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Synthesis and Characterization of Novel 1, 3-Oxazole Derivatives and Study of Their *In-Vitro* Antidiabetic and Antioxidant Activity

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Abstract
A novel series of five derivatives of oxazole were synthesized namely N-4-phenyloxazole-2,4-diamine, 2-Methyl-N-phenyloxazoleamine, N,2-diphenyloxazol-4-amine, N-phenyloxazol-4-amine and 2-Ethyl-N-phenyloxazol-4- by the condensation of acetanilide with different amides in presence of iodine as a catalyst. The synthesized oxazole derivatives were characterized by elemental analysis and ^{1}H NMR. In this study, the *in vitro* anti-oxidant activity and anti-diabetic activity of five derivatives of oxazole were determined. Antioxidant activities of these compounds were evaluated through DPPH assay and hydrogen peroxide assay. The anti-diabetic activities of these compounds were determined by amylase inhibition assay. A_1 derivative shows highest antidiabetic and antioxidant activity. The results obtained showed that the derivatives have anti-oxidant and anti-diabetic activity.

Keywords
Diabetes mellitus, oxazole, alpha-amylase, antioxidant, hydrogen peroxide, DPPH.

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1. INTRODUCTION
Nitrogen and oxygen containing five member heterocyclic compounds have taken huge importance in the field of drug discovery process [1]. Oxazole shows three potential points of substitution such as C-2, C-4 and C-5. Oxazole is shown to have weekly basic aromatic nature**.** The numbering of oxazole is done in the order of the ring starting from the oxygen atom and are designated as 1, 3- oxazole to assign the position of heteroatom in the ring [2]. The acidity of a hydrogen atom decreases in the order C (2) > C (5) > C (4) which shows the reactivity of oxazole [3]. Oxazole contain an oxygen atom at 1 position and a nitrogen atom at the 3 positions of the ring [4].

Oxazole have a wide range of medicinal properties like antimicrobial (M.B. Shukla *et al.*, 2016) [5], anthelmenthic (B.S Rawat *et al*., 2016) [6], Anti-human picornavirus (A. Mai *et al*., 1996) [7], antioxidant (I. Stankova *et al*., 2009) [8], anti-inflammatory (A.K. Sakya *et al*., 2015) [9], anticancer (R.J Dougal *et al*., 2012) [10], Pesticidal activity (A.S. Abdel *et al.,* 2009) [11] and Antiepileptic (A. Ghosh, 2010) [12]. Other than this, oxazole derivatives also have application such as photophysical properties

and Anti corrosion potential against stainless steel (L316) SS (A. Ehsani *et al*., 2016) [13].

In both developed and developing countries diabetes is a very common metabolic disease condition in which there is an abnormal high level of blood sugar which is very complicated disease affecting humans and animals. In diabetic patients the blood sugar level is above 180 mg/dl. Diabetes mellitus is a varied group of disorders that disturb the metabolism of carbohydrate and other body nutrients. It is a defect in which the body's ability to convert glucose (sugar) to energy [14].

An antioxidant are those substances that when present at minimum concentration, compare with those of the oxidizable substrate, considerably delays or inhibits oxidation of the substrate. Oxygen is a very reactive atom that is able to becoming part of potentially damaging molecules commonly called "free radicals" [15]

Antioxidants have been generally divided into two class primary or chain breaking antioxidants and secondary or preventive antioxidants are compounds that retard the rate of oxidation. This may be achieving in a number of ways including exclusion of substrate or singlet oxygen quenching [16]. Primary antioxidants, when present in trace amounts, may either setback or inhibit the initiation step by reacting with a lipid radical or inhibit the propagation step by reacting with the peroxyl or alkoxyl radicals. The antioxidant free radical may further hinder with chain propagation reaction by forming peroxy antioxidant compounds. Chain- breaking antioxidants may occur naturally or they may be formed synthetically as in the case of BHT, BHA, TBHQ (tert butyl hydroquinine) [17].

2. MATERIAL AND METHODS

2.1. Chemicals and regents

All the chemicals were procured by Himalayan Institute of Pharmacy and Research, Dehradun (Manufacturer Central Drug House, New Delhi). Silica gel and fluorescent active TLC plates were purchased from E.Merck. All the solvents were commercially available and used without purification.

2.2. Instruments

The melting points were determined by melting point apparatus using capillary tubes. The entire synthesized compound was checked for thin layer chromatography (TLC) on pre-coated TLC plates and spots were detected through exposure to (Ultra-Violet) UV-lamp chamber at 254nm, 365nm and visible light. The 1H-NMR spectra were recorded in model 400MHz (Buruker) by using Dimethyl sulfoxide (DMSO) as a solvent. Elemental analysis data are in accordance with the theoretically calculated percentage of C, H, N and S. Percentage of C, H, N and S were derived using Perkin Elmer (Punjab University).

2.3. Experimental procedure

[A] Synthesis of N-4-phenyloxazole-2, 4-diamine

The corresponding acetanilide (13.5 gm, 0.1mol) and urea (12 gm, 0.2mol) is transferred in a dry beaker equipped with mechanical stirrer. Then iodine (15 ml, 0.1mol) is added drop wise continuously in the mixture. The resulting mixture is stirred for about 20 minutes at ambient temperature. The solution is boiled for 6-7 hrs. until the reaction is complete. The mixture is then cooled in ice bath, filtered and collected. Neutralize the solution and wash with $NaHCO_3$ solution, extract with diethyl ether (3 times with 15 ml) the aqueous layer is separated and wash 3 times with water (20 ml). The product is dried and recrystallizes with hexane. The compound is purified in column chromatography and TLC is prepared by n-Hexane: Ethyl acetate (8:2). Yield: 86%. **(Scheme 1)** The oxazole derivative exists in crystalline form and white in color.

[B] Synthesis of 2-Methyl-N-phenyloxazole amine

The corresponding acetanilide (13.5 gm, 0.1mol) and acetamide (11.8 gm, 0.2mol) is transferred in a dry beaker equipped with mechanical stirrer. Then iodine (15 ml, 0.1 mol) is added dropwise continuously in the mixture. The resulting mixture is stirred for about 20 minutes at ambient temperature. The solution is boiled for 6-7 hrs until the reaction is complete. The mixture is then cooled in ice bath, filtered and collected. Neutralize the solution and wash with $NaHCO_3$ solution, extract with diethyl ether (3 times with 15 ml) the aqueous layer is separated and wash 3 times with water (20 ml). The product is dried and recrystallizes with hexane. The compound is purified in column chromatography and TLC is prepared by n-Hexane: Ethyl acetate (8:2). Yield: 82%

(Scheme-2) The oxazole derivative exists in crystalline form and white in color.

[C] Synthesis of N,2-diphenyloxazol-4-amine

The corresponding acetanilide (13.5 gm, 0.1mol) and benzamide (24.2 gm, 0.2mol) is transferred in a dry beaker equipped with mechanical stirrer. Then iodine (15 ml, 0.1 mol) is added dropwise continuously in the mixture. The resulting mixture is stirred for about 20 minutes at ambient temperature. The solution is boiled for 6-7 hrs until the reaction is complete. The mixture is then cooled in ice bath, filtered and collected. Neutralize the solution and wash with $NaHCO_3$ solution, extract with diethyl ether (3 times with 15 ml) the aqueous

layer is separated and wash 3 times with water (20 ml). The product is dried and recrystallizes with hexane. The compound is purified in column chromatography and TLC is prepared by n-Hexane: Ethyl acetate (8:2). Yield: 82%.

(Scheme-3) The oxazole derivative exists in amorphous form and cream in color.

[D] Synthesis of N-phenyloxazol-4-amine

The corresponding acetanilide (13.5 gm, 0.1mol) and formamide (9.0 gm, 0.2mol) is transferred in a dry beaker equipped with mechanical stirrer. Then iodine (15 ml, 0.1 mol) is added dropwise continuously in the mixture. The resulting mixture is stirred for about 20 minutes at ambient temperature. The solution is boiled for 6-7 hrs until the reaction is complete. The mixture is then cooled in ice bath, filtered and collected. Neutralize the solution and wash with $NaHCO_3$ solution, extract with diethyl ether (3 times with 15 ml) the aqueous layer is separated and wash 3 times with water (20 ml). The product is dried and recrystallizes with hexane. The compound is purified in column chromatography and TLC is prepared by n-Hexane: Ethyl acetate (8:2). Yield: 80%

(Scheme-4) The oxazole derivative exists in crystalline form and light yellow in color

[E] Synthesis of 2-Ethyl-N-phenyloxazol-4-amine

The corresponding acetanilide (13.5 gm, 0.1mol) and propionamide (14.4 gm, 0.2mol) is transferred in a dry beaker equipped with mechanical stirrer. Then iodine (15 ml, 0.1 mol) is added dropwise continuously in the mixture. The resulting mixture is stirred for about 20 minutes at ambient temperature. The solution is boiled for 6-7 hours until the reaction is complete. The mixture is then cooled in ice bath, filtered and collected. Neutralize the solution and wash with $NaHCO_3$ solution, extract with diethyl ether (3 times with 15 ml) the aqueous layer is separated and wash 3 times with water (20 ml). The product is dried and recrystallizes with hexane. The compound is purified in column chromatography and TLC is prepared by n-Hexane: Ethyl acetate (8:2). Yield: 78%

(Scheme-5) The oxazole derivative exists in crystalline form and white in color.

2.4. In vitro methods employed in antidiabetic studies

Inhibition of alpha-amylase enzyme

Alpha-amylase activity was carried out by starch-iodine method. Alpha- amylase solution (0.025 mg/mL) was mixed with 16mM of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentration of extracts. After incubation at 37 °C for 10 min, starch solution (1%) was added, and the mixture was re-incubated for 1 h. Next, 0.1 mL of 1% iodine solution was added, and after adding 5 mL distilled water, the absorbance was taken at 565 nm. Sample, substrate and α-amylase blank determinations were carried out under the same reaction conditions.

Inhibition of enzyme activity was calculated as (%) = (A-C) X100/ (B-C), where, A= absorbance of the sample, B= absorbance of blank (without α-amylase), and C= absorbance of control (without starch). [18]

2.5. In vitro methods employed in antioxidant studies

2.7.1. 1, 1- Diphenyl-2-picrylhydrazyl (DPPH)

30 ml of methanol was taken in a volumetric flask and about 4.2 mg of DPPH (1, 1-Diphenyl –2-picrylhydrazyl) was dissolved in it; then it was protected from light by covering the test tubes with aluminum foil. 0.1 ml DPPH solution was added to 3ml methanol and absorbance was taken immediately at 517nm. 0.1-0.5 ml of various concentrations of oxazole compounds as well as standard compound (Ascorbic acid) was taken and the volume was made uniformly using methanol. The compound was then further diluted with methanol up to 3ml and to each 0.1 ml DPPH was added. Then the absorbance was measure after 15 min. at 517nm using methanol as blank on UV-visible spectrometer [19-20].

The free radical scavenging activity by DPPH was calculated by using the following formula-

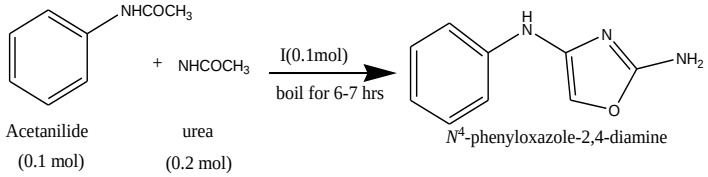
%scavenging activity= [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

2.7.2. Hydrogen peroxide radical scavenging activity

1ml of (1mg/ml) test drug/standard (Ascorbic acid) was added to 0.6ml of hydrogen peroxide solution in phosphate buffer (pH-7.4). After incubating for 10 minutes at 37° C the absorbance was measured at 230nm.Corresponding blanks were taken. The absorbance of hydrogen peroxide in phosphate buffer as control was measured at 230nm. The scavenging effect (%) was measured using equation. Hydrogen peroxide produces hydroxyl radicals in cells. Scavenging hydrogen peroxide radicals by the test compounds is used as a test for antioxidant activity. The reduction of these radicals is seen by the decreased absorbance at 230nm with increasing concentration of the test drug. [21]

Experimental procedure

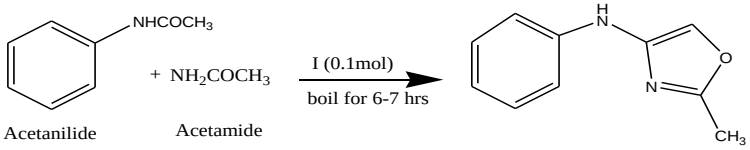
[A] Synthesis of N-4-phenyloxazole-2, 4-diamine



Acetanilide (0.1 mol) | urea (0.2 mol) | N^4-phenyloxazole-2,4-diamine

Scheme 1: Synthesis of N-4-phenyloxazole-2, 4-diamine

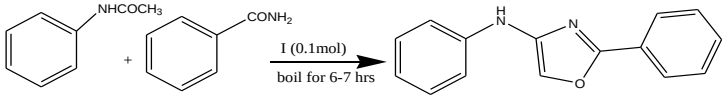
[B] Synthesis of 2-Methyl-N-phenyloxazole amine



Acetanilide (0.1 mol) | Acetamide (0.2 mol) | 2-methyl-N-phenyl oxazoleamine

Scheme 2: Synthesis of 2-Methyl-N-phenyloxazoleamine

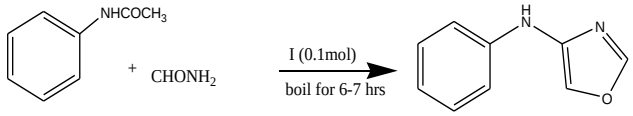
[C] Synthesis of N,2-diphenyloxazol-4-amine



Acetanilide (0.1 mol) | Benzamide (0.2 mol) | *N*,2-diphenyloxazol-4-amine

Scheme 3: Synthesis of N, 2-diphenyloxazol-4-amine

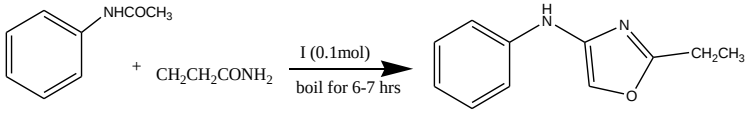
[D] Synthesis of N-phenyloxazol-4-amine



Acetanilide (0.1 mol) | Formamide (0.2 mol) | *N*-phenyloxazol-4-amine

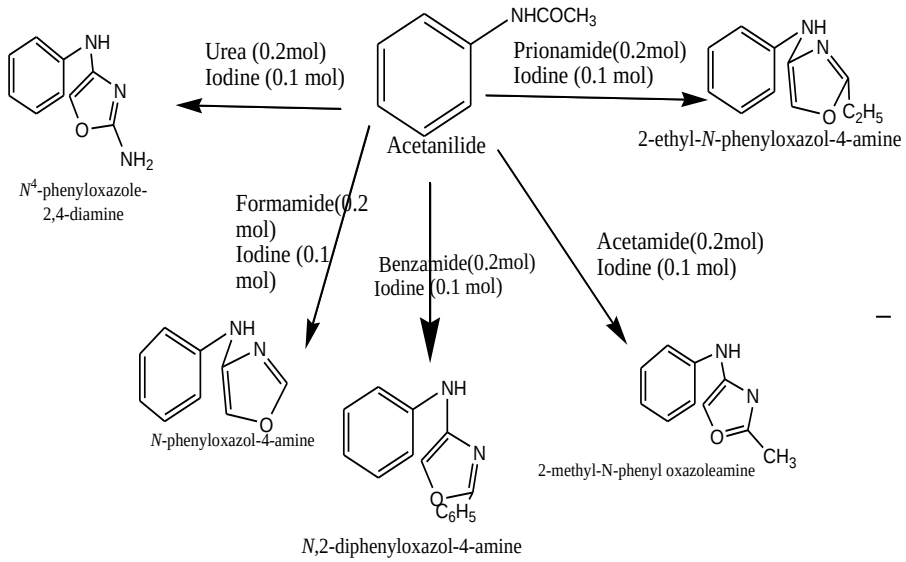
Scheme 4: Synthesis of N-phenyloxazol-4-amine

[E] Synthesis of 2-Ethyl-N-phenyloxazol-4-amine



Acetanilide (0.1 mol) | Propionamide (0.2 mol) | 2-ethyl-*N*-phenyloxazol-4-amine

Scheme 5: Synthesis of 2-Ethyl-N-phenyloxazol-4-amine



Scheme 6: Flowchart of synthesis of oxazole derivative

3. RESULT AND DISCUSSION

3.1. Chemistry and spectral studies

The novel oxazole derivatives are synthesized according to the described process in scheme 6. The structure of all the compounds were evaluated on the basis of ^{1}H NMR and elemental analysis. In the 1H NMR spectra of oxazole, multi signals are assigned to Aromatic protons observed in the range from 7.0 to 7.65 δppm. A sharp singlet is observed for oxazole A_1, A_2, A_3, A_4 and A_5 at 9.9 δppm, 7.03 δppm and 7.4 δppm respectively in spectrum. Methyl group protons appear as singlet at 1.24 δppm. The amine group proton for A_1 is observed as a broad singlet at 7.24 δppm in the spectrum.

3.2. Antidiabetic activity

The *in vitro* antidiabetic activity of synthesized compounds was estimated by using enzyme alpha-amylase in order to check their percentage inhibition. All compounds show dose dependent increase in percentage inhibition. Voglibose is used as a standard. The compound shows lower inhibition as compare to standard drug. The compound A_1 (78%) and A_4 (65%) showed good Antidiabetic activity, indicating that electron donating groups enhanced the Antidiabetic activity. Results are shown in Table-3 and Figure no-1.

3.3. Antioxidant activity

The *in vitro* antioxidant potential of compound was estimated from DPPH and hydrogen peroxide scavenging activity. The reducing value of compound was significantly lower than that of ascorbic acid.

3.3.1. Free radical scavenging activity by 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) assay method

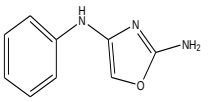
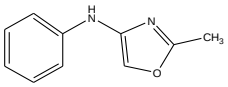
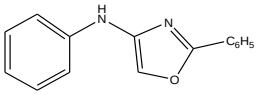
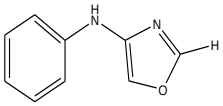
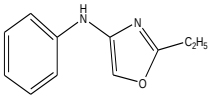
All the compounds (A_1-A_5) were screened by DPPH scavenging assay method. The compounds A_1 (78.06%), A_4 (67%) showed good scavenging effect, indicating that electron donating groups enhanced the antioxidant activity. Table -4 and Figure 2 show the DPPH radical scavenging activity of compound with respect to ascorbic acid which was used as standard compounds in the study. It was observed from the study that the DPPH radical scavenging activity of compounds was significantly lower than that of Ascorbic acid.

3.3.2. Hydrogen peroxide scavenging assay method

All the compounds (A_1-A_5) were screened by hydrogen peroxide scavenging assay method. The compounds A_1 (72.8%), A_4 (68.8%) showed good scavenging effect, indicating that electron donating groups enhanced the antioxidant activity. Figure 3 show the hydrogen peroxide radical scavenging activity of compound with respect to ascorbic acid which was used as standard compounds in the study. It was observed from the study that the hydrogen peroxide radical scavenging activity of compounds was significantly lower than that of Ascorbic acid.

Spectral studies of derivatives

Table 1: ^{1}H NMR data and interpretation of synthesized compounds

Compound No.	Compound structure	δ ppm	Group	Number of H	Interpretation
A_1		9.9 7.0-7.61 7.03 7.3	NH Ar –H CH NH_2	1 5 1 2	Singlet, 1H Multiplet, 5H Singlet, 1H Doublet, 2H
A_2		9.9 7.0-7.61 7.03 2.05	NH Ar –H CH CH_3	1 5 1 3	Singlet, 1H Multiplet, 5H Singlet, 1H Singlet, 3H
A_3		9.9 7.01-7.9 7.20-7.48 7.03	NH Ar-H Ar-H CH	1 5 5 1	Singlet, 1H Multiplet, 5H Multiplet, 5H Singlet, 1H
A_4		9.9 7.0-7.60 7.2-7.6	NH Ar-H CH_2	1 5 2	Singlet, 1H Multiplet, 5H Singlet, 2H
A_5		9.9 7.0-7.60 7.2-7.6 2.59 1.24	NH Ar-H CH_2 CH_2 CH_3	1 5 2 2 3	Singlet, 1H Multiplet, 5H Singlet, 2H Doublet, 2H Singlet, 3H

ELEMENTAL ANALYSIS

Table 2: Elemental analysis of different compounds

Compound No.	Molecular Weight	Molecular Formula	Compound Name	Calculated % found			
				C%	N%	O%	H%
A_1	175.19	$C_9H_9N_3O$	N-4-phenyloxazole-2,4-diamine	61.70	23.99	9.13	5.18
A_2	174.2	$C_{10}H_{10}N_2O$	2-methyl-N-phenyloxazoleamine	68.95	16.08	9.18	5.95
A_3	236.27	$C_{15}H_{12}N_2O$	N,2-diphenyloxazol-4-amine	76.25	11.86	6.77	5.12
A_4	160.17	$C_9H_8N_2O$	N-phenyloxazol-4-amine	67.49	17.49	9.99	5.03
A_5	188.23	$C_{11}H_{12}N_2O$	2-ethyl-N-phenyloxazol-4-amine	70.19	14.88	8.50	6.43

RESULT AND DISCUSSION

Table 3: *in vitro* antidiabetic activity of compounds using alpha amylase

Conc. (mg/ml)	% inhibition of different derivatives and standard					
	A_1	A_2	A_3	A_4	A_5	Standard
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.02	40	25	23	30	36	43
0.04	53	37	30	35	39	59
0.06	66	50	44	49	48	75
0.08	70	55	48	61	52	80
0.10	78	60	52	65	61	93

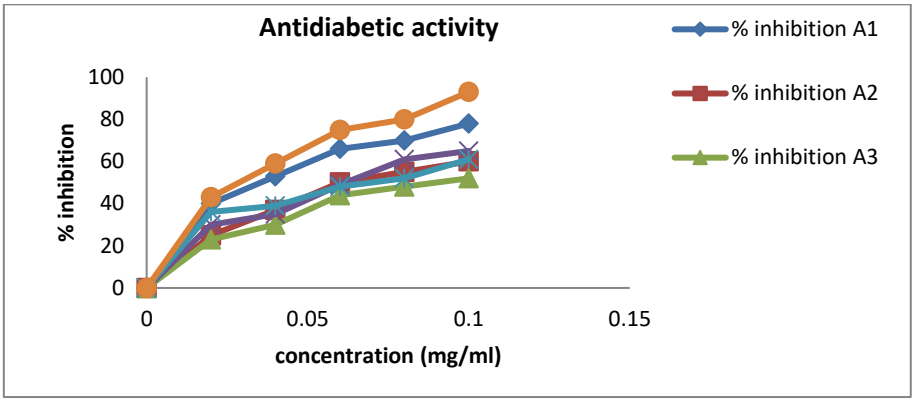


Figure 1: Alpha-amylase enzyme inhibition of different derivative

Table 4: DPPH scavenging activity of different derivatives

Concentration (mg/ml)	% inhibition					
	A_1	A_2	A_3	A_4	A_5	Ascorbic acid
0	0.0	0.0	0.0	0.0	0.0	0.0
0.02	42.44	20.4	32.57	30.6	36.8	56.2
0.04	56.83	24.2	41.27	37.3	41.02	79.2
0.06	64.6	42.5	47.22	48.6	51.32	90.01
0.08	67.03	53.6	54.14	60.32	55.33	95.12
0.10	78.06	60.05	63.44	67.03	62.11	98.42

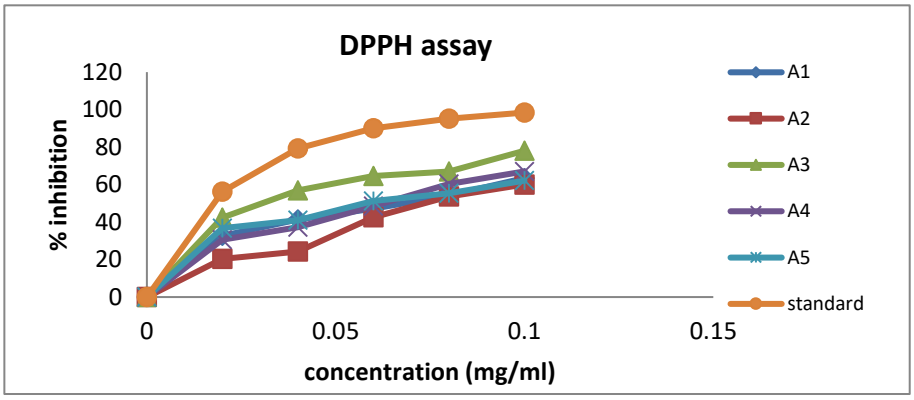


Figure 2: DPPH radical scavenging activity of compounds

Table 5: Hydrogen peroxide scavenging activity of different derivatives

Concentration (mg/ml)	% inhibition of different derivatives with standard					
	A_1	A_2	A_3	A_4	A_5	Ascorbic acid
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.02	36.6	32.4	23.8	33.9	34.00	41.33
0.04	49.3	40.3	35.5	44.00	39.6	58.9
0.06	60.8	56.7	49.5	56.7	48.23	64.6
0.08	68.5	60.1	53.9	60.9	55.09	78.38
0.10	72.8	60.7	67.3	68.8	61.8	93.45

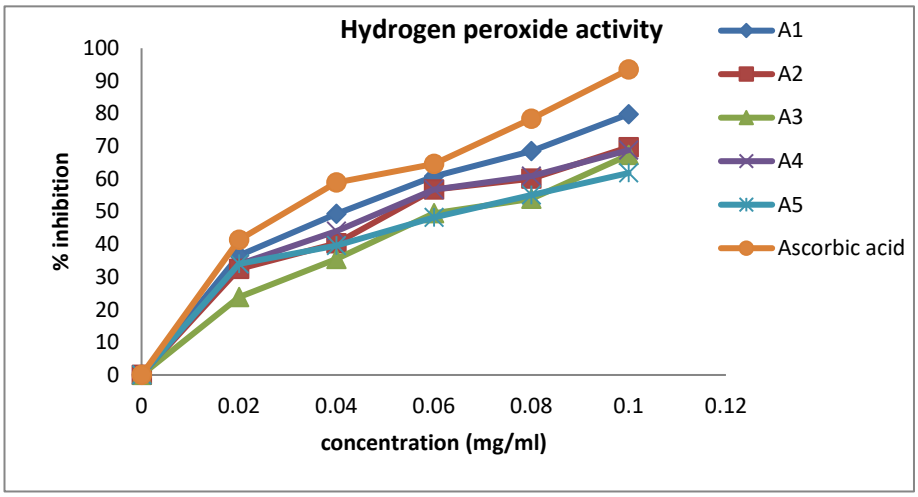


Figure 3: Hydrogen peroxide scavenging activity

CONCLUSION

The present study attempts to investigate in-vitro antidiabetic activity and antioxidant activity of synthesized 1, 3-oxazole and its derivatives. The compound A_1 shows higher percentage practical yield. Total five derivatives A_1, A_2, A_3, A_4 and A_5 were synthesized and tested for them *in vitro* antidiabetic and antioxidant activity. The present findings divulge that all synthesized compounds efficiently inhibit alpha amylase enzymes as *in vitro* in a dose dependent manner. From the result compounds A_1 showed highly significant activity against alpha-amylase enzyme in comparison to standard Voglibose. The result shows that as the concentration of compound increase, the compound showed high significant activity against alpha-amylase enzyme. All tested compound showed low to moderate activity against alpha-amylase. Compound A_1 showed highly significant activity due to presence of electron donating group against DPPH and Hydrogen peroxide in comparison to standard ascorbic acid. The result shows that as the concentration of compound increase, the compound showed high significant activity against DPPH and hydrogen peroxide. The other entire tested compound showed low to moderate activity. The study could be concluded that the synthesized compounds have effective against anti-diabetic and anti-oxidant activity. These 1,3-oxazole derivatives could be selected as lead molecules for further synthetic and biological evaluation.

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