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Advances in Bioresearch

ORIGINAL ARTICLE

Microwave-Assisted Synthesis of Substituted 1,2,4- Triazole and their Antioxidant Activity

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ABSTRACT
The two new compounds containing the 1,2,4- triazole moiety were synthesized from Isoniazid and 3-chlorobenzaldehyde as starting material by multi-step reactions under microwave irradiation. The methodology significantly reduced reaction time and increased yields compared to conventional methods. Triazole is a five membered heterocyclic compound consisting of three nitrogen atoms and two carbon atoms within the ring. There are the two isomers of triazole: 1,2,3-triazole and 1,2,4- triazole, differing arrangement of nitrogen atoms in the ring. 1,2,4-triazole and its derivatives are widely studied due to their broad spectrum of biological activities, such as antimicrobial, antifungal, antiviral and anticancer, antibacterial, analgesic, anticonvulsant, anti-inflammatory, antidepressant, antitubercular and antioxidant properties. These are also important in organocatalysis, agrochemicals and material science. Thus, they have a broad range of therapeutic applications with ever-widening future scope across scientific disciplines. The synthesized compounds were characterized by Fourier Transformed Infrared Spectroscopy (FT-IR), Thin-Layer Chromatography (TLC), Melting Point (M.P). The antioxidant activity of these compounds was measured by the in-vitro free radical scavenging method (DPPH) with Ascorbic Acid as the reference medication. The DPPH assay method is widely used to calculate antioxidant activity because it's a cost-effective and rapid method that can be used to evaluate it easily. Microwave-assisted synthesis is a viable method for generating biologically active triazole derivatives with enhanced antioxidant activity. The result suggested that compound A1 had a higher percentage of inhibition.
Keywords: Antioxidant activity, 1,2,4-Triazole, DPPH, IR, TLC, Isoniazid, 3-Chlorobenzaldehyde.

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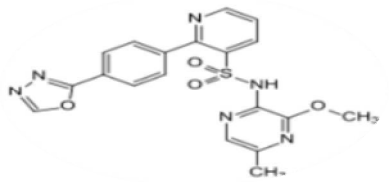
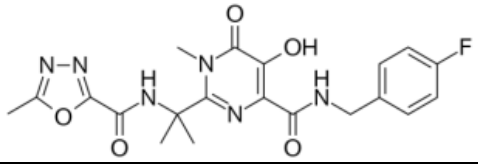
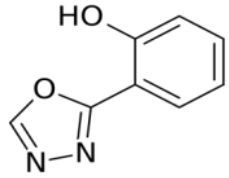
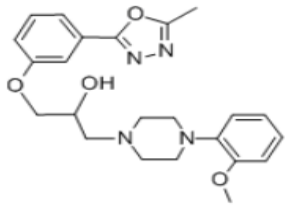
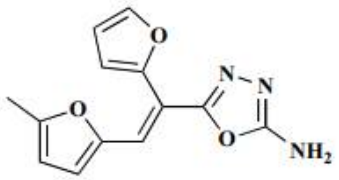
INTRODUCTION

Because of their wide range of uses, heterocyclic compounds play an important role in many sectors. A summary of their importance is provided below: Applications in agriculture (pesticides and herbicides), industry (colorants), and pharmacology (antihypertensive, antiviral, depressive, and anticancer action). Many natural organic compounds, such as pheromones, nucleic acids, and alkaloids, contain heterocyclic structures that contribute to their biological functions. Heterocyclic rings are a common structural element in medicinal chemistry, accounting for more than 67% of identified compounds, according to the Comprehensive Medicinal Chemistry (CMC) database. Heterocyclic chemistry plays a crucial role in the development of pharmaceuticals due to the rings' ability to enhance the bioactivity, stability, and solubility of medicinal compounds.[1] Heterocyclic compounds are defined by a cyclic ring structure containing at least one heteroatom, such as Sulphur, nitrogen, or oxygen.[2] A literature review confirms that certain heterocyclic compounds, particularly five- and six-membered rings with one to three heteroatoms in their nucleus, have a role in the metabolism of all living cells. Numerous heterocyclic derivatives with noteworthy biological activity have been synthesized due to this knowledge. Condensed N-benzylidene and triazole-pyrimidine derivatives demonstrate anti-inflammatory, antifungal, antibacterial, anticonvulsant, anti-allergic, herbicidal, and anticancer properties. The diuretic and antiemetic effects of substituted 1,3,4-oxadiazoles, such as 2-Acetamide-5-phenyl-1,3,4-oxadiazole, are demonstrated. [3]

Oxadiazole is an aromatic heterocyclic compound with a molecular formula $C_2H_2N_2O$. It contains a five-membered ring with two nitrogen atoms and one oxygen atom. Among the four isomers of Oxadiazole, 1,3,4- oxadiazole is particularly noteworthy for its role in pharmaceutical chemistry. [5]
As it has broad biological activity, several inventions were made for the synthesis of substituted 1,3,4 - Oxadiazole derivatives such as the synthesis of a substituted 1,3,4-oxadiazole derivative with 2- (5-thioxo-4,5-dihydro1,3,4-oxadiazol-2-yl) phenyl acetate, synthesis of a new family of 1,3,4-oxadiazole bearing the benzimidazole moieties. Zebotentan, Raltegravir, Furamizole, Pleconaril, Fenadizole, and Oxolamine are the successfully used Oxadiazole nuclei containing a marketed drug that shows various biological activity. [10-14]

Table 1. Oxadiazole Nucleus Containing Marketed Drug

s no.	drug	chemical structure	biological activity
1.	Zebotentan		anticancer drug
2.	Raltegravir		anti-HIV drug
3.	Fenadizole		hypnotic drug
4.	Nesapidil		antihypertensive drug
5.	Furamizole		Antibiotic

MATERIAL AND METHODS
The chemicals used in this work are listed below with their suppliers.

Table 2. List of chemicals and reagents with their origin

Materials	company	Origin
n-hexane	central drug house (cdh)	new delhi, india
chloramine t	central drug house (cdh)	new delhi
dimethylformamide	central drug house (cdh)	new delhi
Ethanol	hemedia pvt. ltd.	mumbai, india
Methanol	hemedia pvt. ltd.	mumbai, india
3-chloro benzaldehyde	central drug house (cdh)	new delhi
Leucine	central drug house (cdh)	new delhi
Glycine	central drug house (cdh)	new delhi
Isoniazid	central drug house (cdh)	new delhi
ethyl acetate	central drug house (cdh)	new delhi
silica gel (60-120)	hemedia pvt. ltd.	mumbai, india

GENERAL MECHANISTIC PATHWAY

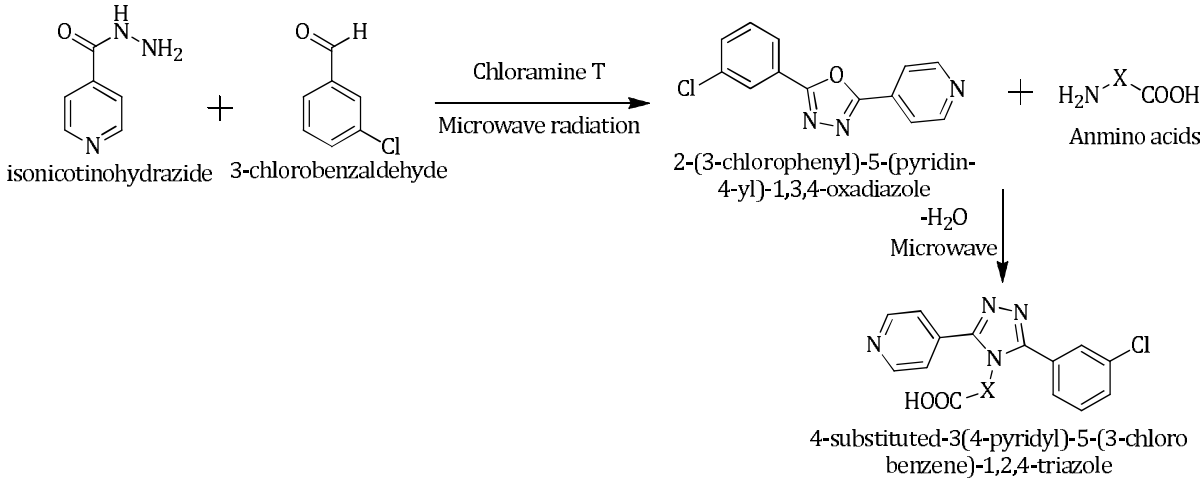


Figure 1. Synthesis of 4-substituted-3(4-pyridyl)-5-(3-chloro benzene)-1,2,4-triazole

Chemical synthesis

The synthesis was achieved by following procedures. Purification of the compound was done by thin-layer chromatography.

1. Synthesis of intermediate I

Isoniazid (0.01 mol) and 3-chlorobenzeldehyde (0.01 mol) were transferred in a dry, clean conical flask. Add 5 drops of dimethylformamide (DMF) into the flask, mix it properly, and fitted with a loose top cap. Then the resulting mixture was heated in a commercial microwave oven at 300 W internally for 30 seconds for 3 minutes. After 3 minutes the reaction mixture was cooled with ice-cold water, filtered and a solid product (intermediate I) was collected. The solid product was washed with water, dried, and recrystallized from ethanol.

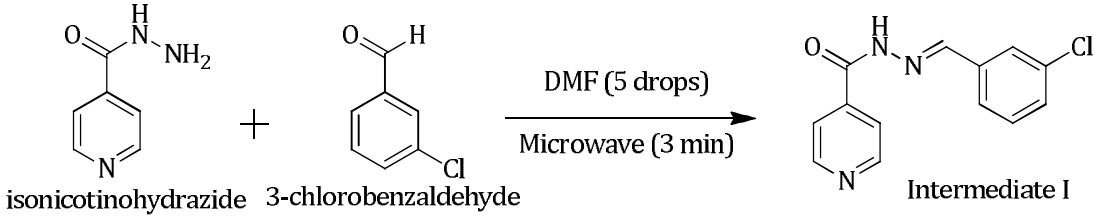


Figure 2. Synthesis of Intermediate I

2. Synthesis of 2-(4-hydroxy phenol)-5-(4-pyridyl)-1, 3, 4-oxadiazole

Intermediate I (0.01 mol) was mixed in a clean conical flask with ethanol (15 ml). To this solution chloramine-T (0.01 mol) was added and fitted with a loose top cap. The mixture was heated in a microwave oven at 300W internally for 30 seconds for 4 minutes. Then the reaction mixture was cooled with cold water, filtered, and a solid product (intermediate I) was collected. The solid product (2-(3-chloro benzene)-5-(4-pyridyl)-1, 3, 4-oxadiazole) was washed with water, dried and recrystallized from methanol.

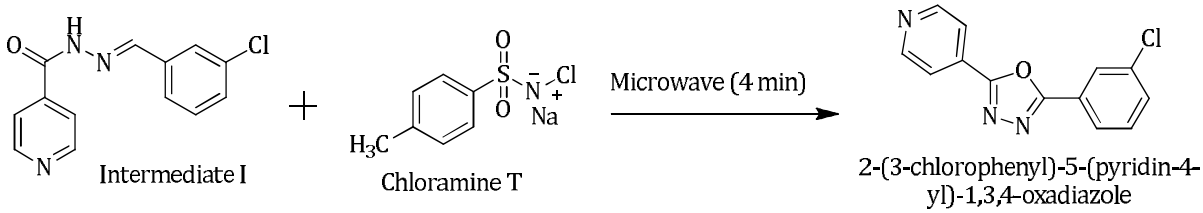
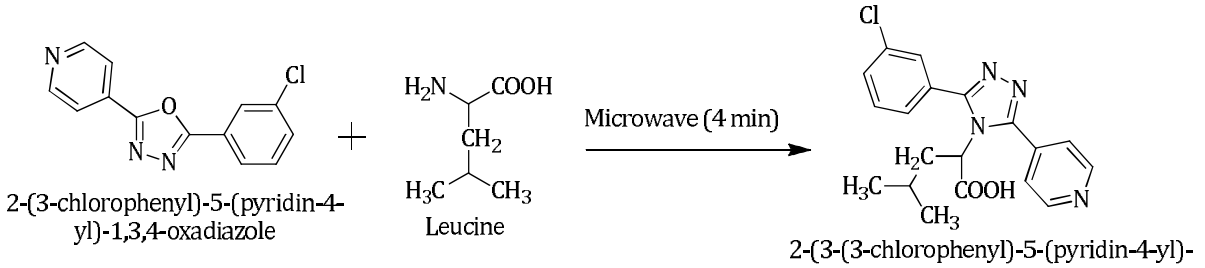


Figure 3. Synthesis of 2-(3-chlorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole

3. Synthesis of compound A1

A mixture of 2-(3-chloro benzene)-5-(4-pyridyl)-1, 3, 4-oxadiazole (0.01 mol) and leucine (0.01 mol) in a dry and clean conical flask, was subjected to microwave oven at 2450 MHz for 3 minutes. Then the reaction mixture was cooled with cold water, and filtered and a solid product was collected which was further recrystallized from ethanol.



2-(3-chlorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole

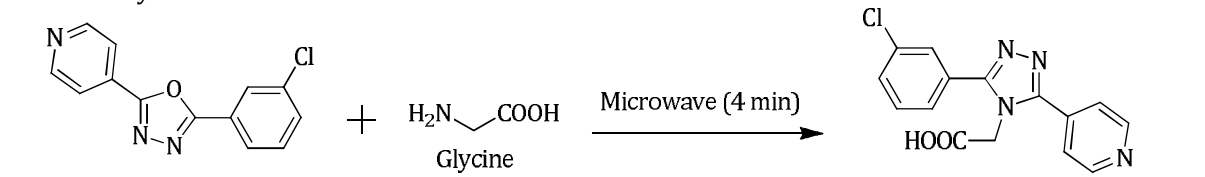
Leucine

2-(3-(3-chlorophenyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-4-yl)-4-methylpentanoic acid

Figure 4. Synthesis of 2-(3-(3-chlorophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-yl)-4-methyl pentanoic acid

4. Synthesis of compound A2

A mixture of 2-(3-chloro benzene)-5-(4-pyridyl)-1, 3, 4-oxadiazole (0.01 mol) and glycine (0.01 mol) in a dry and clean conical flask, was subjected to microwave oven at 2450 MHz for 3 minutes. Then the reaction mixture was cooled with cold water, and filtered and a solid product was collected which was further recrystallized from ethanol.



2-(3-chlorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole

Glycine

2-(3-(3-chlorophenyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-4-yl)acetic acid

Figure 5. Synthesis of 2-(3-(3-chlorophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-yl) acetic acid

Antioxidant activity

In- Vitro, methods were employed in anti-oxidant studies (Hricha Joshi et al. 2020).

1. 9.6 mg of DPPH (1, 1-Diphenyl-2- picrylhydrazyl) was dissolved in 40 ml methanol; it was protected from light by covering the test tubes with aluminum foil.
2. 2 ml of DPPH solution was added to 6 ml of methanol and absorbance was taken immediately at 517 nm for control reading.
3. Various concentrations of substance, to be examined as well as of standard compound (ascorbic acid) were taken and volume was made uniformly using methanol.
4. Each sample was further diluted with methanol up to 6 ml and 0.2 ml of DPPH was added.
5. Absorbance was taken after 15 minutes at 517 nm using methanol as blank on a UV-visible spectrometer.
6. the following formula calculated IC50 for each compound as well as standard preparation.

The DPPH free radical scavenging activity was calculated using the formula:

% scavenging= [absorbance of control – absorbance of test sample/ absorbance of control] X 100.

RESULTS

The synthesis of novel amino acids substituted 1,2,4-triazole derivatives was performed. The synthesis process begins with a microwave-assisted reaction between isoniazid hydrazide and 3-chlorobenzaldehyde, which yields an intermediate product that is then converted into an oxadiazole nucleus through treatment with chloramine-T. The final steps involve substituting different amino acids, which results in the creation of new amino acids substituted 1,2,4-triazoles derivatives.

PHYSIOCHEMICAL PROPERTIES

Table 3. Physiochemical properties of synthesized 1, 2, 4-triazole derivatives (A1- A2)

Sr. No.	Synthesized compound	Appearance	Practical yield
1.	A1	dark brown viscous solid	84%
2.	A2	dark brown viscous solid	80%

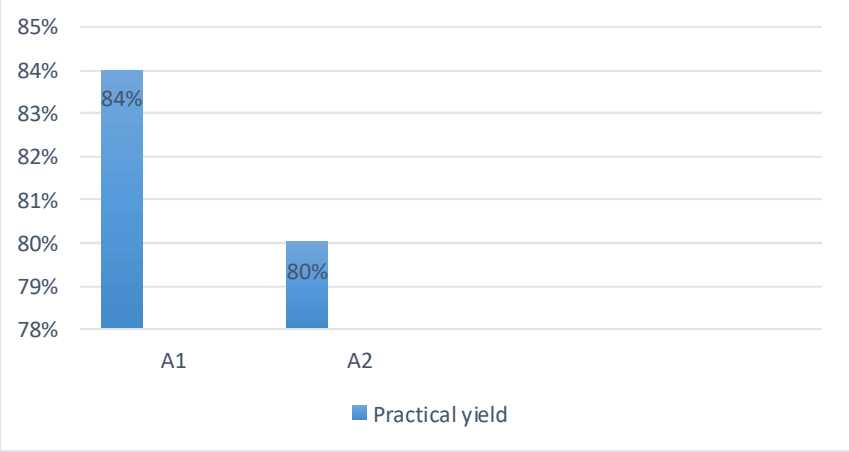


Figure 6. The practical yield of the synthesized compound

SOLUBILITY

The solubility of the compound was done by gravimetric solubility method using various solvent systems, in which a saturated solutions of synthesized compound was obtained by stirring and excess amount of compound in the solvent until the equilibrium was achieved (Table 4).

Table 4. Solubility of synthesized 1, 2, 4-triazole derivatives (A1- A2)

s. no.	Synthesized compound	Solubility
1.	A1	Soluble in ethyl acetate and n-hexane
2.	A2	Soluble in ethyl acetate and n-hexane

pH

The pH determination of the synthesized compound was done Eutech pH meter (Table 5).

Table 5. pH of synthesized 1, 2, 4-triazole derivatives (A1- A2)

s. no.	synthesized compound	pH
1.	A1	5.10
2.	A2	4.99

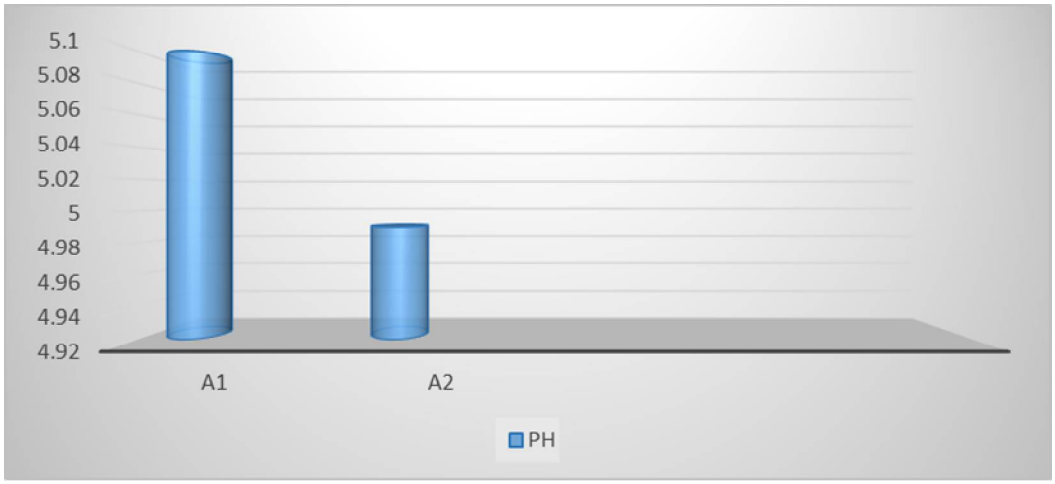


Figure 7: pH of the synthesized compound

MELTING POINT

The melting point of the synthesized compound was determined by the capillary method (Table 6).

Table 6. Melting point of synthesized 1, 2, 4-triazole derivatives (A1- A2)

s. no.	synthesized compound	melting point (°c)
1.	A1	222-226
2.	A2	233-241

OPTICAL ROTATION

The optical activity of the synthesized compounds was done by Rudolph polarimeter (Table 7).

Table 7. Optical rotation of synthesized 1, 2, 4-triazole derivatives (A1- A2)

s. no.	synthesized compound	optical rotation
1.	A1	0.118
2.	A2	0.109

THIN LAYER CHROMATOGRAPHY

Thin-layer chromatography of the synthesized compound was done by using n-hexane: ethyl acetate solvent and visualization was done by UV radiation.

Table 8. Retention factor of synthesized 1, 2, 4-triazole derivatives (A1- A2)

s. no.	synthesized compound	retention factor
1.	A1	0.85
2.	A2	0.72

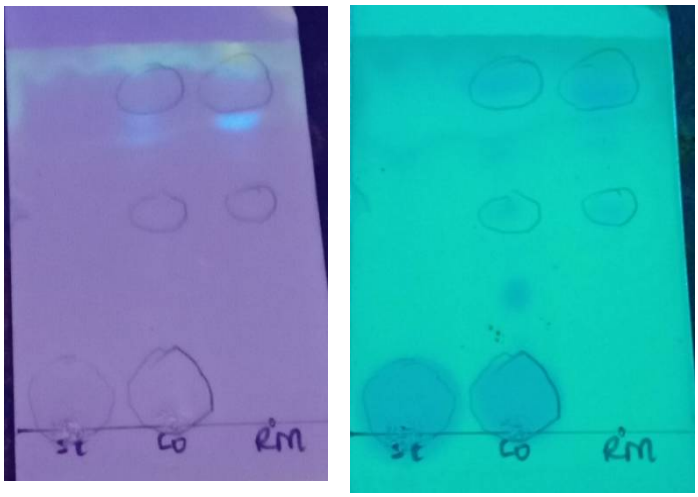


Figure 8: Visualization of TLC plate in UV light

ULTRAVIOLET SPECTROSCOPY

Table 9. Λ_{max} of synthesized 1, 2, 4-triazole derivatives (A1- A2)

s.no.	synthesized compound	Solvent	λ_{max} (nm)
1.	A1	ethyl acetate	277
2.	A2	ethyl acetate	269

INFRA-RED SPECTROSCOPY

IR spectra of Compound A1:

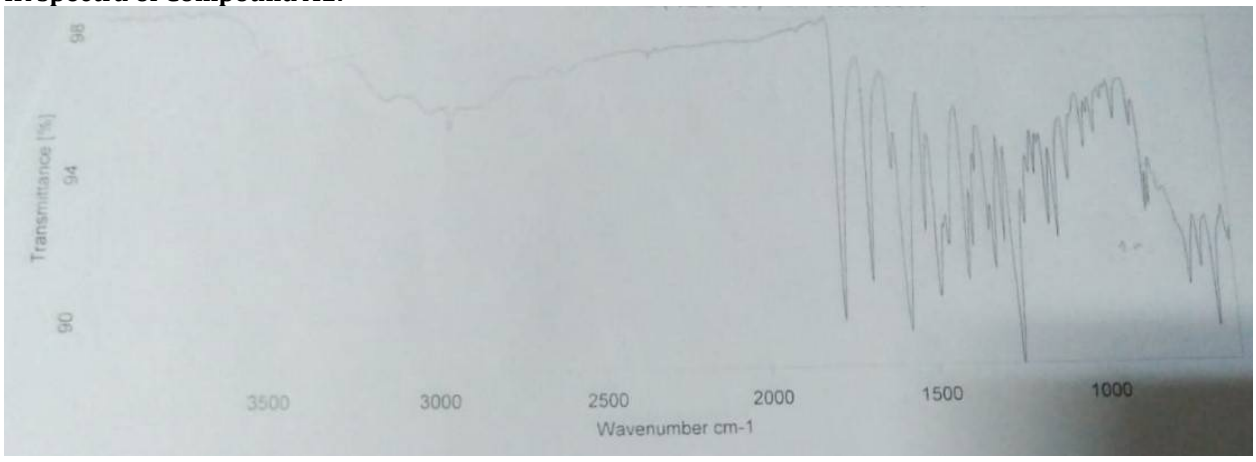


Figure 9: IR spectra of compound A1

IR spectra of Compound A2:

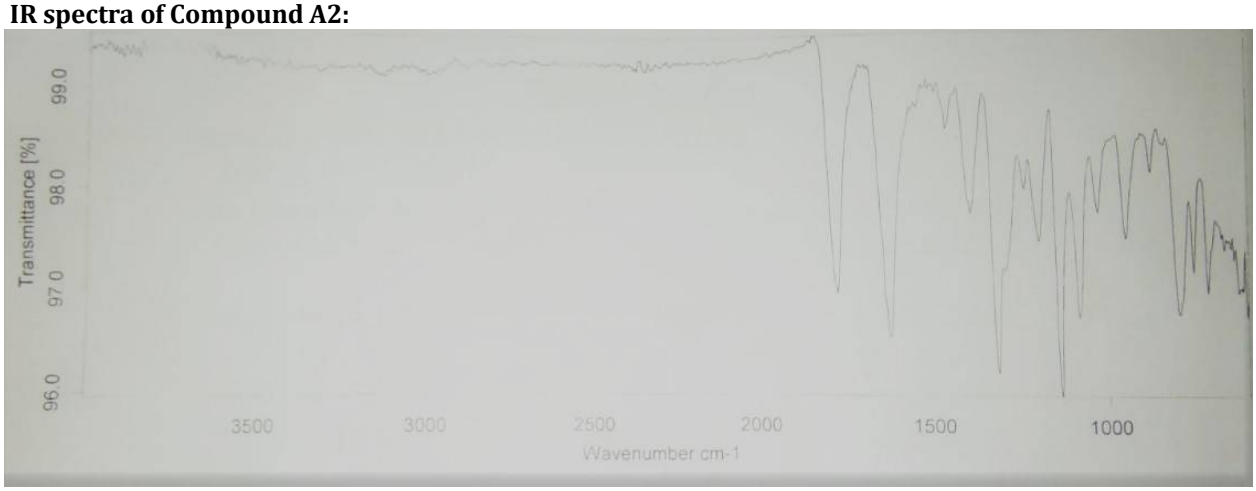


Figure 10: IR spectra of compound A2

ANTIOXIDANT ACTIVITY

Antioxidant activities of synthesized compounds were done by the DPPH method with ascorbic acid used as the standard medication. The UV absorbance of synthesized compounds and reference drugs was tested at various concentrations (Table 10) the free radical inhibition activity (%) of the synthesized chemical and ascorbic acid was then determined using the appropriate formula (Table 11).

Table 10. The absorbance of the synthesized compound and ascorbic acid as antioxidant activity

s. no.	concentration (mg/ml)	A1	A2	standard (ascorbic acid)	Control
1.	0.04	0.295	0.340	0.151	0.489
2.	0.08	0.210	0.285	0.121	0.489
3.	0.12	0.190	0.230	0.099	0.489
4.	0.16	0.162	0.201	0.048	0.489
5.	0.20	0.110	0.138	0.021	0.489

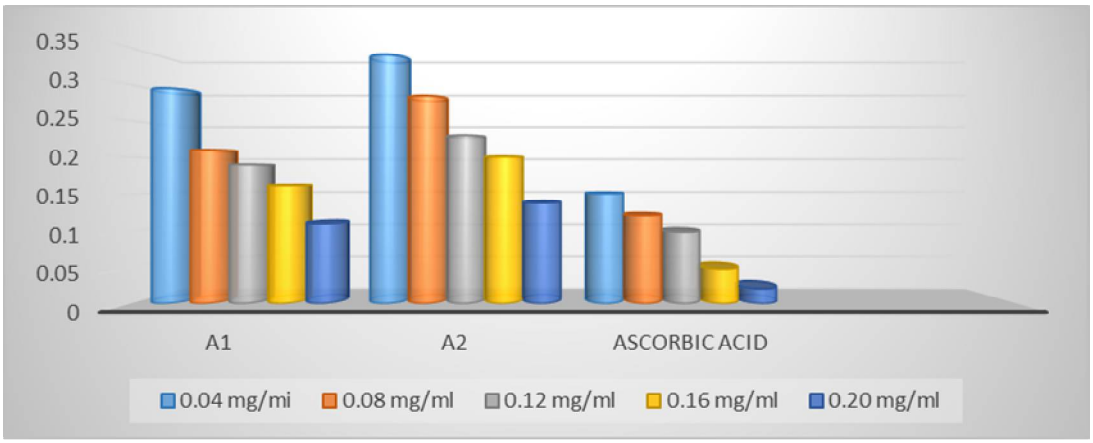


Figure 11: Absorbance of synthesized compound and ascorbic acid

Table 11. Free radical inhibition activity (% inhibition) of the synthesized compound and ascorbic acid

s. no.	concentration (mg/ml)	a1	a2	standard (ascorbic acid)
1.	0.04	40.08	29.45	69.94
2.	0.08	57.67	42.94	77.09
3.	0.12	61.35	54.19	82.82
4.	0.16	67.28	59.92	92.02
5.	0.20	77.91	73.21	96.52

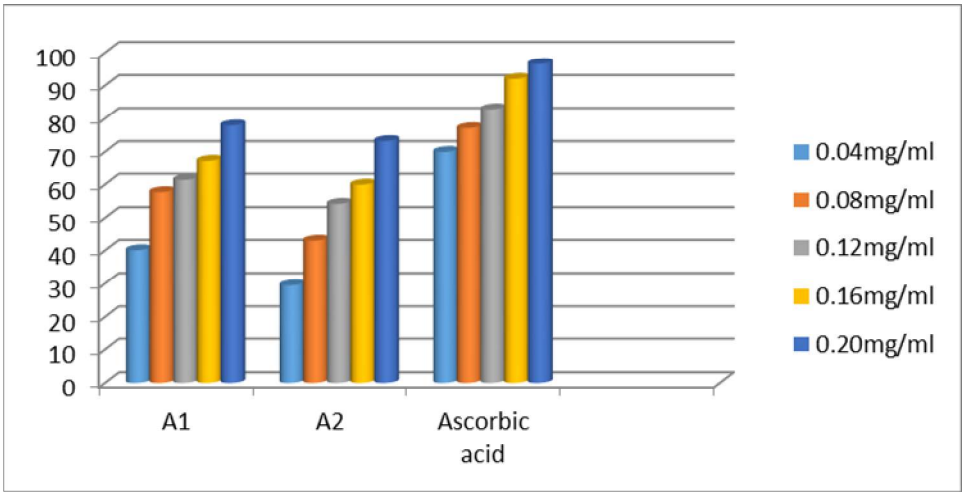


Figure 12: Free radical inhibition activities (% inhibition) of synthesized compound and ascorbic acid

DISCUSSION

A1 and A2 underwent screening for in-vitro antioxidant activities. Ascorbic acid served as the standard; the antioxidant properties of the synthesized compounds were assessed using the DPPH radical scavenging method. The compounds and standard were prepared at varying concentrations and subjected to analysis via UV spectroscopy with DPPH. Results indicated a positive correlation between compound concentration and percentage inhibition.

The current investigation involved the design synthesis of amino acid substituted 1, 2, 4-triazole derivatives originating from 1, 2, 3-oxadiazole nuclei. The synthesized derivatives were evaluated for solubility, pH, melting point, optical rotation, TLC, UV, IR, and antioxidant efficacy. The study inferred that a heterocyclic nucleus like triazole could exhibit antioxidant properties. Compound A1 displayed the highest percentage inhibition in comparison to Compound A2, reinforcing the notion that introducing triazole to a heterocyclic compound can enhance antioxidant activity. These resultant compounds present opportunities for further exploration into their antibacterial and antimicrobial potential.

CONCLUSION

Both compounds A1 and A2 were tested for antioxidant activities using the DPPH method. Results show increasing compound concentration leads to higher inhibition. Amino acid-substituted triazole derivatives were synthesized from oxadiazole nuclei. These derivatives were analyzed for various properties and antioxidant activity. Triazole-containing compounds showed antioxidant effects. Compound A1 had higher inhibition than A2. Substituting a compound with triazole showed antioxidant activity. Further research can explore the antibacterial and antimicrobial effects of these compounds.

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