SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT DPPH SCAVENGING ASSAY OF SOME NOVEL MANNICH BASES OF ISOXAZOLINE DERIVATIVES

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Abstract:
Novel isoxazoline derivatives were synthesized by condensation of varyingly substituted acetophenone with aldehyde in presence of alcoholic sodium hydroxide to get intermediate chalcones, which were further treated with hydroxylamine hydrochloride in presence of sodium hydroxide to get isoxazoline derivatives. The latter were refluxed separately with Isonicotinic acid hydrazide and Sulphanilamide in presence of formaldehyde for 6-10 h to afford corresponding Mannich bases. The structures of synthesized compounds were established on the basis of melting point, TLC, IR, ^{1}HNMR and HRMS. The derivatives were evaluated for the radical scavenging activity compared to the standard Ascorbic acid. The results of antioxidant study show that some of the derivatives posses mild to moderate activity as compared to standard.

Keywords- Chalcone, Isoxazoline, Mannich bases, Antioxidant activity.

I. INTRODUCTION

The isoxazoles are five membered rings containing a nitrogen and an oxygen atom adjacent to each other. The Dihydro derivatives of isoxazoles are called as isoxazolines. Isoxazolines have various methods of preparation. Isoxazolines were synthesized from chalcones represents a class of compounds of great biological importance. Isoxazoline possess a broad spectrum of biological activity. Isoxazoline derivatives have been reported in the literature to possess antifungal, antibacterial, anticonvulsant, anti-inflammatory, antiviral and analgesic activity[1-10]. It serves as an important building block for the synthesis of biologically active molecules. Mannich bases of heterocyclic molecules have been attracting the attention of the synthetic chemists for their wide range of biological activities ranging from antibacterial, antifungal, anticancer, antiparkinson to anticonvulsant and anti–HIV [11-21]. Free radicals can oxidize biomolecules viz. nucleic acids, proteins, lipids, DNA, lead to tissue damage and can initiate degenerative diseases. Oxidative damage plays a significantly pathological role in human diseases such as cancer, emphysema, cirrhosis, atherosclerosis and arthritis [22-23]. Almost all organisms are protected to some extent against free radicals such as peroxide, hydroperoxide and lipid peroxyl damage by enzymes such as superoxide dismutase and catalase or compounds such as ascorbic acid (AA), tocopherols, phenolic acids, polyphenols, flavonoids and glutathione [24]. However, antioxidant supplements or dietary antioxidants may be sources of protection that the body needs to protect against the damaging effects of free radicals [25]. Presently synthetic antioxidants are widely used because they are effective and cheaper than natural antioxidants. By observing the importance of the above said problem, the mannich bases of isoxazoline derivatives were synthesized following the steps given in **Figures 1**.The purity of the compounds was monitored by thin layer chromatography (TLC) and the structures of the products were confirmed by elemental and spectral analysis. All the newly synthesized compounds were screened for their antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay method.

II. Experimental

All the chemicals in the synthesis were purchased from S.D. Fine Chemicals LTD., Mumbai. Melting points were determined by open capillary method on Veego (model: VMP-D) electronic apparatus and are uncorrected. The IR spectra of the synthesized compounds were recorded on Shimadzu 8400-S FT-IR Spectrophotometer using ATR sampling technique.
^{1}H NMR spectra was obtained on Bruker AV III 500 MHz spectrometer spectra in $CDCl_3$ and chemical shifts are given in parts per million, downfield from Tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained from Bruker Impact HD 3050 system instrument at the SPPU, Pune. To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin layer chromatography was performed on microscopic slides (2 x 7.5 cm) coated with silica gel G F_{254}, using Benzene:Methanol (7:3) solvent systems and the spots were visualized under ultra-violet light (254nm) or by exposure to iodine vapours.

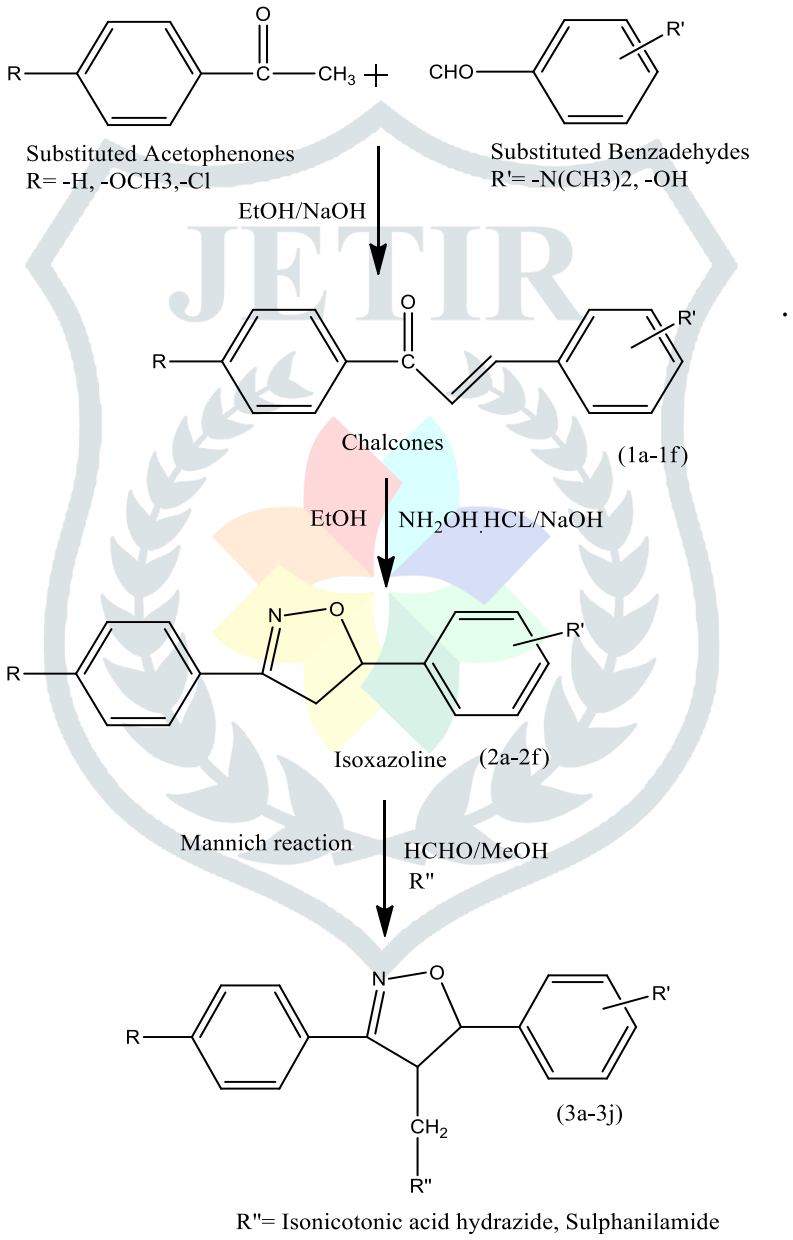


Figure 1 scheme for synthesis of substituted isoxazoline derivatives

2.1 Synthesis of Chalcones (1a-1f)

In an erlenmeyer flask, appropriate acetophenone (0.01 mol) and aromatic aldehyde (0.01 mol) in ethanol were separately dissolved in ethanol. The two were mixed and 10 ml of 40% sodium hydroxide solution was added with stirring. The resulting solution was kept overnight at room temperature. The completion of reaction was monitored by TLC. The contents of mixture was then poured over crushed ice and acidified with dil. HCl. The solid obtained was filtered, dried and recrystallized from ethanol.

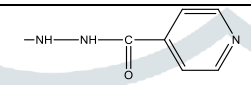
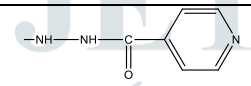
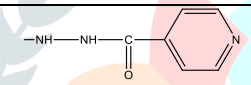
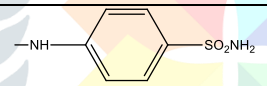
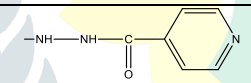
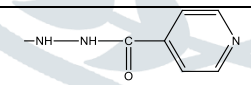
2.2 Synthesis of isoxazolines: (2a-2f)

The isoxazolines were prepared by refluxing a mixture of purified chalcones (0.01mol) and hydroxylamine hydrochloride (0.03 mol) in ethanolic NaOH (0.01 mol) for 6 hrs. The progress of reaction was monitored by TLC. After completion of the reaction, an excess of the solvent was removed by distillation and the resultant mass was poured into ice water with vigorous stirring. The solution was acidified with dilute HCl. It was kept overnight in cool condition. The resultant solid product was filtered, washed with sufficient cold water, dried and purified by recrystallization from ethanol.

2.3 General procedure for the synthesis of substituted isoxazolines (Mannich reaction) (3a-3j)

To a solution of isoxazoline (2) (0.01mol) in methanol (30ml), formaldehyde (0.02mol) and corresponding isonicotinic acid hydrazide/sulphanilamide (0.02mol) were added. The reaction-mixture was refluxed for 6 - 10 h. The progress of reaction was monitored by TLC. The solvent was distilled off and the residue was poured into ice water. The precipitate was filtered off, dried and recrystallised from ethanol.

Table 1. physical characteristics of synthesized derivatives (3a-3j):

Compound Code	R	R'	R''	Molecular Formula	Molecular Weight	Melting Point (0^0C)	Rf Value	% Yield
3a	-H	p-$N(CH_3)_2$		$C_{24}H_{25}N_5O_2$	415.49	88-90	0.54	55
3b	-H	p-$N(CH_3)_2$		$C_{24}H_{26}N_4O_3S$	450.55	161-163	0.72	65
3c	p-OCH_3	p-OH		$C_{23}H_{22}N_4O_4$	418.45	270-275	0.62	60
3d	p-OCH_3	p-OH		$C_{23}H_{23}N_3O_5S$	453.51	175-178	0.82	56
3e	p-OCH_3	p-$N(CH_3)_2$		$C_{25}H_{27}N_5O_3$	445.51	155-159	0.71	53
3f	p-OCH_3	p-$N(CH_3)_2$		$C_{25}H_{28}N_4O_4S$	480.58	156-158	0.80	58
3g	p-Cl	p-OH		$C_{22}H_{19}ClN_4O_3$	422.86	85-87	0.63	63
3h	p-Cl	p-OH		$C_{22}H_{20}ClN_3O_4S$	457.93	75-78	0.62	67
3i	p-Cl	p-$N(CH_3)_2$		$C_{24}H_{24}ClN_5O_2$	449.93	125-130	0.81	59
3j	p-Cl	p-$N(CH_3)_2$		$C_{24}H_{25}ClN_4O_3S$	485.00	132-135	0.72	65

2.4 Free Radical Scavenging Activity (DPPH Assay)

The radical scavenging activity of the synthesized compounds against stable free radical 2.2- diphenyl-2-picrylhydrazyl hydrate (DPPH, Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometric ally. When DPPH reacts with antioxidant compounds, which can donate hydrogen, it is reduced. Following the reduction, its deep violet color in methanol bleached to yellow, showing a significant absorption decrease at 517 nm . Then 3ml of various concentrations (2,4,8,16 and 32 µg/ml) of the compounds (3a-3j) dissolved in ethanol were added to 1ml of ethanol solution of DPPH. (Accurately 4.079 mg of DPPH was weighed and added to 100 ml ethanol). After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm (Shimadzu UV-Vis spectrophotometer) Ascorbic acid was used as the reference compound. All tests and analyses were done in three replicates and the results were averaged. Free radical DPPH inhibition in percentage (AA %) was calculated as follows:

$$\text{Scavenging Effect (\%)} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

2.5 Statistical analysis

Results were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey's test using statistical software package, GraphPad Prism; version 5.03. Values were expressed as a mean ± standard deviation of the mean and (***) $P < 0.001$ was considered as statistically highly significant. Values of $P \leq 0.05$ (∗) and $P \leq 0.01$ (∗∗) were considered statistically significant. To calculate the IC50 values using Microsoft Excel. IC50 was calculated from linear equation relationship, i.e., y = mx + c, (IC_{50}: Half maximal inhibitory concentration)

2.6 Spectral Analysis

3a, N'-((5-(4-(dimethylamino)phenyl)-3-phenyl-4,5-dihydroisoxazol-4-yl)methyl)isonicotino hydrazide, IR (ATR, cm^{-1}): Ar-H Stretch (3051.49), -CO stretch of amide(1664.62), -C=C- (1529.60), -C=N(1614.47), -C-O-N(1323.21), NH stretch (3429.55), -NH stretch of hydrazide (3408.33), Aliphatic –CH stretch (2889.46 & 2908.75), -C=O stretch of amide(1664.62), Ar-CN stretch of amine (1359.86), -N-O stretch of Isoxazole (947.08), -C-N stretch of amine (1230.63), N-N stretch of hydrazide (1166.97), ^1H *NMR (δ ppm):* 2.977 (6H,s, Aliphatic-CH_3), 3.68-3.75 (1H, d, Isoxazole ring –O-CH-CH-C=), 3.34-3.4 (1H, q, Isoxazole ring CH-CH), 6.73-6.77 (2H, d, Aliphatic –CH_2), 5.64-5.7(2H,NH, d, Amine nitrogen), 7.28-7.31(4H, d, Aromatic hydrogen), 7.42-7.46, (5H, d, Aromatic hydrogen), 7.71-7.75, (2H, d, Aromatic hydrogen), 7.63-7.64, (2H, d, Aromatic hydrogen), HRMS m/z (%): $[M]^+$:415

3b, 4-(((5-(4-(dimethylamino)phenyl-4,5,-dihydroisoxazole-4-yl) methyl) amino) benzene sulfon amide, IR (ATR, cm^{-1}): Ar-H stretch (3373.71), -C-O-N (1381.79), -C-N stretch of amine (1230.63), -S=O symmetric Stretch (1159.26), -S=O asymmetric Stretch (1361.79), -NH stretch of sulphonamide (3242.45 & 3373.61), NH bend of sulphonamide (1546.60), -N-O stretch of Isoxazole (952.87), C-N stretch of tertiary Amine (1309.71), ^1H *NMR (δ ppm):* 2.97-2.99 (6H, s, Aliphatic-CH_3), 3.67-3.74 (1H, d, Isoxazole ring –O-CH-CH-C=), 3.35-3.4 (1H, q, Isoxazole ring CH-CH), 6.73-6.76 (2H, d, Aliphatic –CH_2), 5.65-5.70 (1H, t, Amine nitrogen), 9.766 (2H, s, -SO_2NH_2), 7.28-7.32(4H, d, Aromatic hydrogen), 7.42-7.49 (5H, m, Aromatic hydrogen), 7.71-7.75(2H, d, Aromatic hydrogen), 7.46-7.48 (2H, d, Aromatic hydrogen), HRMS m/z (%):$[M]^+$:450.55

3c, N'-((5-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazol-4-yl)methyl) isonico tinohydrazide, IR (ATR, cm^{-1}): Ar-H Stretch (3373.71), -CO stretch of amide(1668.48), -C=C- (1508.38), -C=N(1668.48), -C-O-N(1371.43), -NH stretch of hydrazide (3201.94), -CN stretch (1259.56) , –CH bend (857.49), -C=O stretch of amide(1668.48), -C-O of phenyl alkyl ether (1062.81 & 1259.56), -N-O stretch of Isoxazole (970.23), -CN stretch of amine (1259.56),N-N stretch of hydrazide (1165.04)

3d, 4-(((5-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazol-4-yl)methyl)amino) benzene sulfonamide, IR (ATR, cm^{-1}): Ar-H stretch (3063.06), aliphatic –CH stretch (2864.39), -CONH stretch (1650), -C=C (1600.97), Ar-CN stretch (1311.64), -S=O symmetric stretch (1157.33), -S=O asymmetric stretch (1361.79), -NH stretch of sulphonamide (3244.38 & 3335.03), -NH bend of sulphonamide (1514.17), -C-O-N (1323.21), Ar-OH (3500)

3e, N'-((5-(4-(dimethylamino)phenyl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-yl) methyl)isonico tinohydrazide, IR (ATR, cm^{-1}): Ar-H stretch (3068.85), -NH str. of hydrazide(3408.33), aliphatic –CH str (2845.10), -CONH stretch (1664.62), -C=N str.(1597.11), -C=C(1519.96), -CH bend (750.33), -C-O of phenyl alkyl ether (1058.96 & 1249.91), N-N str. of hydrazide (1180.47), -C-O-N (1311.64), C-N str. of ter. Aromatic amine (1365.65), -CN str. of amine (1232.55), ^1H *NMR (δ ppm):* 2.93-2.97(6H, s, Aliphatic-CH_3), 3.65-3.68(1H, d, Isoxazole ring –O-CH-CH-C=), 3.06-3.67 (1H, q, Isoxazole ring CH-CH), 3.88(3H, s, Methoxy –OCH_3), 6.73-6.75(2H, d, Aliphatic –CH_2), 5.62-5.66(2H NH, d, Amine -N), 6.60-6.75(4H, d, Aromatic hydrogen), 6.91-6.94(4H, d, Aromatic hydrogenl), 7.28-7.32(2H, d, Aromatic hydrogen), 7.60-7.67(2H, d, Aromatic hydrogen), HRMS m/z (%):$[M]^+$:445.5

3f, 4-(((5-(4-(dimethylamino)phenyl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazol-4-yl)methyl) amino) benzenesulfonamide, IR (ATR cm^{-1}): Ar-H stretch (3066.92), aliphatic –CH str.(2839.31), -NH str.(3375.54), -C-O-N (1244.13), -S=O sym. Str. (1155.40), -S=O asym. Str.(1350.22), C-N str. of ter. Amine (1301.99), -C-O of phenyl alkyl ether (1030.02 & 1244.13), ^1H *NMR (δ ppm):* 2.93-2.96 (6H,s, Aliphatic-CH_3), 3.62-3.69(1H, d, Isoxazole ring –O-CH-CH-C=), 3.28-3.35 (1H, q, Isoxazole ring CH-CH), 3.842 (3H, s, Methoxy –OCH_3), 6.71-6.74 (2H, d, Aliphatic –CH_2), 5.59-5.63(1H, s, Amine -N), 6.69-6.73(4H, d, Aromatic hydrogen), 6.9-6.94 (4H, d, Aromatic hydrogen), 7.226-7.27(2H, d, Aromatic hydrogen), 7.63-7.64(2H, d, Aromatic hydrogen) HRMS m/z (%):$[M]^+$: 480

3g, N'-((3-(4-chlorophenyl)-5-(4-hydroxyphenyl)-4,5-dihydroisoxazol-4-yl)methyl)isonico tinohydrazide, IR (ATR, cm^{-1}): Ar-H str.(3063.06), - C-Cl (821.70), NH str.(3240.52), -CONH str.(1670.41), -C=C (1492.95), Ar-OH (3550), -C-O-N (1396.44), - CH bend (746.48), N-N str. of hydrazide (1182.40), CN str. of amine (1307.78), Ar-CN str. of pyridine (1369.50), ^1H *NMR (δ ppm):* 8.89 (1H,s, Aromatic-OH), 3.30-

3.36 (1H, d, Isoxazole ring –O-CH-CH-C=), 3.29-3.34 (1H, q, Isoxazole ring CH-CH), 6.70-6.74 (2H, d, Aliphatic $–CH_2$), 5.65-5.69 (1H, s, Amine -N), 6.53 (1H, s, Amide nitrogen) 7.25-7.29 (4H, d, Aromatic hydrogen), 7.34-7.45 (4H, d, Aromatic hydrogen), 7.54-7.56(2H, d, Aromatic hydrogen), 7.63-7.64(2H, d, Aromatic hydrogen), HRMS m/z (%):$[M]^+$:422

3h, 4-(((3-(4-chlorophenyl)-5-(4-hydroxyphenyl)-4,5-dihydroisoxazol-4-yl)methyl)amino) benzene sulfonamide, IR (ATR, cm^{-1}): C-Cl (821.70), Ar-H (3242.45), C=N (1641.48), C-O-N (1307.78), -NH str. of sulphonamide (3242.45 & 3296.42), -NH bend of sulphonamide (1597.11), -S=O sym str.(1149.61), -S=O asym. Str.(1367.58), -CN str. of amine (1220.98), -C=C (1492.95), ^1H *NMR (δ ppm):* 8.9 (1H, s, Aromatic-OH), 3.67-3.74 (1H, d, Isoxazole ring –O-CH-CH-C=), 3.35-3.4 (1H, q, Isoxazole ring CH-CH), 6.73-6.76 (2H, d, Aliphatic $–CH_2$), 5.65-5.70 (1H, d, Amine nitrogen), 9.7-10 (2H, s, $-SO_2NH_2$), 7.28-7.32(4H, d, Aromatic hydrogen), 7.43-7.46 (5H, m, Aromatic hydrogen), 7.65-7.68 (2H, d, Aromatic hydrogen), 7.63-7.64 (2H, d, Aromatic hydrogen), HRMS m/z (%):$[M]^+$:457.93

3i, N'-((3-(4-chlorophenyl)-5-(4-(dimethylamino)phenyl)-4,5-dihydroisoxazol-4-yl)methyl) isonicotinohydrazide, IR (ATR, cm^{-1}): -C-Cl (827.49), Ar-H (3039.91), aliphatic CH str. (2974.33), -CONH str. of amide (1670.41), -C=N str. (1523.82), -C=C (1491.02) , -C-O-N (1296. 21) , -CN str. of amine (1228.70), -CN str. of amine (1350.22), N-N str. of hydrazide (1165.04) , -NH str. of hydrazide (3201.94) , Ar-CN str. of pyridine (1402.30), ^1H *NMR (δ ppm):* 2.975(6H, s, Aliphatic-CH_3), 3.31-3.36(1H, d, Isoxazole ring –O-CH-CH-C=), 3.64-3.69 (1H, q, Isoxazole ring CH-CH), 6.61-6.73(2H, d, Aliphatic $–CH_2$), 5.66-5.70 (1H, s, Amine -N), 6.52 (1H, s, -NH-NH-), 7.25-7.28(4H, d, Aromatic hydrogen), 7.38-7.45 (4H, d, Aromatic hydrogen), 7.51-7.52(2H, d, Aromatic hydrogen), 7.63-7.64(2H, d, Aromatic hydrogen), HRMS m/z (%):$[M]^+$:449.93

3j, 4-(((3-(4-chlorophenyl)-5-(4-(dimethylamino)phenyl)-4,5-dihydroisoxazol-4-yl)methyl) amino) benzene sulfonamide, IR (ATR, cm^{-1}): -C-Cl(827.49), Ar-H(3084.28), aliphatic CH str. (2850.88), -C=N str. (1595.18), -C=C(1612.54), C-O-N(1315.50), -CN str. of amine(1230.63), -CN str. of amine(1193.98), -NH bend of sulphonamide(1525.74), -S=O sym str. (1149.61), -S=O asym. Str. (1350.22), ^1H *NMR (δ ppm):* 2.977(6H, s, Aliphatic-CH_3), 3.64-3.69(1H, d, Isoxazole ring –O-CH-CH-C=), 3.31-3-36(1H, q, Isoxazole ring CH-CH), 6.75-6.76(2H, d, Aliphatic $–CH_2$), 5.66-5.70(1H, s, Amine -N), 9.977(2H, s, -NH-NH-), 7.27-7.28 (4H, d, Aromatic hydrogen), 7.39-7.47(4H, d, Aromatic hydrogen), 7.64-7.72(2H, d, Aromatic hydrogen), 7.81-7.82(2H, d, Aromatic hydrogen), HRMS m/z (%):$[M]^+$:485

III. Result and Discussion

The antioxidant activity of the synthesized Isoxazole was evaluated using DPPH free radical scavenging assay. The results of antioxidant screening were depicted in Table 2 and Fig. 2. DPPH radical scavenging is considered a good *in vitro* model and is widely used to conveniently assess antioxidant efficacy. 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 colour changes from purple to yellow. This colour 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 2-32 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 3g is probably due to the presence of hydroxyl group in the isoxazole moiety. The order of activity regarding the derivatives 3g>3a> 3h> 3j> When the observed results compared, it observed that the hydroxyl substituted compounds showed more DPPH scavenging activity in comparison to the halogen substituted compounds. The substitution with different substituent on the phenyl of the aldehydic and acetophenic group of chalcone. When the phenyl group on isoxazole is substituted with –OH group (compound 3g, 3a) the compounds exhibited better activity in comparison to substitution with the other groups like halogen which may be due to more reducing potential. Hydroxyl substitution on both moieties of isoxazole has more scavenging effect than substitution on any one moiety. Highest DPPH free radical scavenging activity is shown when 4 hydroxyl substitution in isoxazole moiety. The chlorine substitution in the isoxazole moiety (compound 3h, 3j) and dimethylamine substitution in isoxazole moiety (compound 3b, 3b, 3e, 3f,and 3i) also showed the moderate antioxidant activity but less than hydroxyl substitution. Among the synthesized compounds, compound 3g, 3a, 3h and 3j showed the better or comparable activity in comparison to the standard drug. The compounds with no substitution or less substitution were showed less scavenging effect in comparison to the substituted compounds due to lesser electro negativity. Most of the synthesized compounds were potential lead for antioxidant activity. On the

bases of observed results, it may be concluded that the substitution favours the activity, but the halogen substitution disfavours the scavenging activity. The hydroxyl substitution increases the DPPH free radical scavenging activity of the compounds.

Table 2. results of DPPH assay

Sample	% Scavenging Activity At Different Concentrations (n = 6)					IC_{50}
	2µg/mL	4µg/mL	8µg/mL	16µg/mL	32µg/mL	
3a	21.82***±0.58	34.61***±0.276	48.37***±0.38	56.17±0.74	67.02***±0.77	15.74*±0.48
3b	15.26***±0.79	30.73***±0.40	38.28±0.43	45.37***±0.53	62.90***±0.47	20.84***±0.37
3c	15.26***±0.46	30.73***±0.77	33.12***±0.60	47.38***±1.01	54.86***±0.69	14.56±0.59
3d	16.70***±0.72	24.92±0.70	31.07***±0.74	46.22***±0.63	61.19***±1.23	22.24***±0.69
3e	18.49±0.64	23.66***±0.73	29.98***±0.56	48.76***±0.66	52.84***±0.62	21.33***±0.87
3f	17.48***±0.66	26.53±0.66	30.94***±0.73	39.21***±0.72	50.52***±0.67	21.06***±0.88
3g	17.24***±0.28	32.81***±0.36	44.77***±0.44	57.83**±0.50	72.94***±0.89	15.35±1.00
3h	18.51±0.66	31.16***±0.54	43.89***±0.52	50.24***±0.54	65.21***±0.81	18.42***±0.40
3i	21.19*±0.64	30.73***±0.71	33.12***±0.62	47.38***±0.78	64.91***±0.75	20.06***±0.48
3j	19.22±0.50	29.73***±0.68	46.76***±0.58	49.20***±2.18	61.07***±0.83	19.70***±0.83
Ascorbic Acid	19.70±0.36	25.92±1.19	37.96±0.38	55.77±0.26	89.61±0.36	14.23±0.11

Data were expressed as mean ± SD (n = 6), *P<0.05,**P<0.01, ***P<0.001, vs Ascorbic acid with the same concentration (Tukey-Kramer Multiple Comparisons Test)

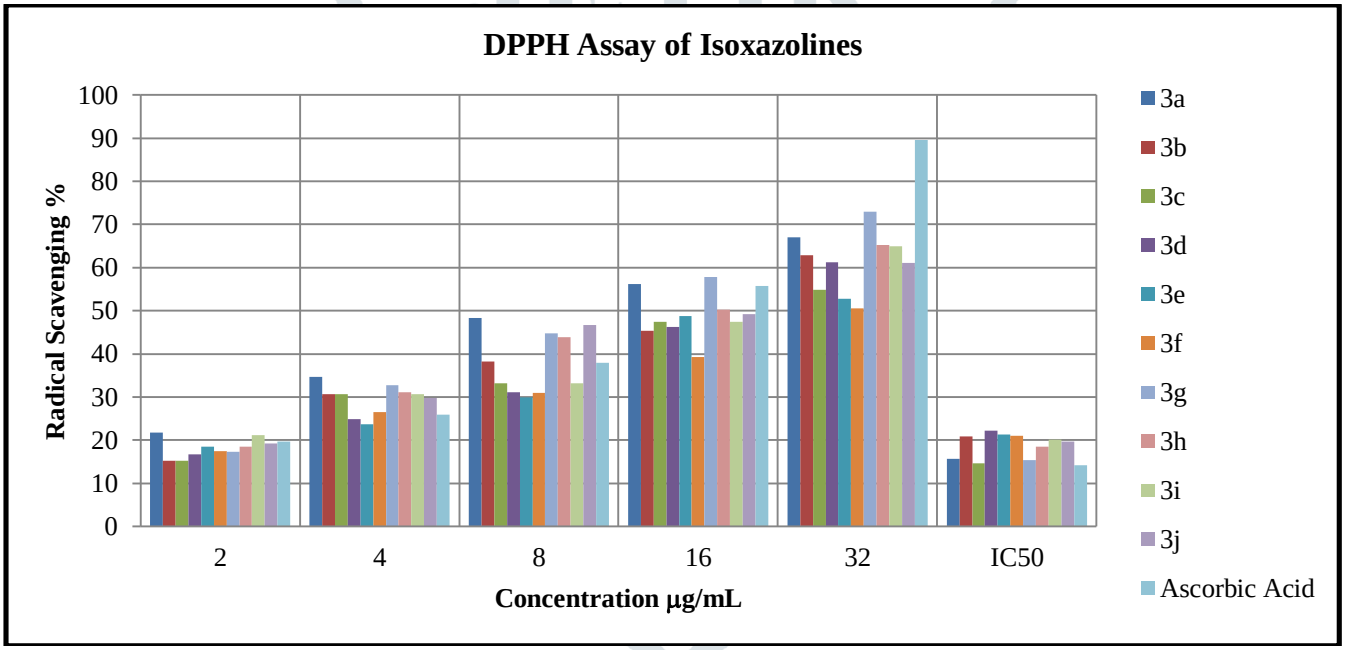


Figure 2. graphical presentation of DPPH assay of isoxazolines

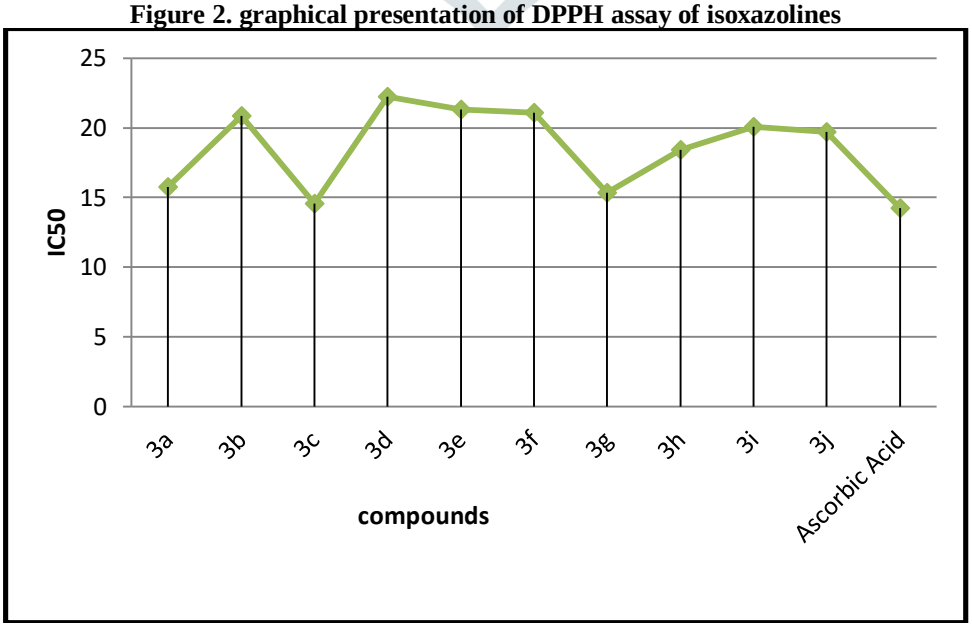


Figure 2. graphical presentation of IC_{50} value DPPH assay of isoxazolines

IV. Conclusion:

The physical and spectral analysis of the isoxazole derivatives were carried out. In vitro Antioxidant activity was carried out by three methods. Compounds **3a, 3i and 3j** have shown at 2 µg/ml, **3a, 3g, and 3h** at 4 µg/ml, **3a, 3g, 3h and 3j** at 8 µg/ml, **3a, 3g, 3h and 3j** at 16 µg/ml and **3a, 3g 3h and 3i** have shown promising antioxidant activity at 32 µg/ml, respectively. In DPPH assay compound 3c (14.56±0.59), 3a (15.74±0.48) 3g with IC50 value of 15.35±1.00 showed significant activity when compared to ascorbic acid with 14.23±0.11. 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.

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