World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 7.409

DESIGN, SYNTHESIS, MOLECULAR DOCKING & IN VITRO ANTIOXIDANT EVALUATION OF ISONIAZID DERIVATIVES

Devika Chandran[1], Harsha S.[1], Vaishak S.[1] and Lirin Mary M. K.[2*]

[1]Students, KVM College of Pharmacy, Cherthala, Alappuzha, India.
[2]Associate Professor, Department of Pharmaceutical Chemistry, KVM College of Pharmacy, Cherthala, Alappuzha, India.

***Corresponding Author: Lirin Mary M. K.**
Associate Professor, Department of Pharmaceutical Chemistry, KVM College of Pharmacy, Cherthala, Alappuzha, India.

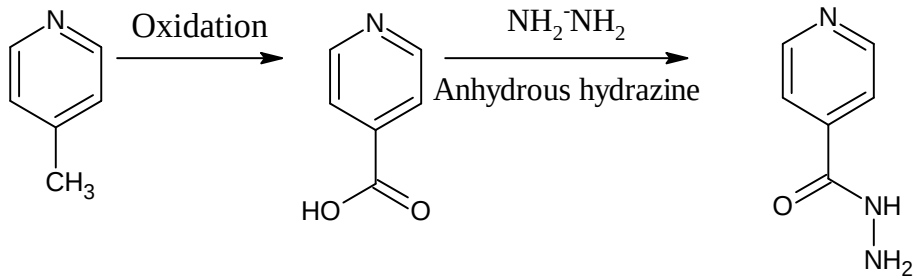
Article Received on 09/07/2024 **Article Revised on 28/07/2024** **Article Accepted on 18/08/2024**

ABSTRACT
This research focuses on the synthesis and analysis of Isoniazid derivatives, emphasizing their potential antioxidant properties in addition to their established antimicrobial effects. The process began with the oxidation of 4-methylpyridine to create Isonicotinic acid, which was then reacted with anhydrous hydrazine to produce Isoniazid. Various derivatives were formed by reacting Isoniazid with different aldehydes. These compounds were analyzed using techniques such as Thin Layer Chromatography, UV and IR spectroscopy, and mass spectrometry. Docking studies were performed to evaluate their binding affinity with cytochrome C peroxidase, and their antioxidant activities were assessed using the DPPH radical scavenging assay. The findings revealed that while all derivatives demonstrated some antioxidant activity, compound 1(a) had the highest inhibition percentage. Nonetheless, their effectiveness was lower than that of the standard antioxidant, Ascorbic acid, indicating that further improvements are needed.

KEYWORDS: Isoniazid, Antioxidant activity, Isonicotinic acid Derivatives, Docking studies, Thin Layer Chromatography, UV spectroscopy, IR spectroscopy, Mass spectrometry, DPPH assay.

INTRODUCTION
Isoniazid, a well-established antitubercular drug, has attracted interest not only for its effectiveness in treating tuberculosis but also for its potential antioxidant capabilities. The antioxidant properties of Isoniazid and its derivatives are particularly valuable in drug development, as oxidative stress is a key factor in many diseases.[1] By neutralizing free radicals and minimizing oxidative damage, Isoniazid derivatives could provide additional therapeutic advantages beyond their antimicrobial properties. Investigating the antioxidant potential of Isoniazid-based compounds in drug design opens up new possibilities for creating multifunctional drugs that target both infections and oxidative stress-related disorders. This dual action enhances the therapeutic value of Isoniazid derivatives, positioning them as promising candidates for the next generation of drugs.[2]

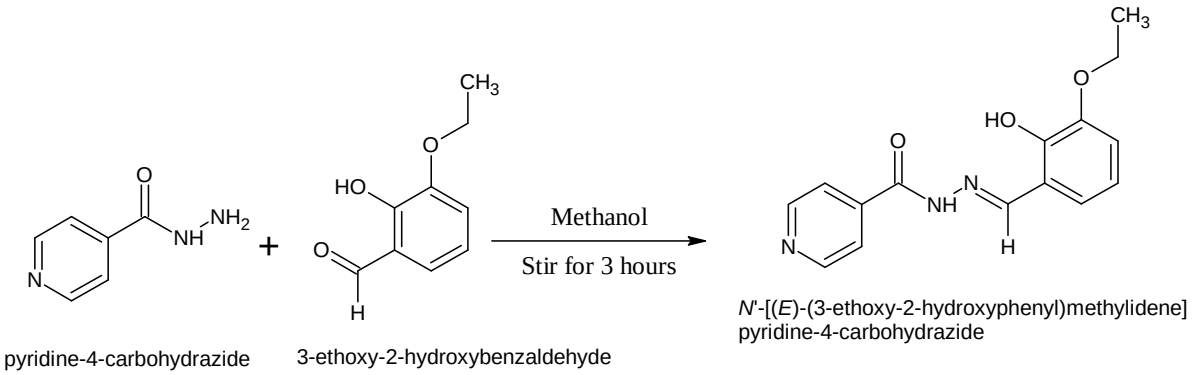
EXPERIMENTAL
Synthesis of Isoniazid
4-methylpyridine is oxidized to obtain Isonicotinic acid. Isonicotinic acid upon heating with anhydrous hydrazine form Isoniazid.[3]



Scheme-1: Synthesis of Isoniazid.

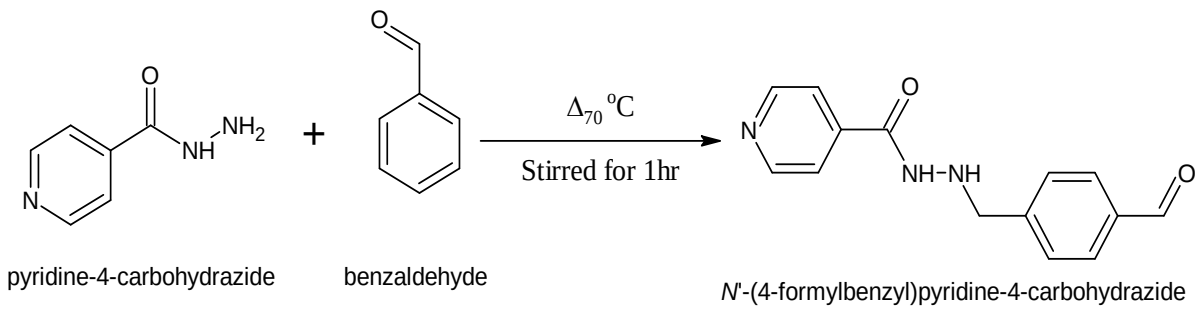
Synthesis of Isoniazid derivatives

Synthesis of 1.6g of 3-ethoxysalicyaldehyde was added to a solution of 1.3g of isoniazid in 30 ml of methanol and stirred for 3 hours. The pale yellowish solid separated was filtered, washed repeatedly with methanol, dried in air and recrystallized from ethanol.[4]



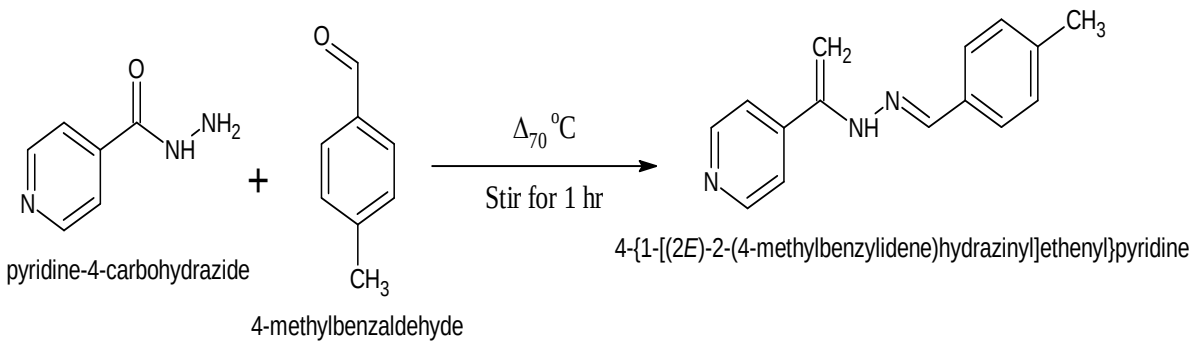
Scheme-2: Synthesis of Compound 1(a)

A mixture of 1.4g of isoniazid, 1ml of benzaldehyde and 10 ml of isopropyl alcohol was stirred thoroughly in a magnetic stirrer. The reaction mass was stirred at 70^0C for 1 hour. The obtained product was cooled and the solid was filtered, recrystallized by ethanol.[5]



Scheme-3: Synthesis of Compound 1(b)

A mixture of 1.4g of isoniazid, 1.2 ml of 4-methylbenzaldehyde and 10ml of isopropyl alcohol. The reaction mass was stirred at 70^0C for 1 hour. The obtained product was cooled and the solid was filtered, recrystallized by ethanol.[5]



Scheme-4: Synthesis of Compound 1(c)

RESULTS AND DISCUSSION

PHYSICAL CHARACTERIZATION

The physical characterization of synthesized compounds is shown in the Table 1.

Table 1: Physical Characterization of Synthesized Compounds.

Compound	Molecular Formula	Molecular Weight (g/mol)	Colour	R_f value	Solubility	Percentage Yield (%)
1(a)	$C_{15}H_{15}N_3O_3$	285.30	Pale yellowish solid	0.9	Moderate soluble in ethanol, dimethyl sulfoxide, Limited soluble in hexane	93%
1(b)	$C_{14}H_{11}N_3O_2$	253.26	White solid	0.84	Soluble in methanol, ethanol, dimethyl sulfoxide, Limited solubility in water	69%
1(c)	$C_{14}H_{13}N_3O$	239.27	White solid	0.8	Soluble in methanol, ethanol, dimethyl sulfoxide, Limited solubility in water	71%

Thin Layer Chromatography[6]

Samples was analyzed using thin-layer chromatography on a silica gel F_{254} with a mobile phase comprising ethyl acetate, acetone, methanol and hexane in a 5:2:2:1 (v/v) ratio. Then the R_f value was calculated.

R_f = Distance travelled by solute / Distance travelled by solvent

DOCKING STUDIES

Docking studies simulate how a small molecule, such as isoniazid derivatives, interacts with a protein like cytochrome C peroxidase to predict their binding affinity and interaction modes.

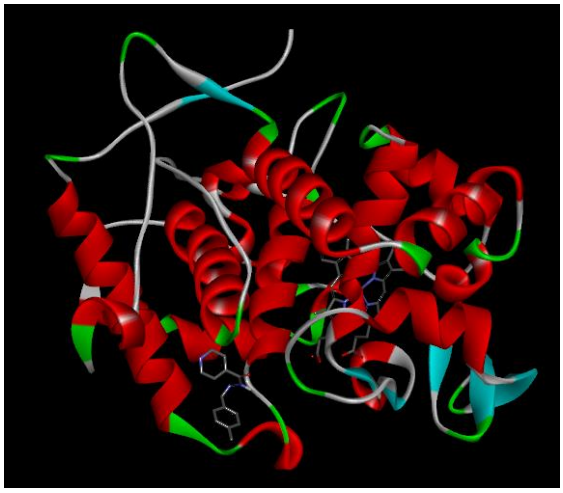
To assess the binding affinity and interaction patterns of isoniazid derivatives with cytochrome C peroxidase (CcP) by calculating the dock score, which aids in determining their potential as enzyme inhibitors or modulators. Obtain and prepare the 3D structures of both isoniazid derivatives and cytochrome C peroxidase.[7]

Prepare the protein by removing water molecules and adding hydrogen atoms, while minimizing and preparing the derivatives for docking. Use docking software (like AutoDock, Dock, or Glide) to model the binding of the isoniazid derivatives to the active site of cytochrome C peroxidase. The software will predict the binding conformations and orientations of the molecules. Determine the dock score, which indicates the binding affinity between the derivatives and cytochrome C peroxidase. A more negative dock score signifies stronger binding. Examine the binding interactions, including hydrogen bonds, hydrophobic, and electrostatic interactions. Compare the dock scores of different derivatives to identify the most promising candidates.[8]

The docking analysis will reveal which isoniazid derivatives are likely to bind most effectively to cytochrome C peroxidase, guiding further experimental validation and optimization efforts.

Compound 1(a)

Compound 1(b)



Compound 1(c)

Figure 1: Docking of Synthesized Compounds.

Table 2: Docking score of synthesized Compounds.

Compound	Docking score
1(a)	-4.02
1(b)	-4.88
1(c)	-6.1

UV SPECTROSCOPY

In UV Spectroscopy, there was a slight variation in the λmax of the compounds 1(a),1(b), 1(c) & the results are given in the Table 3.[9]

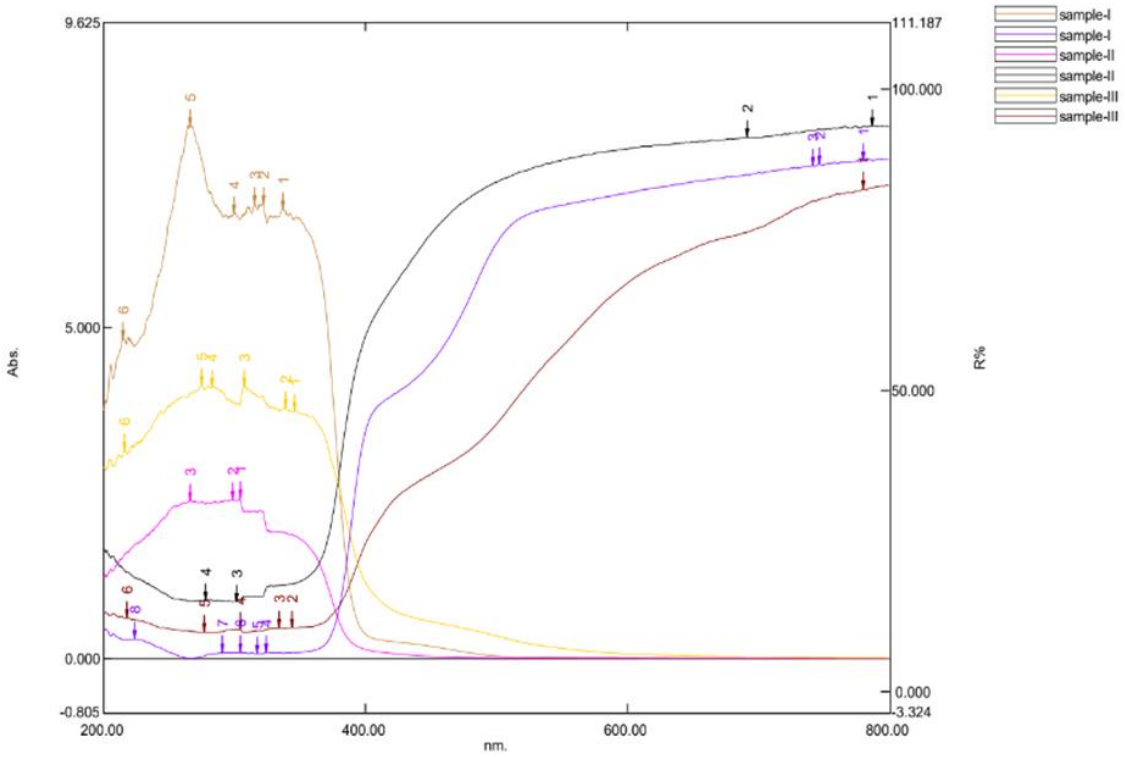


Figure 2: UV Spectrum of Compounds.

Table 3: λ_{max} of Compounds.

Compounds	λmax
1(a)	266nm
1(b)	299nm
1(c)	275nm

IR SPECTROSCOPY

Infrared (IR) spectroscopy is an absorption method widely used in both qualitative and quantitative analysis. Spectrum include electromagnetic radiation that can alter the vibrational and rotational states of covalent bonds in organic molecules.[10]

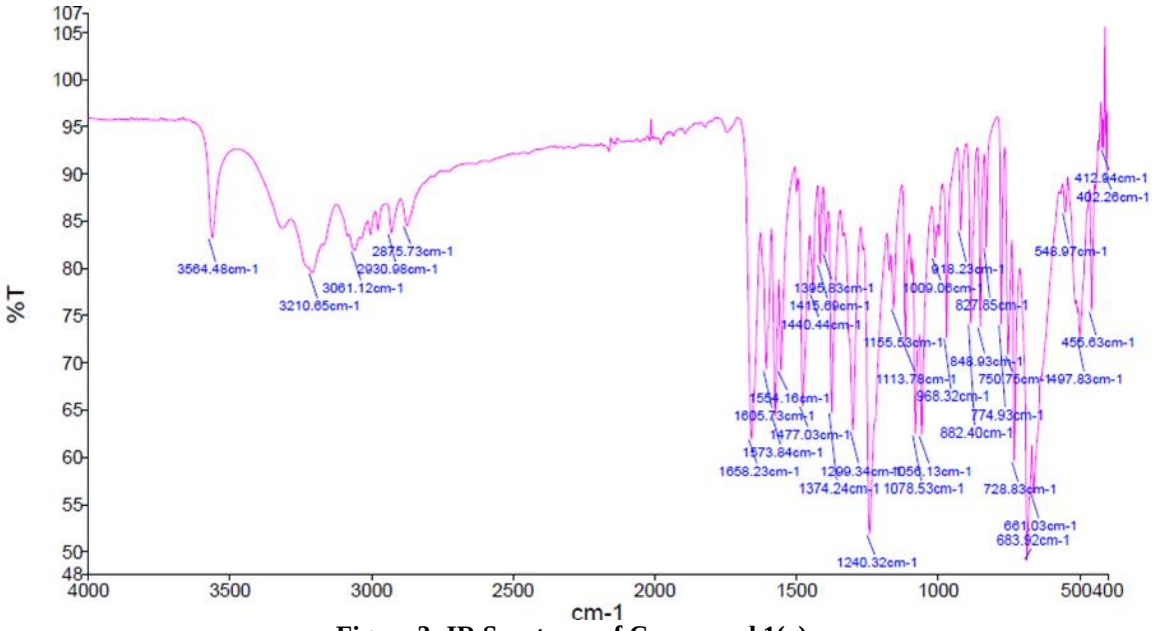


Figure 3: IR Spectrum of Compound 1(a)

IR Spectrum Interpretation of compound 1(a): Phenolic aromatic ring : 3564.48 cm^{-1}, Amide : 1605.7 cm^{-1}, C=N: 1658.23 cm^{-1}, C-O: 1240.32 cm^{-1}, Aromatic ring: 2930.98 cm^{-1}

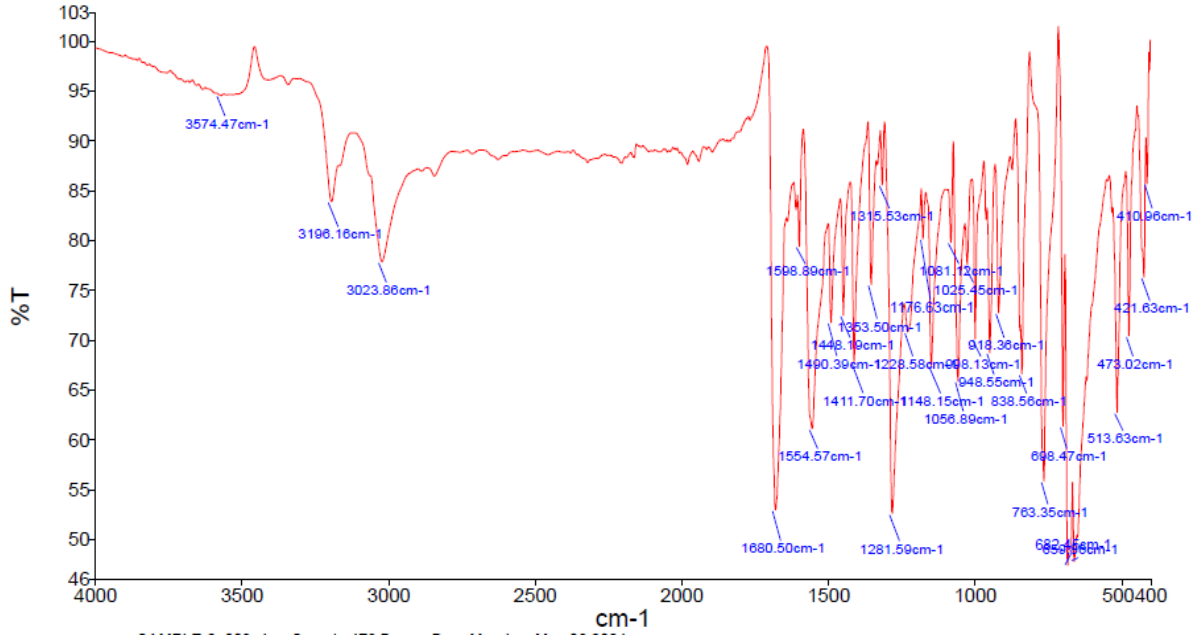


Figure 4: IR Spectrum of Compound 1(b)

IR Spectrum Interpretation of compound 1(b): C=N: 1680.50 cm^{-1}, Aromatic-aldehyde: 3023.86 cm^{-1}, N-H3196.16 cm^{-1}, CH_3: 1315.53 cm^{-1}

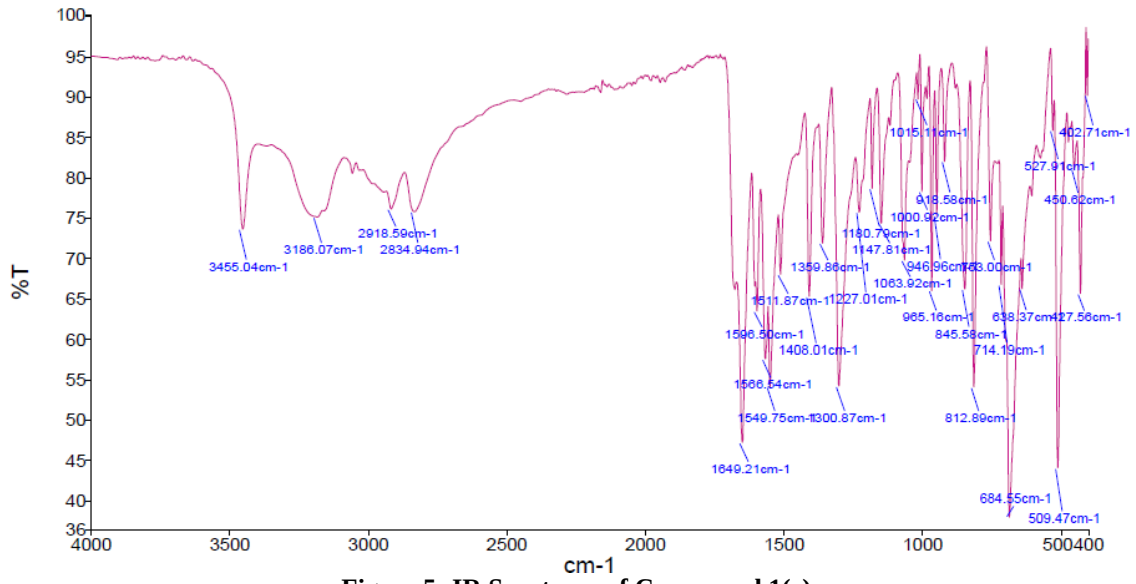


Figure 5: IR Spectrum of Compound 1(c).

IR Spectrum Interpretation of compound 1(c): Aromatic ring: 2918.59 cm^{-1}, C=N: 1649.21 cm^{-1}, Amide :1596.50 cm^{-1}, C=O:1015.11 cm^{-1}, CH_3: 1359.86 cm^{-1}

MASS SPECTROSCOPY

In mass spectrum, which represents the distribution of ions as a function of their mass-to-charge ratio, would provide information about the molecule's mass and fragmentation pattern under ionization. The mass spectrum of isoniazid typically shows peaks corresponding to the molecular ion and its fragments, allowing researchers to identify and characterize the compound.[11]

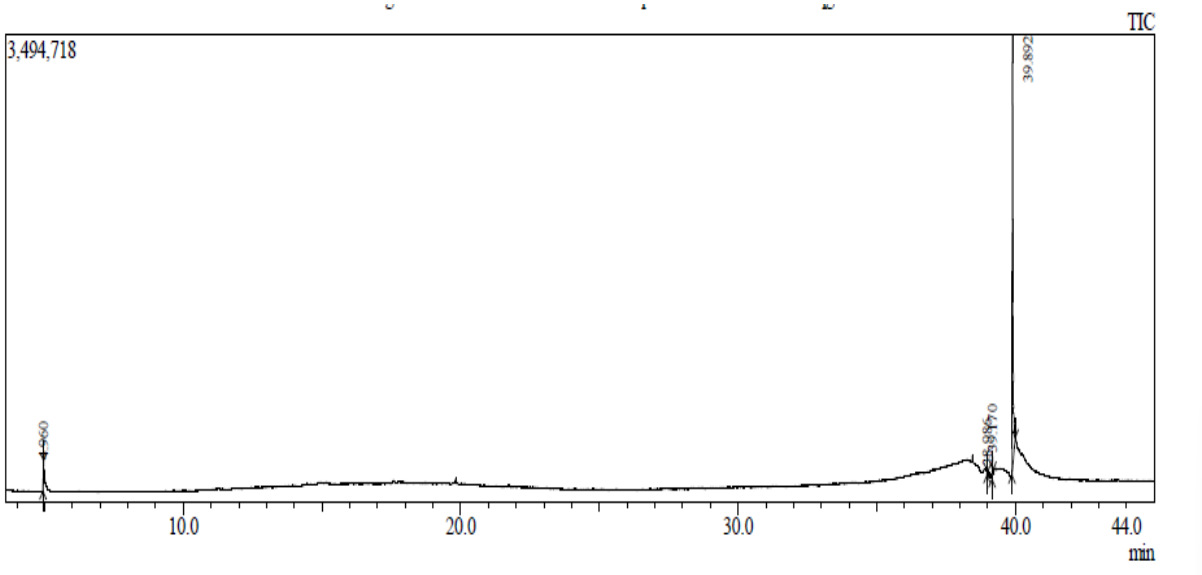


Figure 6: Mass Spectroscopy of Compound 1(a).

ANTIOXIDANT ACTIVITY

DPPH RADICAL SCAVENGING ASSAY

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al [2001].[12] The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

PRINCIPLE

1, 1-diphenyl-2-picryl hydrazyl is a stable free radical with pink colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

DPPH + [H-A] → DPPH-H + (A)

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

REAGENT PREPARATION

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of methanol.

PROCEDURE

Different concentrations of sample such as 12.5µg/mL-200µg/mL from stock solution(10mg/ml) were made up to a final volume of 20µl with DMSO and 1.48ml DPPH (0.1mM) solution was added. A control without the test compound, but an equivalent amount of distilled water was taken. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm (SHIMADZU(UV-1900i) UV-VIS spectrophotometer). 3ml of DPPH was taken as control.

CALCULATION

Percentage of inhibition = $\frac{control-test}{control} X100$

RESULTS

Table 4: Results of DPPH assay of the synthesized compound.

Concentrations (µg/mL)	Absorbance	Percentage of inhibition
Control	0.3554	00
Compound 1(a)		
12.5	0.2557	28.05289
25	0.2256	36.52222
50	0.1985	44.14743
100	0.1643	53.77039
200	0.1322	62.80247
Compound 1(b)		
12.5	0.2801	21.18739
25	0.2458	30.83849
50	0.2156	39.33595
100	0.1863	47.58019
200	0.1601	54.95216
Compound 1(c)		
12.5	0.2871	19.21778
25	0.2663	26.19583
50	0.2276	35.95948
100	0.2004	43.61283
200	0.1734	51.20990

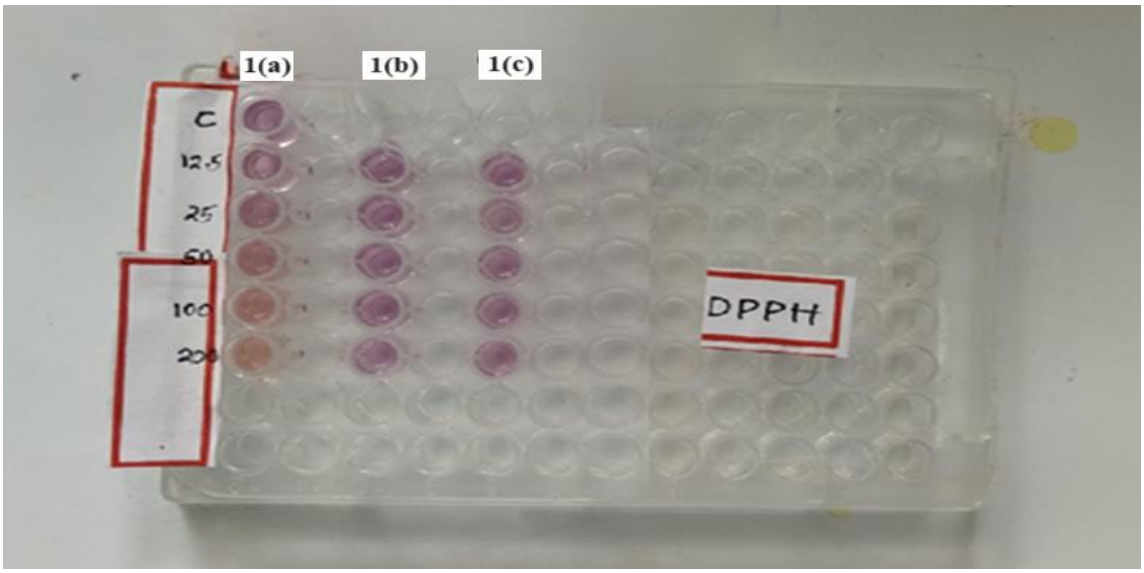


Figure 7: DPPH Assay Plate.

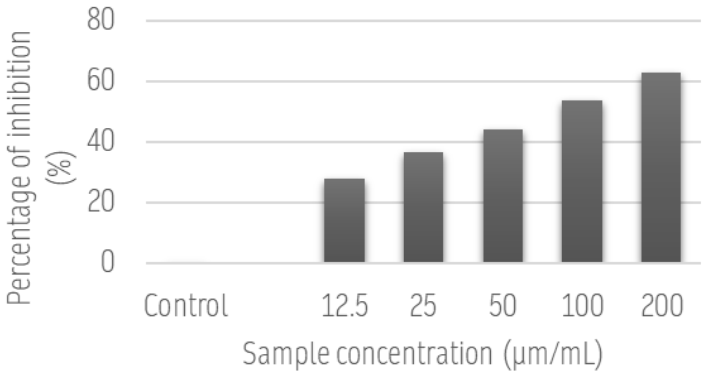


Figure 8: Graphical representation of DPPH Radical scavenging Assay of 1(a). Along Y axis, Percentage of inhibition (%). Along X axis Concentration of sample (µg/mL).

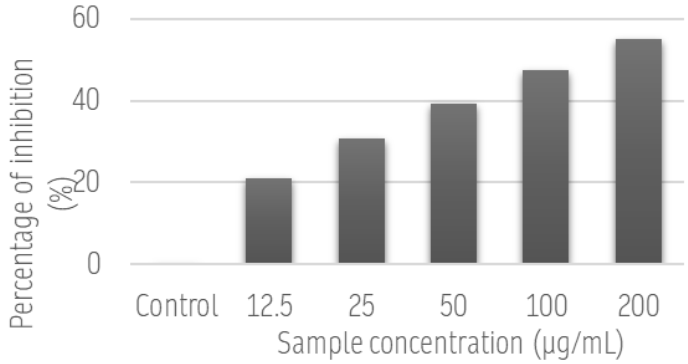


Figure 9: Graphical representation of DPPH Radical scavenging Assay of 1(b). Along Y axis, Percentage of inhibition (%). Along X axis Concentration of sample (µg/mL).

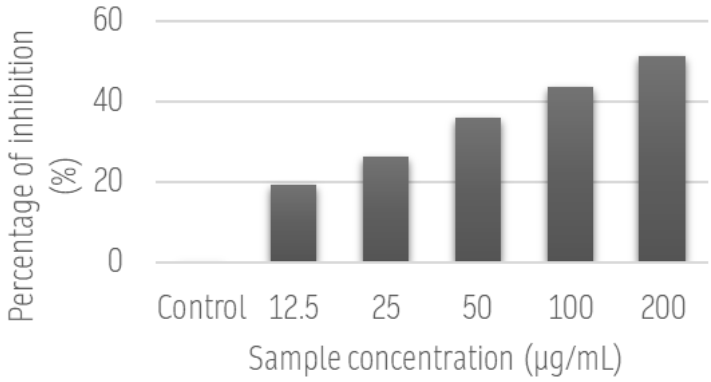


Figure 10: Graphical representation of DPPH Radical scavenging Assay of 1(C). Along Y axis, Percentage of inhibition (%). Along X axis Concentration of sample (µg/mL).

IC_{50} Value- 1(a): 85.43µg/mL (Calculated using ED 50 PLUS V1.0 Software)
IC_{50} Value- 1(b): 150.15µg/mL (Calculated using ED 50 PLUS V1.0 Software)
IC_{50} Value- 1(c): 190.61µg/mL (Calculated using ED 50 PLUS V1.0 Software)

Table 5: Antioxidant characteristics based on IC_{50} Value.[13]

IC_{50} value (µg/mL)	Antioxidant activity
< 50	Very strong
50 -100	Strong
101 -250	Medium
250 -500	Weak
> 500	Not active

CONCLUSION

The Isoniazid derivatives synthesized in this study exhibited antioxidant activity, with compound 1(a) displaying the highest inhibition percentage among them. Despite this, all the derivatives showed lower antioxidant activity compared to Ascorbic acid. These results indicate that while the Isoniazid derivatives have promising antioxidant properties, further refinement is necessary to improve their efficacy. The study

underscores the potential of these derivatives in drug design, particularly for developing drugs that combine antimicrobial and antioxidant properties.

REFERENCES

1. O'Connor C, Patel P, Brady MF. Isoniazid: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan https://www.ncbi.nlm.nih.gov/books/NBK557617/
2. Sarah Zargarnezhad, Ahmad Gholami, Mehdi Khoshneviszadeh, Seyedeh Narjes Abootalebi, Younes Ghasemi; Antimicrobial Activity of Isoniazid in Conjugation with Surface-Modified Magnetic Nanoparticles against Mycobacterium tuberculosis and Non mycobacterial Microorganisms, 11 August 2020 https://doi.org/10.1155/2020/7372531
3. Textbook of Medicinal Chemistry, volume-II, K Illango, P Valentina, 51-57.
4. Pahlavani E, Kargar H, Sepehri Rad N. A Study on Antitubercular and Antimicrobial Activity of Isoniazid Derivative. Zahedan J Res Med Sci, 2015; 17(7): -. https://doi.org/10.17795/zjrms1010.
5. S. L. Gaonkar, S. S. Hakkimane, B. R. Bharath, V. P. Shenoy, U. N. Vignesh and B.R. Guru: Stable isoniazid derivatives: in silico studies, synthesis and biological assessment against Mycobacterium tuberculosis in liquid culture. Rasayan J. Chem, 2022 July-September; 13(3): 1853-1870.
6. Chen, Yu-Jen. The solubility enhancement and the stability assessment of rifampicin, isoniazid and pyrazinamide in aqueous media, 2000.
7. Bakan A, Kapralov AA, Bayir H, Hu F, Kagan VE, Bahar I. Inhibition of Peroxidase Activity of Cytochrome c: De Novo Compound Discovery and Validation. Mol Pharmacol, 2015 Sep; 88(3): 4 21-7. doi: 10.1124/mol.115.097816. Epub 2015 Jun 15. PMID: 26078313; PMCID: PMC4551054.
8. lexander N. Volkov, Peter Nicholls, Jonathan A.R. Worrall: The complex of cytochrome c and cytochrome c peroxidase: The end of the road?, Biochimica et Biophysica Acta (BBA) - Bioenergetics, 2011; 1807(11): 1482-1503, https://doi.org/10.1016/j.bbabio.2011.07.010.
9. Instrumental Method of Analysis, 7th edition, Willard Merritt Dean Settle, 118-339.
10. Fundamentals of Analytical Chemistry, 8th edition, Skoog, West, Holler, Croach, 707-868.
11. Zheng, Yunliang, Xu, Nana, Hu, Xingjiang, Zhang, Qiao, Liu, Yanpeng, Zhao, Qingwei, Development and Application of a LC-MS/MS Method for Simultaneous Quantification of Four First-Line Antituberculosis Drugs in Human Serum, *Journal of Analytical Methods in Chemistry*, 2020; 8838219, 10 pages, 2020. https://doi.org/10.1155/2020/8838219
12. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF, Antioxidant activity of extracts from Acasia confuse bark and heartwood. J Agric Food Chem, 2001; 49: 3420-3424.
13. Kusumawati, Anggun & Farhamzah, Farhamzah & Alkandahri, Maulana & Sadino, Asman & Agustina, Lilis & Apriana, Sukma. (2021). Antioxidant Activity and Sun Protection Factor of Black Glutinous Rice (Oryza sativa var. glutinosa).
14. W K Damdoom, Oday H R Al-Jeilawi: synthesis, characterization of Formazan derivatives from isoniazid and study their antioxidant activity and molecular docking, Russian Journal of Bioorganic Chemistry, 50(1): 86-94.
15. Fengping Chennai, Like Zhou, Bijia Zhou, Siyao Zhang, Xiulan Ma, Hui Zhou, Xun Tuo, Elucidation on the interaction between transferrin and ascorbic acid. A study based on spectroscopic analysis, molecular docking technology and antioxidant evaluation. Journal of Molecular liquids, 2022; 119413(360).
16. Sagar. B. Kedare, R.P. Singh. Genesis and development of DPPH method of antioxidant assay. Pub Med Central, 2011; 48(4): 412-422.
17. Fereidon Shahidi, Ying Zhong. Measurement of anti-oxidant activity. Journal of Functional Foods, 2015; 18: 757-781.
18. Koli, B. P., Gore, R. P., & Malasane, P. R. (2023). Green and efficient three-component synthesis of novel isoniazid pyrazoles, molecular docking, antioxidant and antitubercular evaluation. *Synthetic Communications*, *53*(18): 1506–1519. https://doi.org/10.1080/00397911.2023.2235624