [15] activities. 1,3,4-oxadiazole derivatives and their mannich bases were reported to possess anticancer and antioxidant activity. Furthermore certain thiazole derivatives are well known for their antioxidant. With the aim of finding a promising antioxidant agents, which may have improved efficacy and fewer side effects compared with existing antioxidants, we considered it of interest to synthesize some new derivatives by incorporating thiazole moiety with mannich bases of 1,3,4-oxadiazole analogues to investigate their antioxidant property.

Due to presence of an extra heteroatom there is inductive effect which makes oxadiazole a weak base. There are two pyridine like nitrogen (-N=) present in oxadiazole. The conjugated diene character in oxadiazole is mainly due to reduction in aromaticity of oxadiazole ring. Since the electron density on the carbon atom is less electrophilic substitution at same carbon atom in the oxadiazole ring is less content but the electron releasing groups in oxadiazole can undergo association. Replacement of hydrogen atom by nucleophiles has been seen in nucleophilic substituted oxadiazoles [16].

2. Materials and Methods

Chemistry

Synthetic Procedure

Synthesis of P-Chloro Phenacyl Bromide. (A)

To a cold solution of p-nitro acetophenone (0.01 mol) in chloroform (25ml), bromine (0.012 mol) in chloroform (10ml) was gradually added for about 30minutes with continuous stirring were the temperature maintained is 0-5^{0}C. The mixture of reaction was brought to the room temperature after the addition was complete. For another 60minutes the mixture was stirred and the stirring till hydrogen gas evolution ceases. Under reduced pressure the solvent was eliminated and recrystallized the residue from ethanol to afford to get pure p-chlorophencylbromide [17].

Synthesis of 4-(4′-Chlorophenacyl)-1,3-Thiazole-2-Carboxylate. (B)

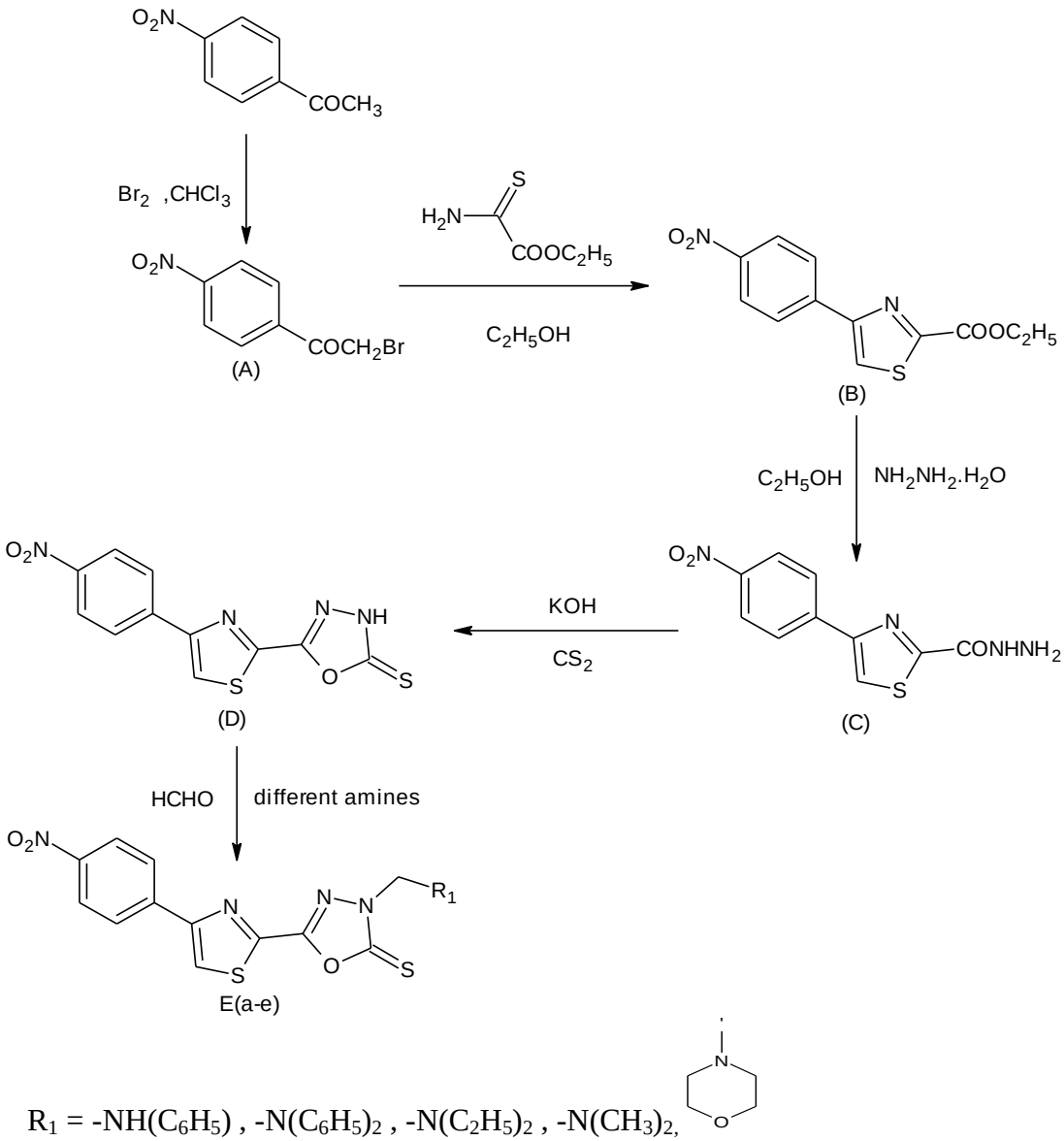
In a round bottom flask a mixture of ethyl thiooxamate (1 equivalent weight), p-nitrophencylbromide (1.1 equivalent weight) and ethanol 10-15ml were taken, and refluxed for 2 hr, the ethanol was distilled off under vacuum and it was neutralized with sodium bicarbonate.

Ethyl acetate is used to extract the mixture, and then washed with water. Under vacuum the solvent was eliminated. Crude product obtained was recrystallized from ethanol [18].

Synthesis of 4-(4′-Chloro Phenacyl)-1,3-Thiazole-2-Carbohydrazide. (C)

Compound-3 was prepared by refluxing 4-(4′-nitrophenacyl)-1,3-thiazole-2-carboxylate (0.015 mol) with hydrazine hydrate (1.6mL) in ethanol (20mL) for 5 h. After cooling the mixture the product obtained was recrystallized from DMF: ethanol mixture (6:1) [19, 20].

Scheme of synthesis:



Synthesis of 5-[4-(4′-Chloro Phenacyl)-1,3-Thiazole-2yl]-3-(2-Methyl Substituted) -1,3,4-oxadiazole-2-thione. E(a-e)

Formaldehyde 40% (0.003mol) was added to a solution of 5-[4-(4′-nitrophenacyl)-1,3-thiazole-2yl]-3-(2-methyl substituted) -1,3,4-oxadiazole-2-thione (0.003mol) in absolute ethanol (10ml). An ethanolic solution (2ml) of the different primary or secondary amine (0.003 mol) was added portion wise to the above reaction mixture, stirred for 3 hours at room temperature, and left overnight in a refrigerator. The precipitate formed was filtered, dried, and crystallized from DMF: Water (5:2) [20].

Pharmacological Study Results

Antioxidant activity results:

DPPH ASSAY (2, 2-diphenyl -1-picrylhydrazyl)

The DPPH assay was used to study radical scavenging activity of test samples according to Scherer and Godoy [21]. The measurement is taken at 517 nm. After the addition of an antioxidant the decrease in the absorption of the DPPH solution was taken. Standard taken is Ascorbic acid.

Procedure

Using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) antioxidant potency of the test samples and standard were evaluated. 3.9mL of DPPH in methanol solution (0.2 mM) is added to methanol solutions of samples or standards (0.1ml) in concentrations of (20, 40, 60, 80, 100 µg/ml). 0.1mL of methanol added to 3.9mL of DPPH solution is the control. Triplicate of the procedure were done. In the dark, 90-min incubation is done at room temperature, then absorbance was measured at 517 nm. Without test compound, but an equivalent amount of methanol taken is the control [21].

Ascorbic acid was used as standard. The results obtained from DPPH assay is shown in table 2. IC_{50} values for standard and test samples were determined using ED50 plus V 1.0 software. Percentage inhibition is calculated using the formula;

% scavenging activity = $\frac{control-test}{control} X100$

Nitric Oxide Scavenging Activity

Nitric oxide scavenging activity can be estimated by the use of Griessre action (Garrat, 1964). The compound sodium nitroprusside produce NO by decomposing in aqueous solution at pH 7.2. In aerobic condition, NO reacts with oxygen to produce stable products (nitrate and nitrite). The quantities of which can be determined using Griess reagent. The completion between scavengers of nitric oxide and oxygen leads to decrease in production of nitrite ions.

Procedure

Griessre action is used to measured Nitric oxide scavenging activity, spectrophotometrically. Different concentration of test sample (20, 40, 60, 80, 100 µg mL^{-1}) in methanol is mixed with sodium nitroprusside (5mmol L^{-1}) in phosphate buffered saline (pH-7.4) and incubated for 30 minutes at 25°C. Instead of test sample equivalent amount of methanol is taken and used as control. 1.5mL of Griess reagent is used to dilute 1.5mL of the incubated solution after 30 minutes. Absorbance was evaluated at 517nm with reference to standard and percentage-scavenging property was evaluated [22].

Ascorbic acid was used as standard. The results obtained from nitric oxide scavenging assay is shown in table 3. IC_{50} values for standard and test samples were determined using ED50 plus V 1.0 software. Percentage inhibition is calculated using the formula;

% scavenging activity = $\frac{control-test}{control} X100$

Hydrogen Peroxide Radical Scavenging Assay

By the action of oxidase enzymes, *in vivo* hydrogen peroxide is generated. Through the reduction product called hydroxyl radical (OH•) hydrogen peroxide is scavenged.

Here hydrogen peroxide is scavenged by the test sample, which is the basis of this method. Using phosphate buffer solution (pH 7.4), hydrogen peroxide solution (2mmol/l) was prepared. To hydrogen peroxide solution (0.6 ml) test samples (20, 40, 60, 80, 100 µg/ml) were added. After 10 min absorbance of hydrogen peroxide was evaluated against blank at 230 nm which containing phosphate

buffer without hydrogen peroxide. The reference compound is ascorbic acid, which is used for comparison [23].
Ascorbic acid was used as standard. The results obtained from hydrogen peroxide assay is shown in table 4. IC_{50} values for standard and test samples were determined using ED50 plus V 1.0 software. Percentage inhibition is calculated using the formula;

% scavenging activity = $\frac{control-test}{control} X100$

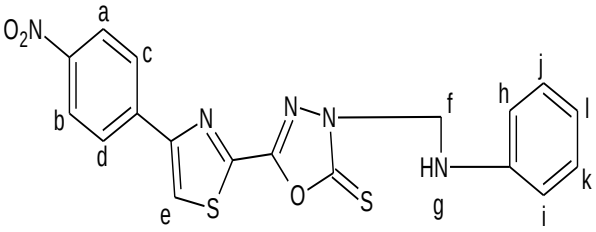
Data are the mean of three or more experiments and reported as mean ± standard error of the mean (SEM)

3. Result and Discussion

Physical data: Physical data like Molecular formula, Melting point, Solubility and R_f value were evaluated for the mannich derivatives and is shown in table 1.

Spectral Analysis:
The spectral analysis like UV, IR, ^{1}H NMR and Mass were done to identify the structure of the mannich derivatives. The spectral analysis like UV, IR, ^{1}H NMR and Mass were done to identify the structure of the mannich derivatives. The instrument model for UV spectra is SHIMADZU UV-2600. IR spectra were analyzed using BRUKER VERTEX FT-IR spectrophotometer. BRUKER 400Mhz is used to obtain ^{1}HNMR. MASS spectra were obtained using the instrument, LCMS THERMO LCQ DECA XP.

Compound Ea

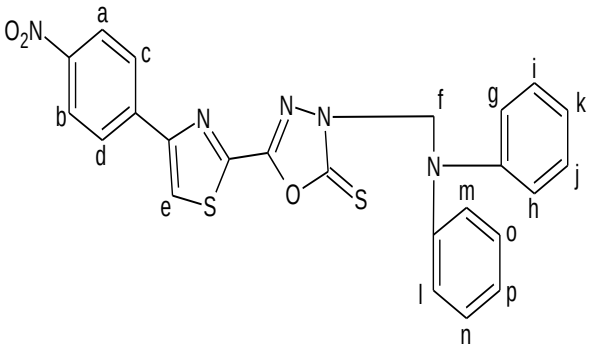


IR Spectra (cm^{-1}): 3166.00(-NHstr); 3083.32(Ar- CH); 1648.10(HC=N); 1627.66(-NHbent); 1499.83(Ar C=C); 1545.70(C-NO_2); 1260.12(C=S); 1218.18(C-O-C); 1178.08(C-N); 733.74(C-S).
NMR Spectra: ^{1}H NMR, δ(ppm)(DMSO): 8.10(d, 2H, Ar CH at a,b); 7.71(d, 2H, Ar CH at c,d); 6.91(s, 1H, CH of thiazole at e); 5.50(s, 2H, CH_2 at f); 3.90(s, 1H, NH at g); 6.80(d,2H, Ar CH at h,i); 7.10(t, 2H, Ar CH at j,k); 6.41(t, 1H, Ar CH at l); solvent peaks at 2.49 and 3.31. UV-λmax (nm):361.

Table 1. Physical characteristics of compounds

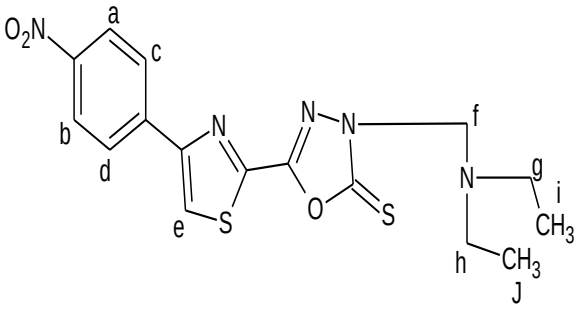
Sample Code	Physical data				
	Molecular Formula	**Melting Point**	**Solubility**	**% yield**	**R_f value**
Ea	$C_{19}H_{14}N_5O_3S_2$	169^{0}C -172^{0}C	Ethanol &DMSO	59%	0.55
Eb	$C_{24}H_{17}N_5O_3S_2$	189^{0}C-191^{0}C	Ethanol &DMSO	61%	0.60
Ec	$C_{16}H_{17}N_5O_3S_2$	184^{0}C -186^{0}C	Ethanol &DMSO	48%	0.67
Ed	$C_{14}H_{13}N_5O_3S_2$	151^{0}C -154^{0}C	Ethanol &DMSO	52%	0.57
Ee	$C_{16}H_{15}N_5O_4S_2$	195^{0}C -198^{0}C	Ethanol &DMSO	57.5%	0.51

Compound Eb



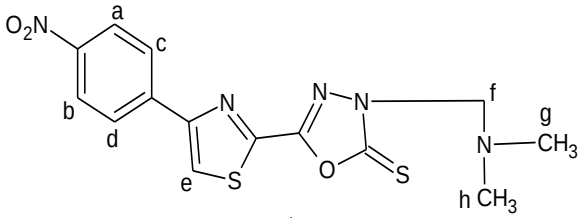
IR Spectra (cm^{-1}): 3106.93 (Ar-CH str); 1522.48 (Ar C=C); 1683.88 (HC=N); 758.39 (C-S); 1439.40 (NO_2); 1165.12(C-O-C).
NMR Spectra: ^{1}H NMR, δ(ppm)(DMSO): 8.30(d, 2H, Ar CH at a,b); 8.01(d, 2H, Ar CH at c,d); 7.10(s, 1H, CH of thiazole at e); 4.10(s, 2H, CH_2 at f); 7.31(d, 4H, Ar CH at l,g,m,h); 6.51(t, 4H, Ar CH at n,o,j,i); 6.91(t, 2H, Ar CH at p,k). Solvent peaks at 2.49 and 3.31. UV-λmax (nm):392.

Compound Ec



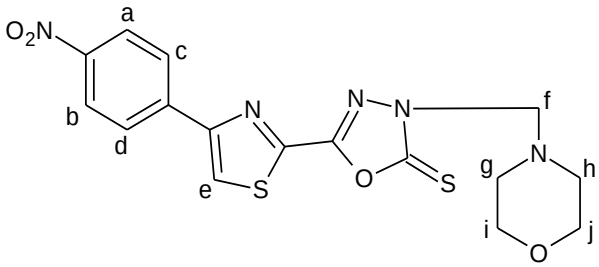
IR Spectra (cm^{-1}): 3100.33 (Ar-CH str); 2924.62 (Ali-CH); 1542.16 (Ar C=C); 1686.88 (HC=N); 839.81 (C-N); 788.12 (C-S); 1445.13 (NO_2); 1169.23(C-O-C).
NMR Spectra: ^{1}H NMR, δ(ppm)(DMSO): 8.21(d, 2H, Ar CH at a,b); 7.15(d, 2H, Ar CH at c,d); 6.90(s, 1H, CH of thiazole at e); 5.46(s, 2H, CH_2 at f); 2.94(m, 4H, CH_2-N-CH_2 at g,h); 1.12(t, 6H, CH_3 at i,j) solvent peaks at 2.49 and 3.31. UV-λmax (nm):279.

Compound Ed



IR Spectra (cm^{-1}): 3109.91(Ar-CH); 2998.31(Ali-CH); 1528.18(HC=N); 1528.18 (ArC=C); 1440.22(C-NO_2); 1269.10(C=S); 1178.10(C-O-C); 819.09(C-N); 754.30(C-S).
NMR Spectra: ^{1}HNMR, δ(ppm)(DMSO): 8.29(d, 2H, Ar CH at a,b); 7.92(d, 2H, Ar CH at c,d); 6.89(s, 1H, CH of thiazole at e); 5.45(s, 2H, CH_2 at f); 2.02(s, 6H, CH_3-N-CH_3 at g,h); solvent peaks at 2.49 and 3.31. UV-λmax (nm): 267. Mass spectra: Molecular weight=363.

Compound Ee



IR spectra(cm^{-1}): 3086.77(Ar-CH); 2929.96 (Ali- CH); 1623.43 (HC=N); 1536.24 (ArC=C); 1451.70(C-NO_2); 1270.17(C=S); 1245.24(C-O-C); 1170.27(C-N); 785.77(C-S).
NMR Spectra: ^{1}H NMR, δ(ppm)(DMSO): 8.25(d, 2H, Ar CH at a,b); 7.71(d, 2H, Ar CH at c,d); 6.90(s, 1H, CH of thiazole at e); 5.49(s, 2H, CH_2 at f); 2.15(t, 4H, CH_2-N-CH_2 at g,h); 3.72(t, 4H, CH_2-O-CH_2 at i,j) solvent peaks at 2.49 and 3.31.UV-λmax (nm):243.

Antioxidant activity results:

The derivatives prepared were evaluated for antioxidant activity by three methods like DPPH assay, Nitric oxide scavenging assay and Hydrogen peroxide scavenging assay. The compounds were tested in five different concentrations.

Table 2. DPPH assay results

Sample	% Scavenging Activity At Different Concentrations					**IC_{50}**
	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
Ea	45.06±0.183	47.89±0.471	51.21±0.391	53.59±0.304	55.75±0.592	56.3
Eb	38.11±0.481	44.08±0.371	45.08±0.421	48.44±0.536	51.14±0.362	91.2
Ec	40.38±0.457	43.52±0.378	64.06±0.382	65.67±0.298	67.43±0.193	**53.2**
Ed	37.15±0.461	40.93±0.362	53.59±0.304	64.74±0.296	66.85±0.562	54.9
Ee	40.93±0.362	44.34±0.462	45.50±0.524	46.80±0.653	55.75±0.592	89.1
Standard	49.38±0.515	67.03±0.541	75.78±0.223	91.92±0.561	95.34±0.111	**21.3**

Standard drug used is ascorbic acid. IC_{50} values in µg/ml for samples were determined using ED50 plus V 1.0 software. Data are the mean of three or more experiments and reported as mean ± standard error of the mean(SEM).

Table 3. Nitric oxide scavenging assay results:

Sample	% Scavenging Activity At Different Concentrations					**IC_{50}**
	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
Ea	23.29±0.362	40.63±0.654	29.94±0.521	47.19±0.657	52.26±0.550	91.4
Eb	21.49±0.483	24.28±0.477	29.10±0.399	41.70±0.307	50.13±0.590	95.7
Ec	24.76±0.453	31.88±0.622	47.78±0.362	50.11±0.298	52.67±0.183	**76.2**
Ed	22.36±0.431	26.95±0.370	43.77±0.321	52.01±0.331	54.29±0.481	89.3
Ee	20.16±0.183	23.32±0.371	29.32±0.426	38.78±0.533	50.04±0.362	99.1
Standard	47.53±0.624	63.44±0.521	84.28±0.623	90.53±0.411	93.56±0.221	**25.2**

Standard drug used is ascorbic acid. IC_{50} values in µg/ml for samples were determined using ED50 plus V 1.0 software. Data are the mean of three or more experiments and reported as mean ± standard error of the mean (SEM).

Table 4. Hydrogen peroxide radical scavenging assay results:

Sample	% Scavenging Activity At Different Concentrations					**IC_{50}**
	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
Ea	42.61±0.113	44.24±0.365	46.77±0.654	47.71±0.304	50.00±0.547	83.7
Eb	30.83±0.123	39.57±0.438	40.66±0.554	47.69±0.309	52.27±0.525	85.1
Ec	30.82±0.462	44.24±0.365	55.19±0.382	56.61±0.246	58.42±0.424	**49.2**
Ed	32.38±0.461	41.71±0.378	47.29±0.434	54.98±0.435	57.81±0.221	67.8
Ee	30.65±0.362	30.83±0.462	34.09±0.524	42.61±0.153	50.46±0.142	87.4
Standard	44.53±0.526	64.65±0.653	71.47±0.36	89.22±0.621	96.19±0.456	**26.9**

Standard drug used is ascorbic acid. IC_{50} values in µg/ml for samples were determined using ED50 plus V 1.0 software. Data are the mean of three or more experiments and reported as mean ± standard error of the mean (SEM).

Conclusion

The physical and spectral analysis of the mannich derivatives were carried out. In vitro Antioxidant activity was carried out by three methods. In DPPH assay compound Ec with IC_{50} value of 26.7 µg/ml showed significant activity when compared to ascorbic acid with IC_{50} 21.3µg/ml. In Nitric oxide and Hydrogen peroxide scavenging methods compound Ec showed maximum activity when compared to other derivatives but was not significant to the result obtained in DPPH method. It is well established that organic molecules incorporating an electron-donating group can act as free radical trapping agents and are capable of opposing oxidative

challenges. Compound **Ec** showed maximum antioxidant activity in all the three methods. This may be due to the presence of chloro substitution at 4' position of phenyl ring and the diethyl group attached to amine. Both chloro and diethyl group are electron releasing which are important in radical scavenging activity.

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