Environmentally benign Synthesis of novel 5-amino-isoxazole-4-carbonitriles by employing Glucose coupled Fe_3O_4(Glu.@Fe_3O_4) as an organocatalyst in aqueous-ethanolic medium and their Antioxidant Potential analysis

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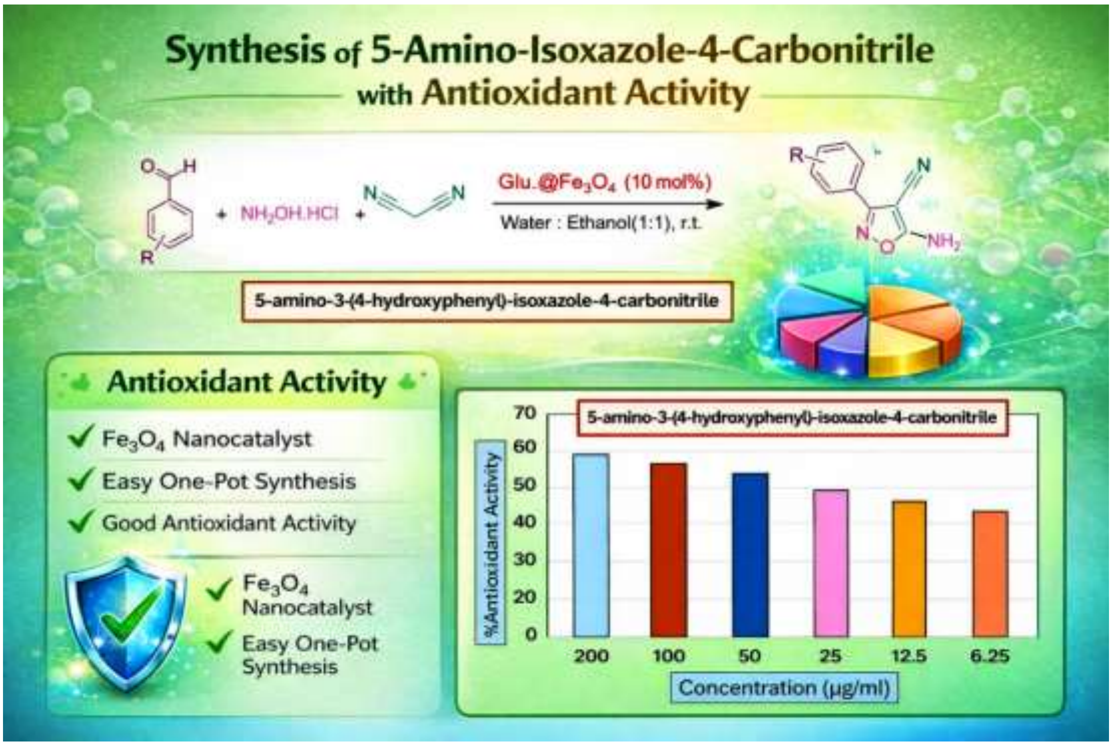


Abstract: An innovative approach has been developed to synthesize 5-amino-isoxazole-4-carbonitrile analogues in a green environment at optimum room temperature. The synthetic process has been carried out in a one-pot multicomponent fashion by employing different aromatic aldehydes, malanonitrile and hydroxylamine hydrochloride in presence of glucose coupled iron oxide (Glu.@Fe_3O_4) as an organocatalyst and water: ethanol(1:1) as solvent. The target heterocyclic scaffolds have been ascertained by FT-IR and NMR spectroscopy. The prepared organocatalyst has been characterized and structurally verified by performing FTIR and X-ray diffraction analysis. The proposed mechanistic process is efficient compared to previously known protocols in terms of short reaction duration, absence of column chromatography, use of ecofriendly solvents and so on. The antioxidant potential of synthesized compounds have been analyzed using in vitro DPPH assay method and have depicted their scavenging radical characteristics.

Keywords: green environment, room temperature,one-pot multicomponent fashion, organocatalyst, antioxidant potential.

Introduction:

The remarkable biological activity of isoxazole heterocycles makes them extremely interesting. Recent years have seen a significant amount of pharmaceutical and clinical research on the treatment of pain.[1,2] Isoxozole-containing pharmacoactive compounds are crucial to medicinal chemistry, according to a thorough analysis of this heterocyclic scaffold. In typical practice, anti-inflammatory, analgesic, and chemotherapeutic medications are analyzed.[3] Additionally, there is proof that small compounds with anti-retroviral activity that

target host elements crucial for HIV-1 replication can be developed using innovative synthetic techniques.[4] The five-membered aromatic heterocycles known as isoxazoles possess adjacent nitrogen and oxygen atoms.
The structure of 5-amino-isoxazole-4-carbonitrile has been depicted in **Figure 1**.

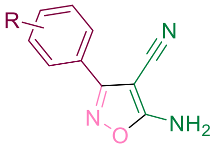


Figure 1: Structure of 5-amino-isoxazole-4-carbonitrile

A wide range of therapeutically intriguing drug candidates, such as AMPA receptor agonists, antithrombin activity, anti-rhinovirus agents, antidepressants, antinociceptive activity, antibacterial activity, anticonvulsant activity, nonnucleoside HIV-1 reverse transcriptase inhibitors, antitumor, cox-1/cox-2 inhibitors, HDAC inhibitors, antitubulin activity, 5-HT reuptake inhibitors, adrenoceptor antagonists, humoral immune response inhibitors, and neurological disorders like schizophrenia.

Cycloisomerization of α, α-acetylenic oximes ;[5] cycloaddition of aldoxime and alkynes ;[6] reaction of alkyl nitriles and α-chlorooximes ;[7] 1,3-dipolar cycloaddition of in situ generated nitrile oxides and terminal acetylenes;[8] addition of hydroxylamine to α-cyano ketones;[9] and four-component coupling of a terminal alkyne, hydroxylamine, and carbon monoxide[10] are a few newly developed isoxazole synthetic techniques.

Furthermore, multicomponent reaction of active methylene compounds, aldehydes and hydroxylamine derivatives were well assessed under different conditions [11-17].

In view of the above discussion, we have devised a novel strategy for synthesizing 5-amino-isoxazole-4-carbonitrile by incorporating aromatic aldehydes, malanonirtile and hydroxylamine hydrochloride using (Glu.@Fe_3O_4) as an organocatalyst in water:ethanol(1:1) as solvent .

Experimental

Materials and methods

Commercial sources have provided quality analytical chemicals and reagents for the procedure, which can be employed readily without the necessity to undergo further purification. All the precursors, comprising hydroxylamine hydrochloride, malononitrile and aromatic aldehydes, have been purchased from TCI and Sigma-Aldrich. For determining the melting points, the unrectified open glass capillary procedure was used.
The 400 FT spectrophotometer, BRUKER AVANCE II (1H NMR at 400 MHz and 13C NMR at 100 MHz), was used to analyze the structure identification of the synthesized target moeity. Using TMS as the internal standard and $CDCl_3$ as a solvent, the chemical shift results

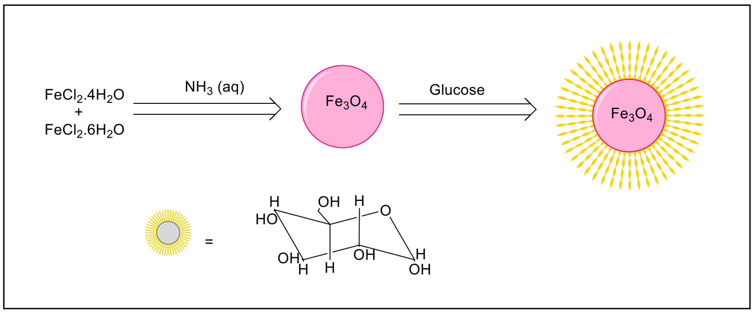
are expressed in δ(ppm). The studies were monitored using twenty 0.25 mm thick silica gel G/UV-254 pre-coated TLC sheets.

Catalyst preparation [Glu.@Fe_3O_4]

A conventional co-precipitation mechanistic process was employed to prepare Superparamagnetic Fe_3O_4 nanoparticles.

In a 150 ml round bottom flask,added $FeCl_3.6H_2O$ (10 mmol) alongwith $FeCl_2.4H_2O$ (5 mmol) salts and dissolved it in 45 milliliters of distilled water. At room temperature, the reaction mixture was vigorously agitated for two minutes at 950 rpm. After that, drops of ammonia solution (25% w/w) were added to create a basic medium (pH of about 11).Stirring occurred forcefully at room temperature for a duration of 1 hour on the acquired black suspension and it was subsequently refluxed for 6 hours duration. An external magnet was applied to isolate the prepared magnetic Fe_3O_4 nanoparticles and washed multiple times using distilled water followed by ethanol.The synthesized Fe_3O_4 nanoparticles were transferred to a flask containing mixture of glucose (20 mmol) dissolved in distilled water(40 ml). The ultrasonic radiations were passed through the mixture during the time span of 20 minutes. After being separated from the basic medium, the resultant superparamagnetic nanoparticles (Glu.@Fe_3O_4) were repeatedly cleaned with distilled water, ethanol, and then left to dry overnight at 60°C.

(This synthetic procedure was previously used by me to prepare the catalyst for my research paper).[18]



Scheme 1: Catalyst synthetic procedure

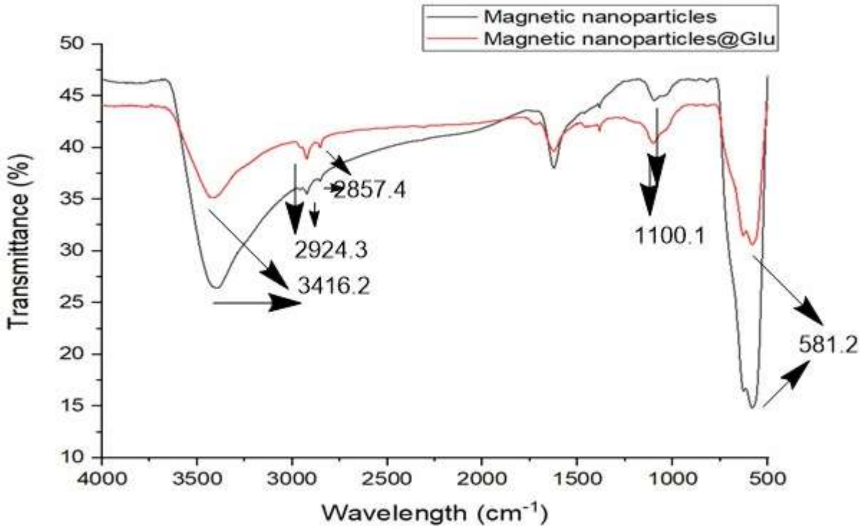
General Procedure:

An equimolar mixture (1 mmol) of malononitrile, hydroxylamine hydrochloride and aromatic aldehyde was added to a 50 ml round bottom flask along with 10 mol% (Glu.@Fe_3O_4) as an organocatalyst and 3ml each of water and ethanol,i.e., water : ethanol(1:1).The reaction mixture was stirred for the required time. Upon completion of reaction as indicated by TLC (hexane: ethyl acetate = 7:3), the reaction mixture was filtered, washed with cold water, then with cold water-ethanol mixture and recrystallized from ethanol to attain the pure product.

Spectral information of all compounds

FT-IR Spectra of [Glu.@Fe_3O_4]

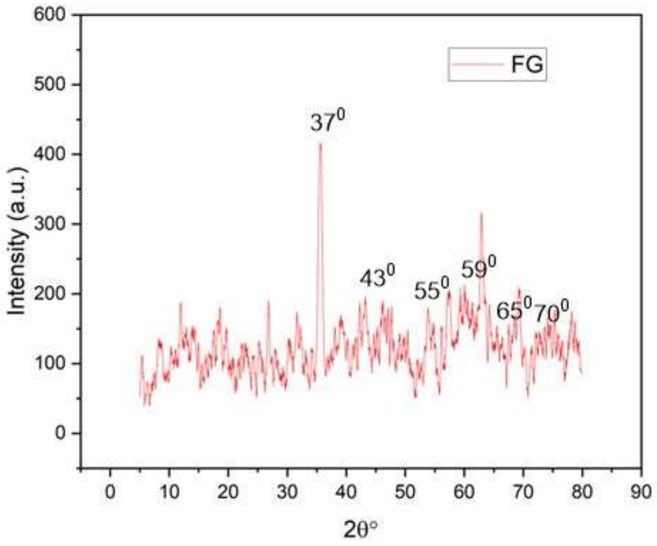
The broad signal at 3416.2 cm^{-1} in the FT-IR spectra of Fe_3O_4 and Fe_3O_4 encapsulated with glucose (Glu.@Fe_3O_4) is indicative of the ─OH functional group. They are associated with the stretching vibrations of C(sp^3)-H , C(sp^2)-H , and Fe-O bonds , respectively, and peak locations are 2924.3, 2857.4, 1100.1, and 581.2 cm^{-1}.



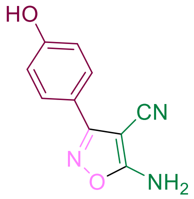
FTIR Spectra of Glu.@Fe_3O_4

XRD analysis of [Glu.@Fe_3O_4]

The prepared Glu.@Fe_3O_4 was examined to evaluate the crystalline structure.The six peaks (2θ = 37°, 43°, 55°, 59°, 65° and 70°) are representative of Fe_3O_4 crystalline structure. Therefore, it is clear that the glucose coating has no effect on the magnetic nanoparticles' structure.

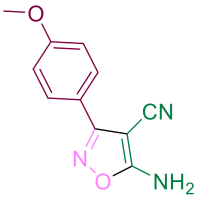


XRD pattern of Glu@Fe_3O_4



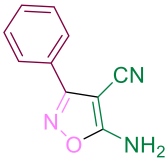
4a) 5-amino-3-(4-hydroxyphenyl)-isoxazole-4-carbonitrile

IR (KBr) ν (cm^{-1}): 3434(OH), 3335 (NH_2), 2228 (C≡N), 1612 (C=N), 1296 (C–O–N) cm−1 ; ^1H NMR (400 MHz, $CDCl_3$) δ: 6.95 (d, 2H,Ar-H), 7.85 (d, 2H, Ar-H), 8.25 (s, 2H, NH_2), 11.06 (s, 1H, OH); 13C NMR (100 MHz, $CDCl_3$) δ: 75.53, 114.60 (C≡N), 115.51 , 117.03, 123.21 , 134.30 , 160.90, 164.30;



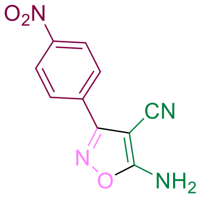
4b) 5-amino-3-(4-methoxyphenyl)-isoxazole-4-carbonitrile

^1H NMR (400 MHz, $CDCl_3$) δ:7.55 (d, 1H, Ar-H), 7.03(d, 1H, Ar-H), 6.20(s,2H,NH_2), 3.81 (s,3H, OMe) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.26, 160.95, 158.50, 128.90,118.20, 117.60, 114.50,102.60, 56.40ppm.



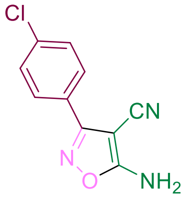
4c) 5-amino-3-(phenyl)-isoxazole-4-carbonitrile

IR (KBr) v (cm^{-1}): 3452, 2224, 1634, 1450, 1384, 1217,958, 755,677,616, 519; ^{1}H NMR (400 MHz, CDCl$_3$) δ:7.89 (d, 1H, Ar-H), 7.77(d, 1H, Ar-H), 7.54(d,1H, Ar-H),7.25(s,2H,NH$_2$)ppm; ^{13}C NMR (100 MHz, CDCl$_3$) δ: 165.26, 159.85, 135.55,134.25,130.48,129.30,125.00, 113.35 ppm.



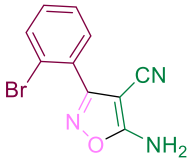
4d)5-amino-3-(4-nitrophenyl)-isoxazole-4-carbonitrile

IR (KBr) v (cm^{-1}): 3417, 3379 (NH$_2$), 2220 (C≡N), 1603 (C=N), 1541, 1361 (NO$_2$), 1289 (C–O–N) cm−1 ;^{1}H NMR (400 MHz, CDCl$_3$) δ: 8.22 (d, 1H, Ar-H), 7.73(d, 1H, Ar-H), 7.26(s,2H,NH$_2$)ppm; ^{13}C NMR (100 MHz, CDCl$_3$) δ: 156.86, 148.09, 138.13,131.40,130.93,127.48,124.66, 111.28 ppm.



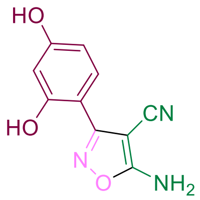
4e) 5-amino-3-(4-chlorophenyl)-isoxazole-4-carbonitrile

IR (KBr) v (cm^{-1}): 3438 (NH$_2$), 2227 (C≡N), 1637 (C=N), 1595, 1317 (NO$_2$), 1299 (C–O–N) ^{1}H NMR (400 MHz, CDCl$_3$) δ: 8.75 (d, 1H, Ar-H), 8.11(d, 1H, Ar-H), 7.82(d,1H,Ar-H), 7.71(d,1H,Ar-H), 7.49(d,1H,Ar-H),7.34(d,1H,Ar-H),7.25(s,2H,NH$_2$)ppm; ^{13}C NMR (100 MHz, CDCl$_3$) δ: 158.18, 149.42, 140.95,135.97,128.88,128.30, 113.35ppm.



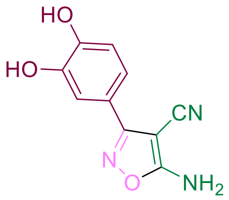
4f) 5-amino-3-(2-bromophenyl)-isoxazole-4-carbonitrile

IR (KBr) v (cm^{-1}): 3447,1635,1482,1435,1384,1318, 1279,1116, 1025,972,930,869,748,704,629,521;^{1}H NMR (400 MHz, CDCl$_3$) δ: 8.52 (d, 1H, Ar-H), 7.82(d,1H,Ar-H), 7.56(d,1H,Ar-H), 7.32(d,1H,Ar-H),7.22(s,2H,NH$_2$)ppm; ^{13}C NMR (100 MHz, CDCl$_3$) δ: 167.41, 149.40, 141.38,133.04,130.92,129.26,127.56,124.20, 107.85 ppm.



4g) 5-amino-3-(2,4-dihydroxyphenyl)-isoxazole-4-carbonitrile

^{1} H NMR (400 MHz, $CDCl_3$) δ: 11.50(s,1H,OH), 9.42(s,1H,OH), 7.43(d,1H,Ar-H), 6.56(d,1H,Ar-H), 6.24(d,1H,Ar-H),6.10(s,2H,NH_2)ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.60, 160.58,159.25, 153.40,133.85,117.64, 115.30,109.60, 105.80,104.70 ppm.



4h) 5-amino-3-(3,4-dihydroxyphenyl)-isoxazole-carbonitrile

^{1} H NMR (400 MHz, $CDCl_3$) δ: 11.98(s,1H,OH), 9.20(s,1H,OH), 7.43(d,1H,Ar-H), 6.70(d,1H,Ar-H), 6.38(d,1H,Ar-H),6.59(s,2H,NH_2)ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.80, 160.98,157.35, 153.59,133.64,117.48, 114.74,109.28, 105.70,102.70 ppm.

Result and Discussion:

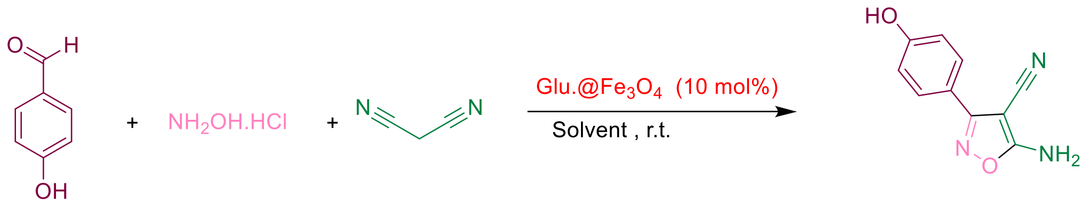
Initially, the model reaction was performed by using 4-hydroxybenzaldehde, malononitrile and hydroxylaminehydrochloride in presence of solvent at room temperature with varying amounts of the catalyst. It was observed that 5 mol% of catalyst led to lower yield of 80% . The best results were procured with 10 mol% of catalyst and an yield of 94%. On increasing the catalytic amount to 15%, gave similar results and further increasing it to 20 mol% led to decrease in yield to 89%.**(Table 1).**

Table 1: Catalyst Loading

S. No.	**Catalyst amount (mol%)**	**Time(min.)**	**Yield%**
1	5	35	80
2	**10**	**15**	**94**
3	15	15	94
4	20	20	89

Thus, in order to develop 5-amino-isoxazole-4-carbonitrile analogues(4a-4h) , we incorporated the most optimal reaction conditions (**Table 2**,**Entry 4**) for analyzing substrate scope. The variation of several solvent used and various reaction parameters affecting the yield % is explained.

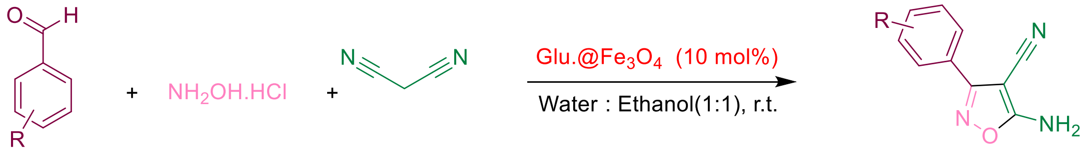
Table 2: Optimization of Solvents

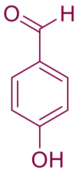
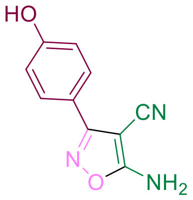
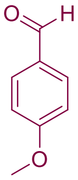
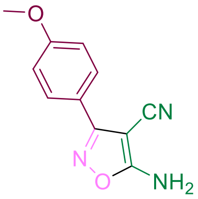


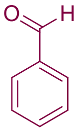
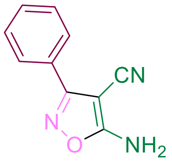
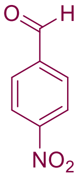
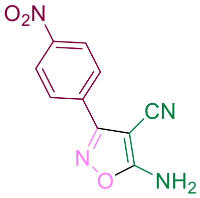
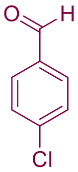
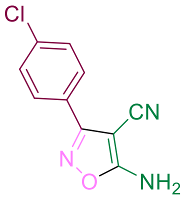
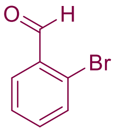
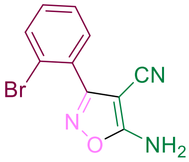
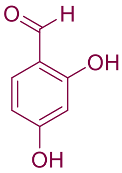
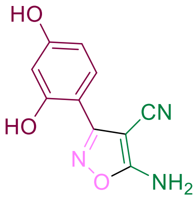
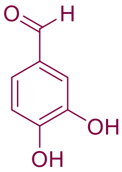
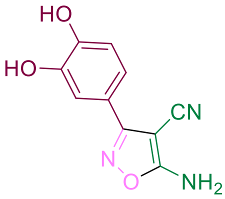
S.No.	Solvent	Catalyst	Temperature(°C)	Time(min.)	Yield%
1	Ethanol	10 mol%	r.t.	30	85
2	Water:Ethanol(9:1)	10 mol%	r.t.	80	80
3	Water:Ethanol(3:2)	10 mol%	r.t.	40	82
4	**Water:Ethanol(1:1)**	**10 mol%**	**r.t.**	**15**	**94**
5	Water:Ethanol(1:1)	10 mol%	45	20	88
6	Water	10 mol%	r.t.	35	86
7	Neat	10 mol%	r.t.	120	40

Once the reaction conditions have been selected, the substrate scope is evaluated. It was noticed that both electron donating and electron withdrawing group substituted aldehydes gave excellent output. The maximum yield has been obtained with 4-hydroxybenzaldehyde as the precursor.

Table 3 : Substrate Scope



S.No.	R	Time (min.)	Product	Yield%
1	 **1a**	**15**	 **4a**	**94**
2	 1b	15		91

			4b	
3	 1c	20	 4c	85
4	 1d	18	 4d	89
5	 4e	19	 4e	86
6	 4f	22	 4f	83
7	 4g	17	 4g	88
8	 4h	18	 4h	87

Recyclability of the catalyst:

Following the successful completion of the reaction, superparamagnetic nanocatalyst was isolated from the solution, as often accomplished by using an external magnet. It was then repeatedly cleaned with water, dried, and utilized for subsequent reaction sequences. The organonanocatalyst was reused until the yield percentage declined and the results were subpar.

Thus, the produced organocatalyst's recyclability was examined (Figure 2). We see a decline in the catalyst's catalytic activity after it exhibits outstanding results for five rounds in a row.

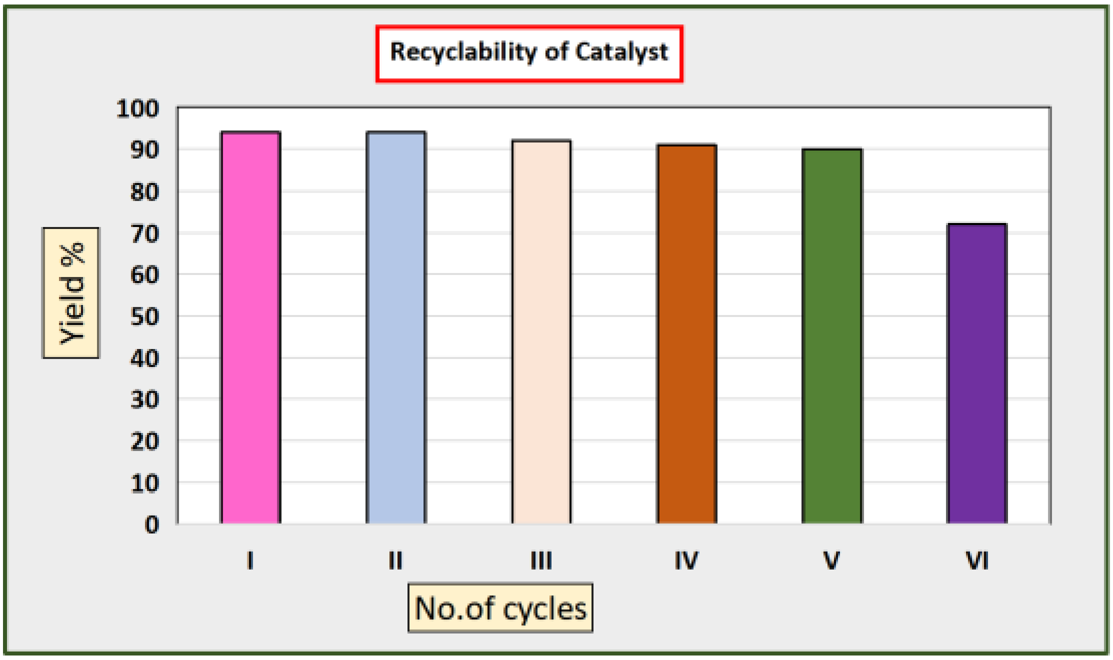
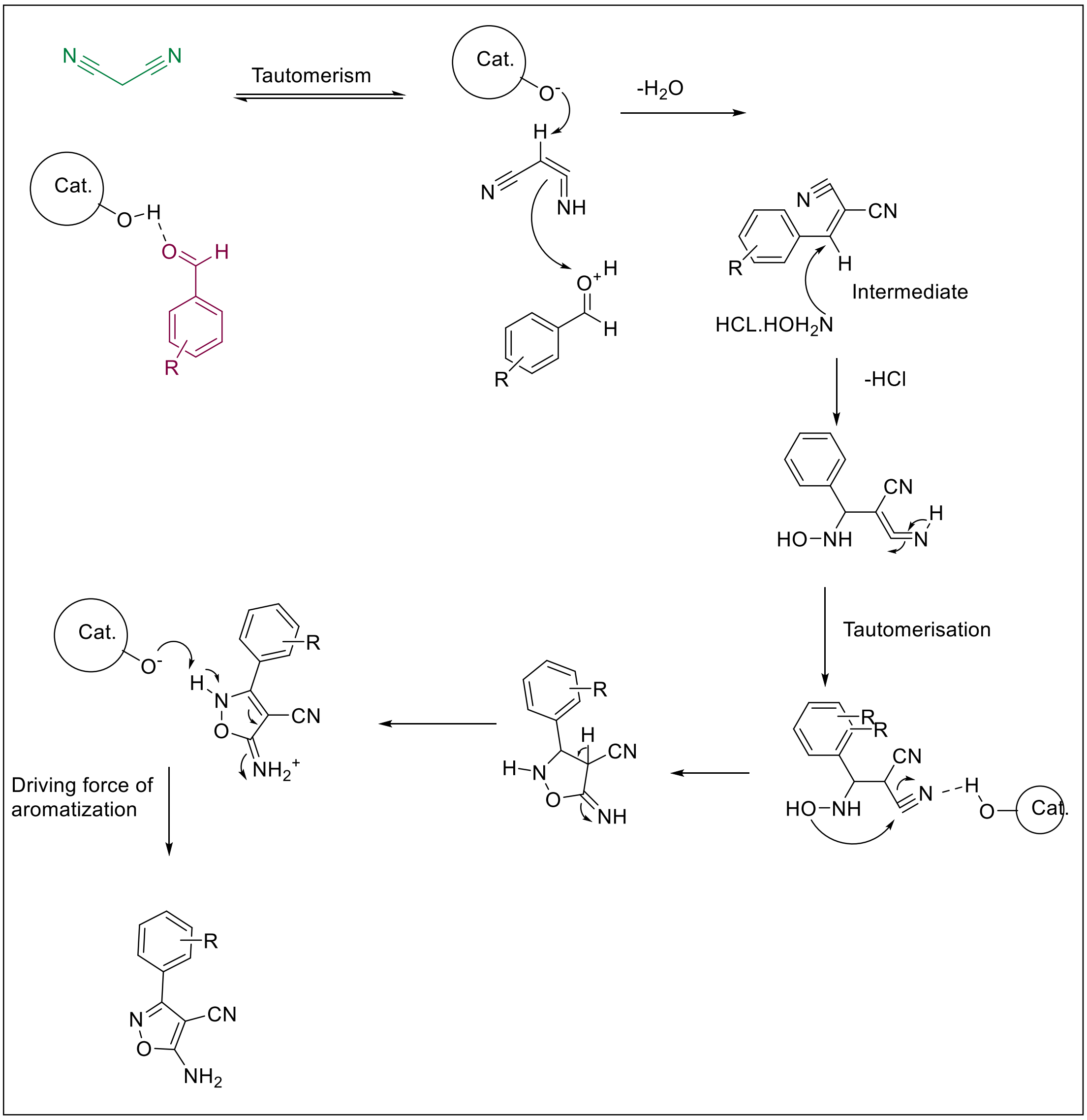


Figure 2: Recyclability of catalyst.

Plausible reaction mechanism:

Firstly, the mild acidic nature of (Glu.@Fe_3O_4) initiates knoevenagel condensation in between active methylene compound malononitrile and carbonyl group of aldehyde. Then the hydroxylamine hydrochloride attacks the daouble and subsequent tautomerisation followed by aromatization led to the formation of 5-amino-isoxazole-4-carbonitrile.



Scheme 3 : Reaction mechanism

Table 4: Comparison of previously reported catalyst with respect to 4a

S.No.	Conditions	Catalyst	Time(min)	Yield%	References
1	EtOH,reflux	DABCO	1.5-15	65-85	19
2	CH_3CN, rt	-	3600-9000	70-93	20
3	Aq.EtOH, hv	CH3CO2Na	5-10	61-89	21
4	H_2O,rt	KP	30-150	85-96	22
5	H_2O,rt	Boric acid	50-1440	82-95	23
6	**H_2O:EtOH(1:1),rt**	**Glu.@Fe_3O_4**	**15-22**	**83-94**	**Present work**

Antioxidant activity

The % Anti-oxidant activity or % inhibition of few of the synthesized compounds 4a-4e was calculated by performing the experiments uing the DPPH assay technique.

Materials and Methods:

The compounds 4a - 4e were synthesized in the laboratory and was evaluated for their anti oxidant activity. Methanol AR was purchased from Loba Chemie Pvt. Ltd. , Mumbai India.Gallic acid which is taken as a reference has been bought from CDH Pvt. Ltd. , New Delhi, India.

The instrument employed to record the absorbance of the sample was UV-visible spectrophotometer.

1, 1-Diphenyl-2-picrylhydrazyl (DPPH* Assay) (Pandey et al., 2014)

DPPH* was used as a radical scavenger and gallic acid as the standard. DPPH* has an odd electron due to which it gives maximum absorption at 517 nm and the procedure follows the decrease in absorption and showed the antioxidant activity of that compound.

The formula used for the calculation is:-

% Inhibition of DPPH* activity = (A-B)/A * 100

Where A= absorbance of control, B= absorbance of sample

Experimental Procedure:

DPPH* scavenging assay

Stock solutions (1mg/10 mL) of synthesized compounds 4a - 4e was prepared in methanol. The stock solution of DPPH* was also prepared at the concentration of 2mg/100 mL in methanol.

Different concentrations such as 2 mL, 1.5 mL, 1 mL, 0.5 mL, 0.25 mL, 0.125 mL of compound 4a was taken out in the test tube separately and made up the volume up to 2 mL by adding methanol respectively. Now add 2 mL DPPH* solution in each test tube and make the volume up to 4 mL and kept the solutions for 30 minutes in dark. After the incubation, the absorbance of all the samples was recorded by UVspectrophotometer. Similarly, performed further experiments for compounds 4b - 4e and gallic acid which is used as a standard reference.

Control was prepared by mixing 2 ml each of DPPH and methanol and kept for 30 minutes and subsequently, its absorbance was recorded.

Result and Discussion:

The % anti-oxidant activity of compound 4a-4e is determined using the experimental procedure described above. The absorbance was recorded for multiple concentrations of the stock solution of the sample and absorbance of control was recorded.

The % anti-oxidant activity was then calculated using the formula already discussed.

It was observed from the data depicted in Table 5 and Figure 2 that the maximum antioxidant activity was determined when the concentration of the sample 5-amino-3-(4-hydroxyphenyl)-isoxazole-4-carbonitrile (4a) was maximum of 200 µg/ml.

Table 5: %Antioxidant activity of 5-amino-3-(4-hydroxyphenyl)-isoxazole-4-carbonitrile

S.No.	Concentration (µg/ml)	Absorbance (nm)	% Antioxidant activity
1	200	0.263	58.3
2	100	0.273	56.7
3	50	0.288	54.3
4	25	0.334	46.9
5	12.5	0.337	46.5
6	6.25	0.354	43.8

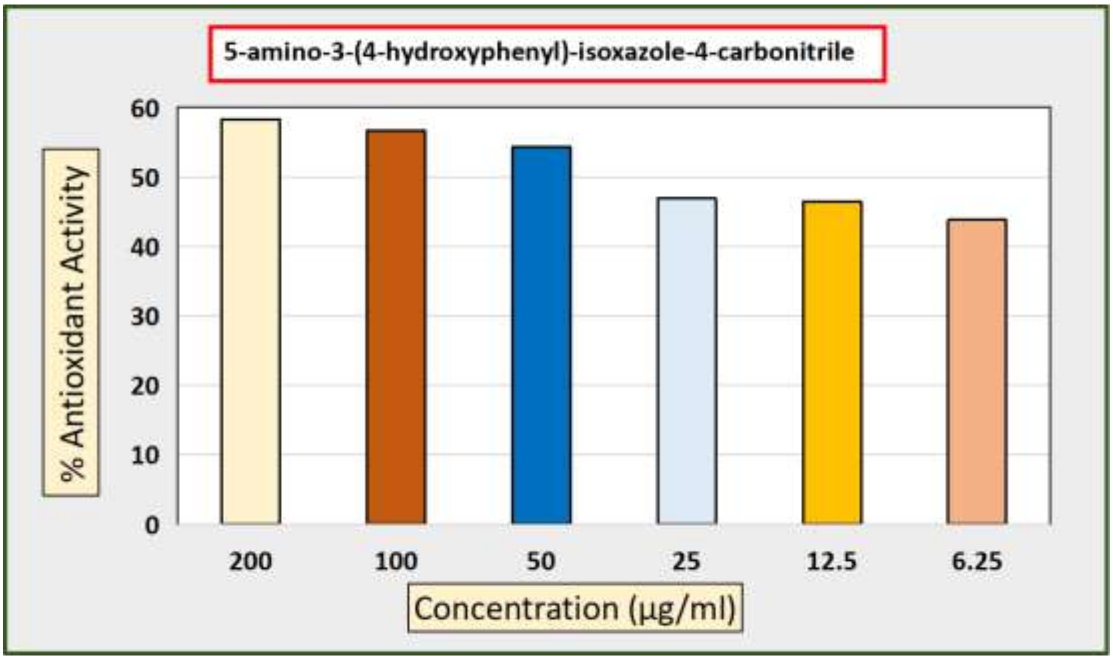


Figure 2: % Antioxidant activity of 5-amino-3-(4-hydroxyphenyl)-isoxazole-4-carbonitrile vs Concentration (µg/ml)

It was observed from the data depicted in Table 6 and Figure 3 that the maximum antioxidant activity was determined when the concentration of the sample 5-amino-3-(4-methoxyphenyl)-isoxazole-4-carbonitrile (4b) was maximum of 200 µg/ml.

Table 6: %Antioxidant activity of 5-amino-3-(4-methoxyphenyl)-isoxazole-4-carbonitrile

S.No.	Concentration (µg/ml)	Absorbance (nm)	% Antioxidant activity
1	200	0.350	44.4
2	100	0.353	43.9
3	50	0.380	39.7
4	25	0.386	38.7
5	12.5	0.399	36.7
6	6.25	0.556	11.7

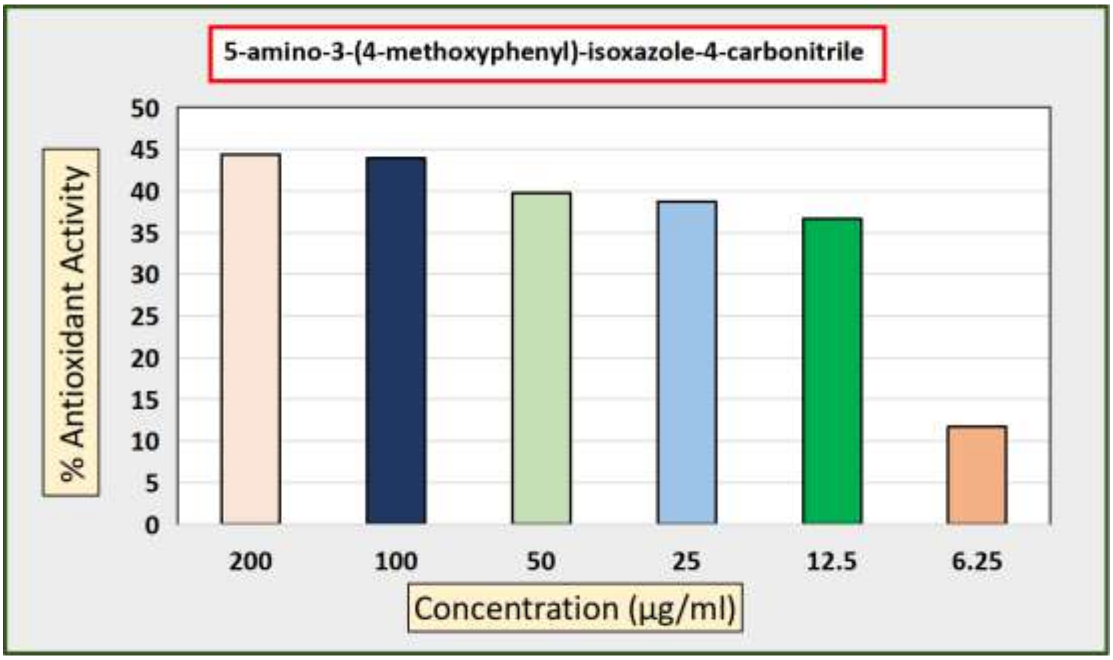


Figure 3: % Antioxidant activity of 5-amino-3-(4-methoxyphenyl)-isoxazole-4-carbonitrile vs Concentration (µg/ml)

It was observed from the data depicted in Table 7 and Figure 4 that the maximum antioxidant activity was determined when the concentration of the sample 5-amino-3-(4-nitrophenyl)-isoxazole-4-carbonitrile (4d) was maximum of 200 µg/ml.

Table 7: %Antioxidant activity of 5-amino-3-(4-nitrophenyl)-isoxazole-4-carbonitrile

S.No.	Concentration (µg/ml)	Absorbance (nm)	% Antioxidant activity
1	200	0.320	49.2
2	100	0.328	47.9
3	50	0.330	47.6
4	25	0.362	42.5

5	12.5	0.429	31.9
6	6.25	0.431	31.6

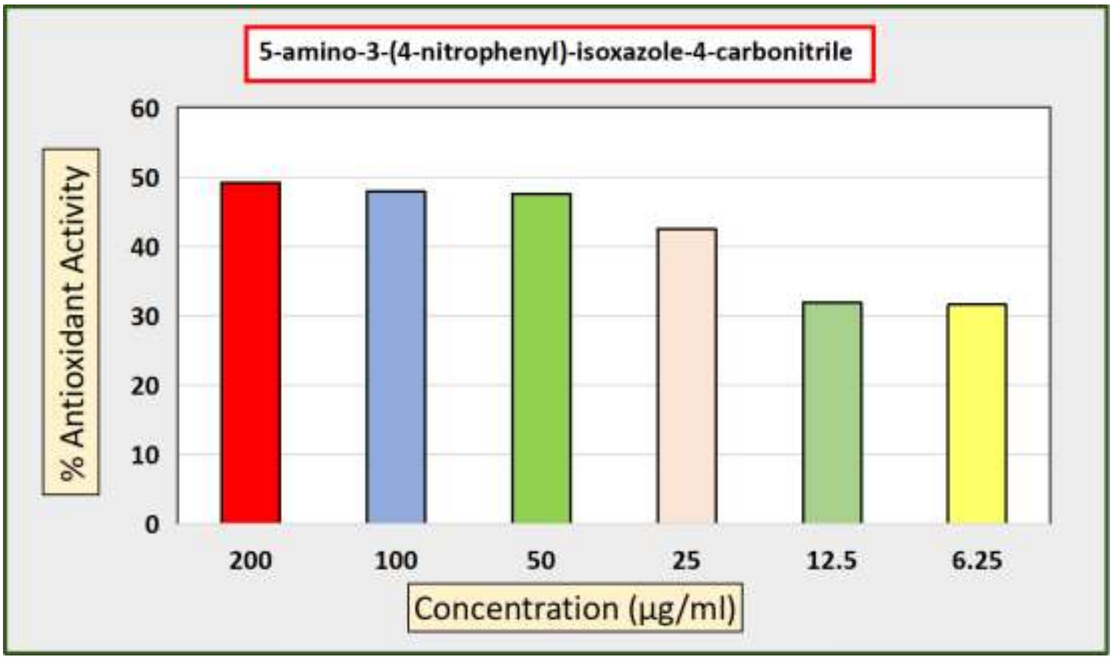


Figure 4: % Antioxidant activity of 5-amino-3-(4-nitrophenyl)-isoxazole-4-carbonitrile vs Concentration (µg/ml)

It was observed from the data depicted in Table 8 and Figure 5 that the maximum antioxidant activity was determined when the concentration of the sample 5-amino-3-(4-chlorophenyl)-isoxazole-4-carbonitrile (4e) was maximum of 200 µg/ml.

Table 8: %Antioxidant activity of 5-amino-3-(4-chlorophenyl)-isoxazole-4-carbonitrile

S.No.	Concentration (µg/ml)	Absorbance (nm)	% Antioxidant activity
1	200	0.234	62.9
2	100	0.317	49.7
3	50	0.352	44.1
4	25	0.377	40.2
5	12.5	0.385	38.9
6	6.25	0.377	37.6

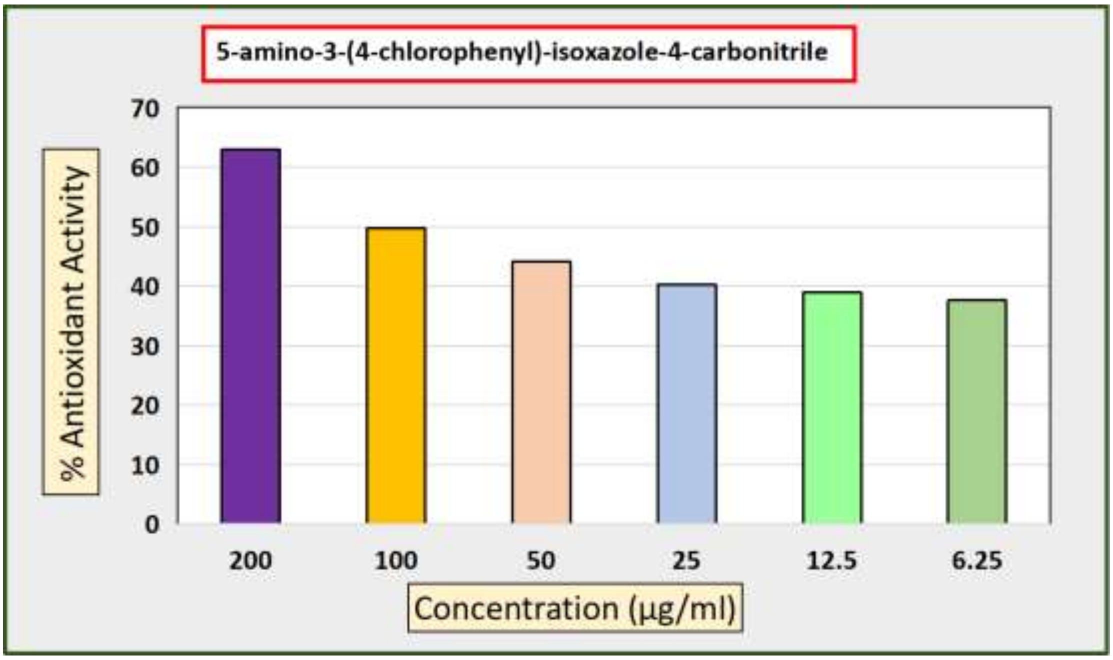


Figure 5: % Antioxidant activity of 5-amino-3-(4-chlorophenyl)-isoxazole-4-carbonitrile vs Concentration (µg/ml)

It was observed from the data depicted in Table 9 and Figure 6 that the maximum antioxidant activity was determined when the concentration of the sample 5-amino-3-(2-bromophenyl)-isoxazole-4-carbonitrile (4e) was maximum of 200 µg/ml.

Table 9: %Antioxidant activity of 5-amino-3-(2-bromophenyl)-isoxazole-4-carbonitrile

S.No.	Concentration (µg/ml)	Absorbance (nm)	% Antioxidant activity
1	200	0.366	41.9
2	100	0.405	35.7
3	50	0.410	34.9
4	25	0.414	34.3
5	12.5	0.422	33.0
6	6.25	0.431	31.6

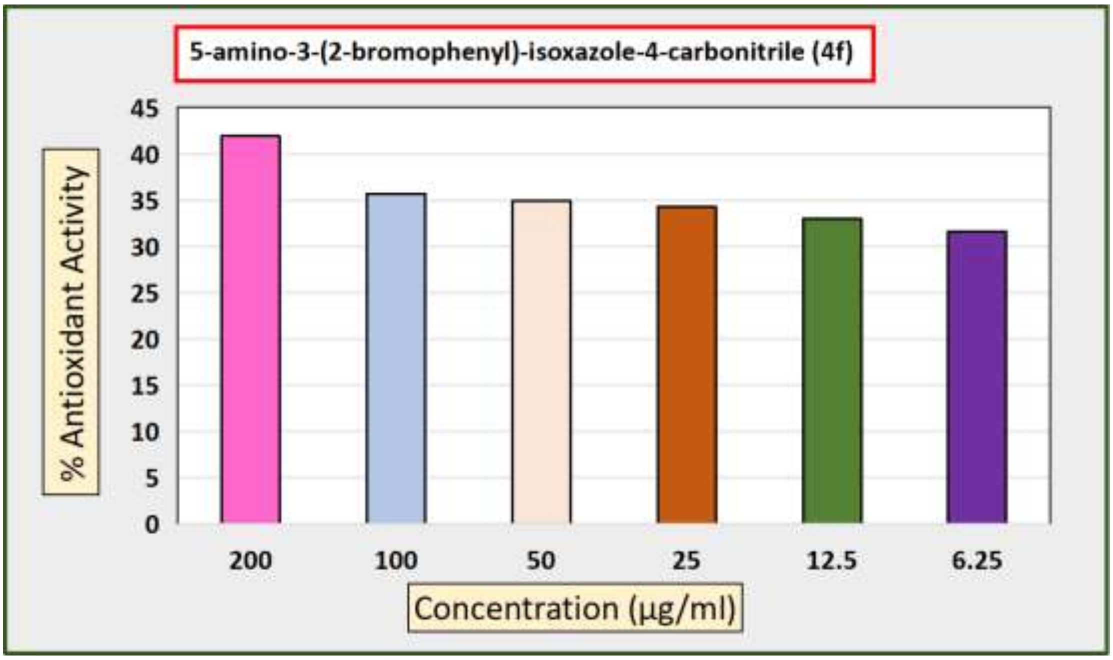


Figure 6: % Antioxidant activity of 5-amino-3-(2-bromophenyl)-isoxazole-4-carbonitrile vs Concentration (µg/ml)

The standard reference used is gallic acid and it also exhibited maximum antioxidant activity at maximum concentration of 200 µg/ml and is illustrated in **Table 10** and **Figure 7**.

Table 9: % Antioxidant activity of Standard Reference Gallic Acid

S.No.	Concentration (µg/ml)	% Antioxidant Activity
1	200	95.7
2	100	95.1
3	50	94.8
4	25	94.2
5	12.5	93.9
6	6.25	89.3

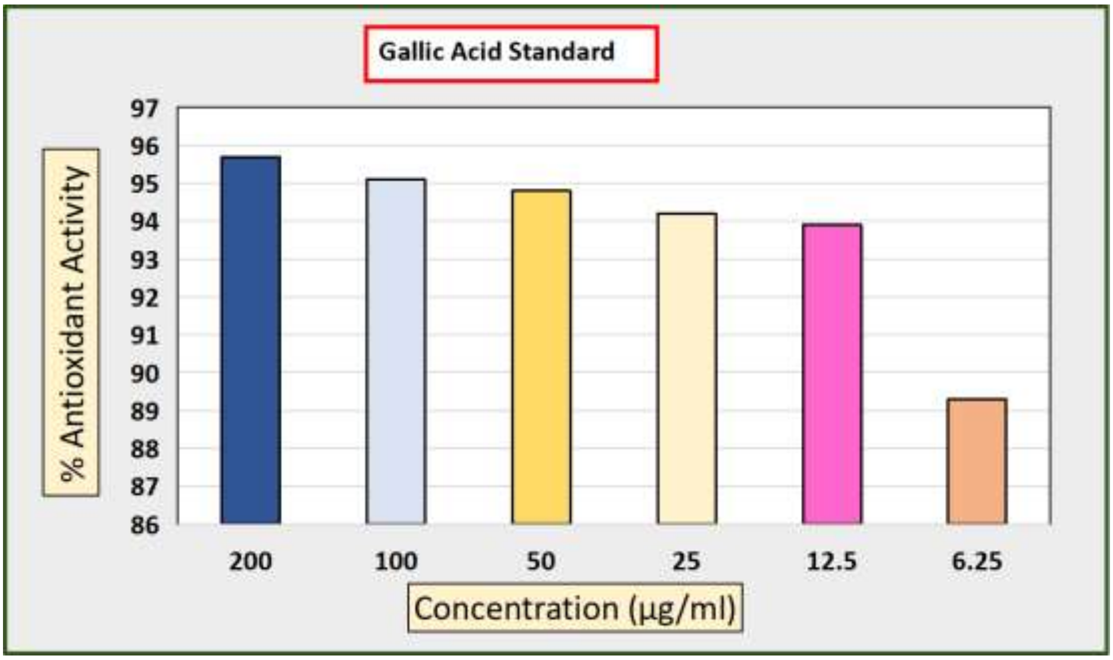


Figure 6 : % Antioxidant activity of Standard Reference Gallic Acid vs Concentration (µg/ml)

On comparing all the compound's antioxidant activity with the standard reference gallic acid's value, it was found that 4e exhibited the maximum antioxidant activity. The result is illustrated in **figure 8**.

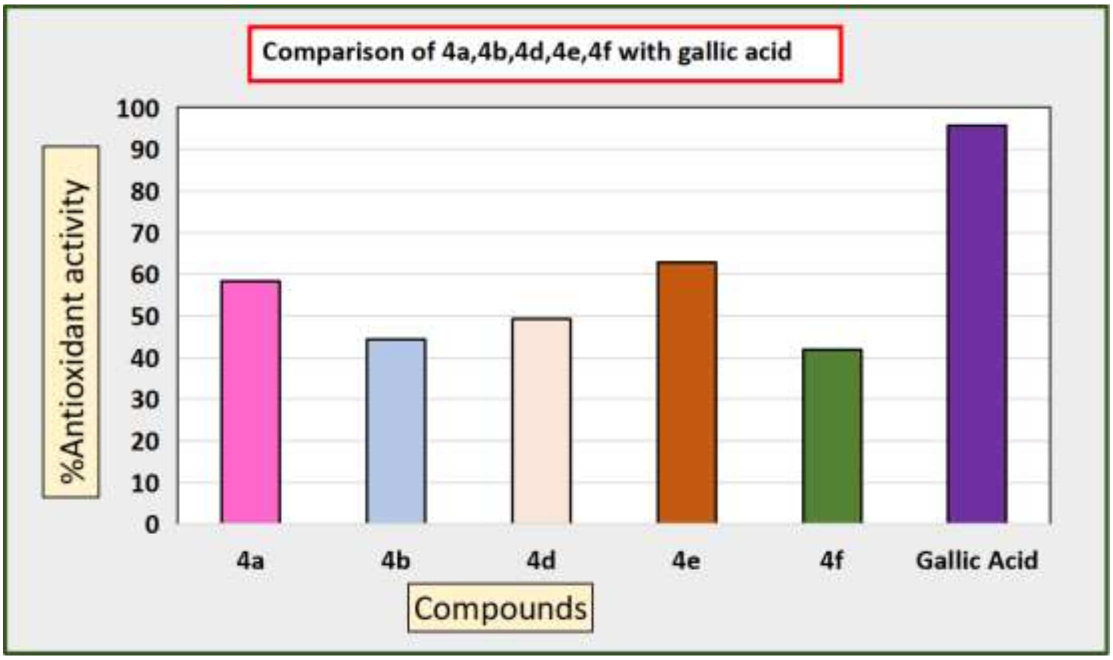


Figure 8: Comparison of %antioxidant activity of compounds with respect to gallic acid.

Conclusion

A novel strategy has been developed to synthesize 5-amino-isoxazole-4-carbonitrile derivatives by using an organocatalyst in green reaction conditions. The method is beneficial compared to previous ones in terms of short reaction time with maximum yield, use of non hazardous solvents and easy purification processs.The anti oxidant activity of synthesized compounds have been evaluated and thus this compound proves to be a scavenging radical which can be further studied for medicinal purposes.

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Confict of Interest:

There is no potential conflict of interest.

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