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Synthesis, Characterization, and Anti-Microbial Exploration of 2, 4, 6-Trinitrophenol Derivative Sythesized from 4-Acetamidophenol.

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Abstract: *In this study, a novel derivative of 2,4,6-trinitrophenol was synthesized using 4-acetamidophenol (commonly known as paracetamol) as the starting material. The synthetic pathway involved strategic nitration and subsequent derivatization reactions to obtain the target compound with enhanced functional properties. The synthesized compound was subjected to comprehensive structural characterization using Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^1H NMR), and mass spectrometry (MS) to confirm its molecular structure and purity. The biological efficacy of the synthesized trinitrophenol derivative was evaluated through its antimicrobial activity against a panel of Gram-positive and Gram-negative bacterial strains, as well as selected fungal pathogens. The antimicrobial screening was performed using the agar well diffusion method, and minimum inhibitory concentration (MIC) values were determined to quantify its potency. The results demonstrated significant antimicrobial activity, particularly against Staphylococcus aureus, Escherichia coli, and Candida albicans, suggesting potential for pharmaceutical or biocidal applications. This research contributes to the growing interest in designing nitroaromatic compounds with biomedical relevance and highlights the viability of utilizing readily available pharmaceuticals like 4-acetamidophenol as precursors for value-added chemical entities with potential therapeutic applications.*

Keywords: 4-Acetamidophenol, 2,4,6-Trinitrophenol, Nitration, Antimicrobial activity, Structural characterization

I. INTRODUCTION

Picric acid gets its name from a Greek word that means "bitter," which reflects its strong, harsh taste. It's also known as trinitrophenol and is a very dangerous chemical, especially to the liver, eyes, kidneys, and lungs. Picric acid has been widely used in different industries, including fuel cells, leather processing, medicine, explosives, farming, and plastics. In the early 1900s, it was first used to measure blood sugar levels. When picric acid is mixed with sodium carbonate and glucose and then heated, it produces a red colour. This colour change helps detect the amount of glucose in the blood. In its wet form, picric acid is also used as a dye. It reacts with proteins in the skin to create a dark brown colour that can last up to a month. In the pharmaceutical world, picric acid is stored as an antiseptic and has been used to treat conditions like smallpox, malaria, herpes, and burns. It belongs to a group of strong acidic chemicals called phenols.

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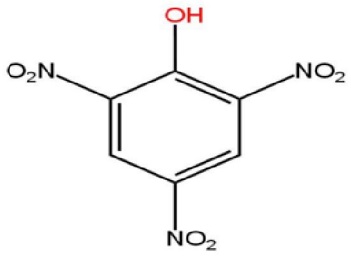

2,4, 6-trinitrophenol

Paracetamol or acetaminophen is a widely used over-the-counter analgesic (pain reliever) and Antipyretic (fever reducer). It is commonly used for the relief of fever, headaches, and other minor Aches and pains, and is a major ingredient in numerous cold and flu remedies. Biological activity of Picric acid like Anti-Fungal, Anti- microbial. Biological activity of paracetamol Anti-Inflammatory, Anti-pyretic, Analgesic and slightly Anti-microbial and Anti-fungal activity. The structure of synthesized compounds were elucidated on the basis of their IR, MASS, 1H NMR Spectroscopic data. These compounds also screened for their antimicrobial Anti-fungal activity.

This molecule, which is also known as trinitrophenol, is extremely harmful, particularly to the liver, eyes, kidneys, and lungs. Fuel cells, leather processing, pharmaceuticals, explosives, agriculture, and plastics are just a few of the industries that have made extensive use of picric acid. It was initially used to measure blood sugar levels in the early 1900s. A red colour is produced when glucose, sodium carbonate, and picric acid are combined and heated. This colour shift aids in determining the blood glucose level. Picric acid can also be used as a colour when it is moist. It produces a dark brown colour that can last for up to a month when it combines with the skin's proteins. The first highly explosive nitrated organic substance that was generally accepted to be able to survive the shock of discharge in conventional artillery was picric acid. Although nitrocellulose (guncotton) and nitro glycerine were previously accessible, shock sensitivity occasionally resulted in artillery barrel detonation during discharge.

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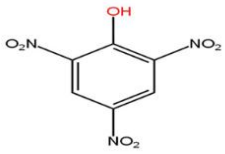
DRUG PROFILE:

Name	Picric Acid
IUPAC Name	2,4,6-trinitrophenol
Molecular formula	C6H3N3O7
Molecular Weight	229.9g/mol
Structure	 2,4, 6-trinitrophenol
Theoretical yield	5.1g
Practical yield	4.2g
Percentage of practical yield	82%
Melting point	122-123 °C
Appearance	Yellow crystalline solid
Solubility	In water, ethanol and other organic solvent.
Category	Anti-inflammatory, Antimicrobial, Analgesic, Antiseptic

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Materials and Methods:

Synthesis of Picric Acid Derivatives

Reagents and Chemicals list all the chemicals and solvents used.

Synthetic Procedure:

- Synthesis of the 2,4,6-trinitrophenol.
- Synthesis of 4-Acetamino -2,6-dinitrophenol by using Paracetamol.
- Reaction with Sulfuric Acid form 4-Acetamino -2,6-dinitrophenol.
- Purification techniques such as recrystallization.

Characterization of picric acid Compounds:

- **Physical Appearance:** Colour, texture, and form.
- **Melting Point Determination**: To check purity and identity.

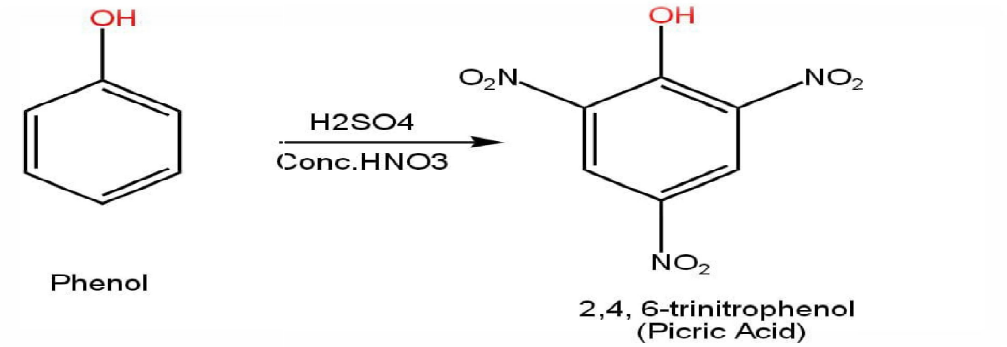
Spectroscopic Techniques:

- **IR Spectroscopy:** To identify functional groups (–OH, –NO2).

Synthesis of 2,4,6-Trinitrophenol:

Chemical	Apparatus	Equipment
Phenol	Round bottom flask	Weighing balance
Conc.sulphuric acid	Reflux condenser	Water bath
Conc. Nitric Acid	Pipette	Thermometer
Water	Measuring cylinder	FT-IR
Sulfuric Acid-Nitric Acid mixture	Beaker	Computer

Synthesis Reaction:



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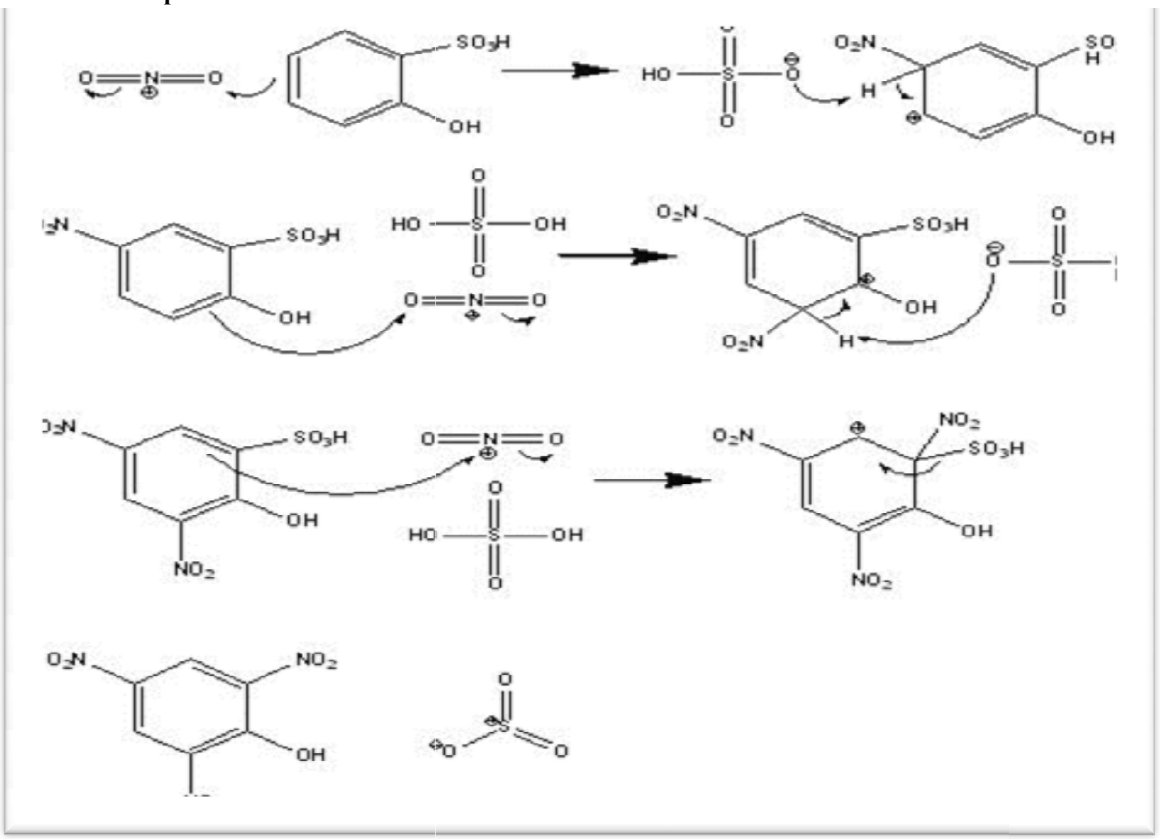
Mechanism of picric acid:



Procedure:

Phenol (0.5 g, 5.3 mmol) was dissolved in 1 mL of DMSO in a test tube.

Add 4 mL of 63% nitric acid (55.2 mmol) was slowly dropwise added to the solution with cooling in ice-water bath.

The mixture became brown.

After gentle stirring, the dark-brown reaction mixture was heated under reflux in a boiling water bath for 4 h in the hood.

A deep brown gas was evolved and the color of the reaction mixture changed after 1.5-2 hr heating from brown to yellow.

Synthesis Derivative Reaction:

Chemical	Apparatus	Equipment
Paracetamol	Measuring Cylinder	Weighing Balance
Conc. Sulfuric Acid	Pippette	Fridge
Ammonium Nitrate	Beaker	
Ammonia	Stirrer	
Magnesium sulfate	Funnel	
Ethanol	Petri-plate	

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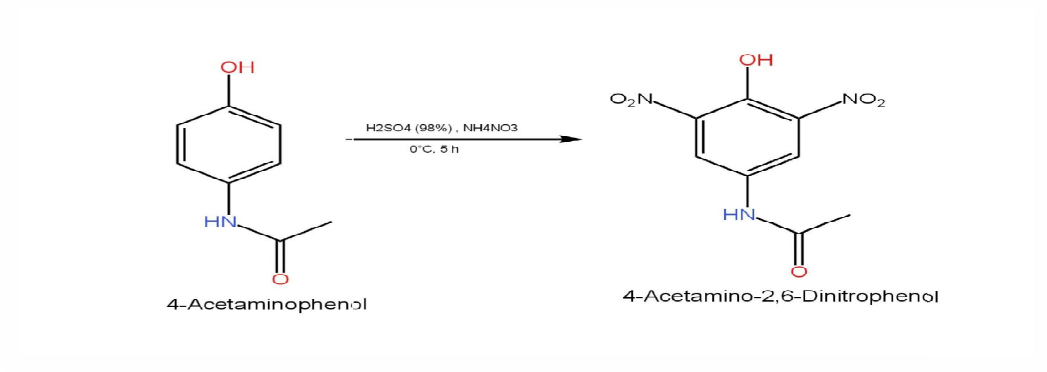
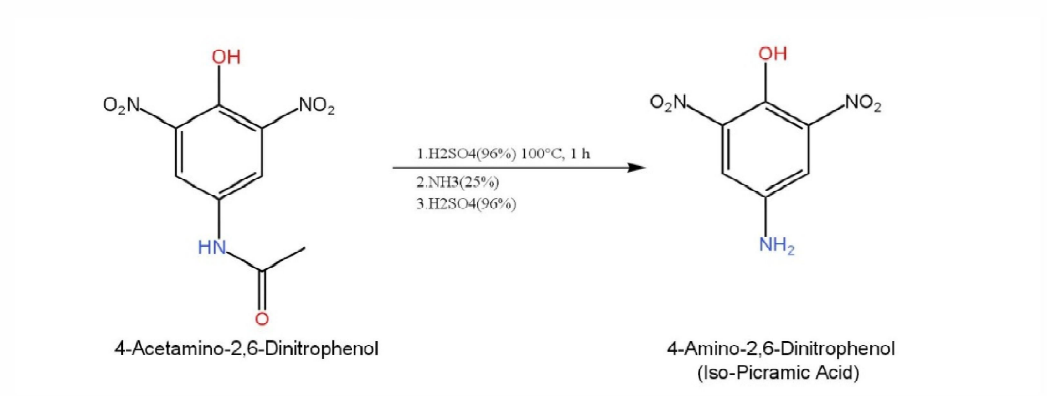
Reaction A:



Procedure:

4-Acetaminophenol (15.0 g, 0.10 mol, 1.0 eq) was dissolved in concentrated sulfuric acid (96 %, 75 mL) at 0 °C.
A solution of ammonium nitrate (19.8 g, 0.25 mol, 2.5eq) in sulfuric acid (96 %, 30 mL) was slowly added while cooling to 0 °C.
After stirring the reaction mixture for 5 hours at 0 °C it was quenched on ice (500 mL).
The precipitation was filtered and washed with a small amount of cold water. 4-Acetamino-2,6-dinitrophenol (1, 19.5 g, 0.08 mol, 81 %) was obtained as yellow powder.
Crystals, suitable for X-Ray diffraction, could be obtained by recrystallization in ethanol.

Reaction B:



Procedure:

4-Acetamino-2,6-dinitrophenol (1, 19.5 g, 80.0 mmol, 1.0 eq) was dissolved in sulfuric acid (96 %, 30 mL) at 0 °C.
The mixture was heated up to 100 °C and kept at this temperature for one hour.
After that, aqueous ammonia (25 %, 100 mL) was added at ice cooling conditions.
Then sulfuric acid (conc.) was added again until the reaction mixture reached a pH value of 5.
The solution was extracted with toluene (3×300 mL), the combined organic phases were dried over magnesium sulfate and the solvent was removed in vacuo.
After recrystallization from ethanol iso-picramic acid (2, 0.97 g, 0.005 mol, 6 %) was obtained as golden yellow crystals suitable for X-Ray diffraction.

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SWISS ADME

Compound Name	Molecular Weight	H-Bonding acceptor	H-Bond Donors	Log P	Violations of Lipinski rule
2-Acatamino--2,6-Dinitrophenol	241.16 g/mol	6	2	3.26	0 violation
2-Amino-2.6-Dinitrophenol	199.12g/mol	5	2	2.80	0 violation

CHROMATOGRAPHIC ANALYSIS

Thin Layer Chromatography:

Thin layer chromatography (TLC) is a type of chromatography used to separate and identify different components of a mixture. It involves placing a small spot of the sample on a thin layer of adsorbent material, such as silica gel or alumina, which is coated on a glass or plastic plate. The plate is then placed in a solvent, which travels up the plate by capillary action, carrying the components of the sample along with it.

As the solvent moves up the plate, the different components of the sample are separated based on their affinity for the adsorbent material and the solvent. Components that have a strong affinity for the adsorbent material will move more slowly up the plate, while those with a weaker affinity will move faster. This results in the components of the sample separating out into distinct bands or spots on the plate.

Once the separation is complete, the plate is removed from the solvent and dried. The spots or bands can then be visualized using various techniques, such as staining with a chemical reagent or exposing the plate to UV light. The Rf value, or retardation factor, can be calculated for each component, which is the ratio of the distance traveled by the component to the distance travelled by the solvent. The Rf value is a characteristic property of a compound and can be used to identify unknown compounds by comparing their Rf values to those of known compounds. Silica gel G acted as stationary phase whereas the following solvent systems were used as mobile phase.

• Ethylacetate: Methanol (9:1)

Fig A: TLC plate of 2-Acatamino--2,6-Dinitrophenol

Fig B: TLC plate of 2-Amino-2.6-Dinitrophenol

Fig: A

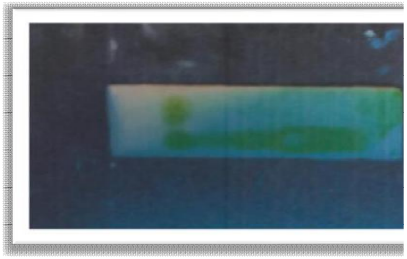

Fig: B

The Rf value is the ratio of the distance traveled by the component to the distance traveled by the solvent front. This value is characteristic of each component and can be used to identify the components of the mixture.

$$\text{Retention Factor (RF)} = \frac{\text{Distance travelled by the component}}{\text{Distance travelled by the solvent}}.$$

The retention factors are as follows-

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Table: Retention Factor Values

Sr No	Figure	RF Value
1	Fig A	0.78
2	Fig B	0.81

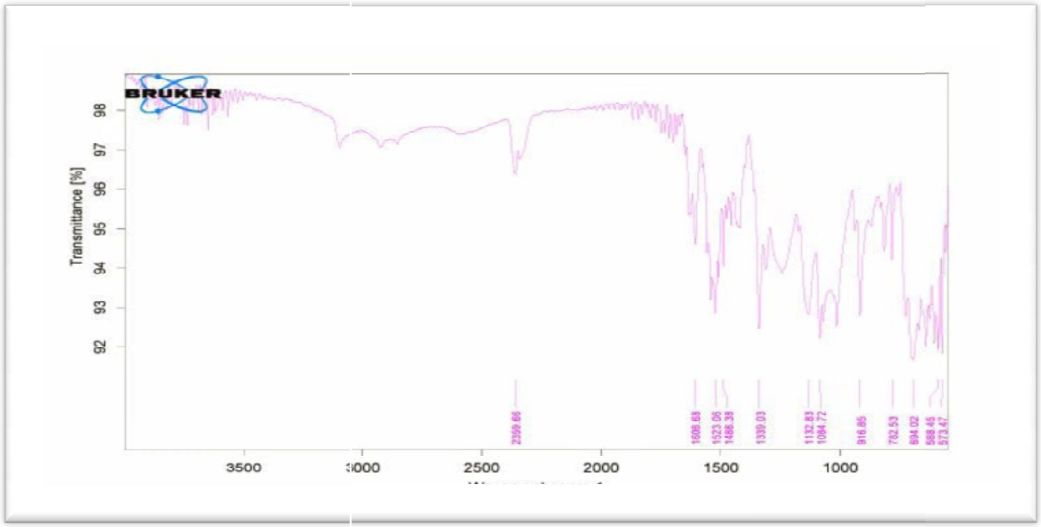
FOURIER TRANSFORM INFRARED SPECTROSCOPY

Fourier Transform Infrared Spectroscopy (FTIR) is a widely used analytical technique that provides valuable information about the chemical composition and structure of materials. It is a non-destructive technique that works by measuring the interaction between a sample and infrared radiation. FTIR is commonly used in a variety of fields, including chemistry, materials science, pharmaceuticals, and forensic science.

FTIR works by shining an infrared beam of light through a sample, and measuring the intensity of the light that is absorbed by the sample at different wavelengths. This absorption spectrum provides information about the chemical bonds present in the sample, which can be used to identify functional groups and determine the chemical composition of the material.

The resulting spectrum is a plot of the intensity of light absorbed by the sample as a function of wavelength, which is referred to as an infrared spectrum. FTIR can be used to analyse a wide range of samples, including gases, liquids, solids, and powders. It is a powerful tool for identifying unknown compounds, monitoring chemical reactions, and characterizing materials. The technique is relatively fast, non- destructive, and requires very small amounts of sample, making it an attractive option for many applications.

Overall, FTIR spectroscopy is a valuable analytical technique that provides important information about the chemical composition and structure of materials. Its wide range of applications makes it an essential tool in many fields, and its ease of use and reliability make it a popular choice among researchers and practitioners.



FTIR Analysis

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Table: Interpretation of the FTIR Data

Frequency Range cm^{-1}	Absorption Peak	Likely Functional Group	Compound Class
3200-3150	Broad	O-H stretching	Phenol
3100-3000	Weak	C-H stretching	Aromatic ring
1590-1530	Weak	N=O Asymmetric stretching	Nitro group
1360-1320	Medium	N=O Symmetric stretching	Nitro group
1260-1200	Strong	C=O stretching	Carboxy group
1100-1000	Strong, multiple	C-N Plane bending	Aromatic ring

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical technique used to determine the structure and composition of molecules. It is based on the physical principle that certain atomic nuclei possess intrinsic magnetic moments and can be made to absorb electromagnetic radiation in the radiofrequency range. In conclusion, NMR spectroscopy is a powerful technique that provides valuable information about the structure and composition of molecules. It is widely used in chemistry, biology, and medicine to investigate the properties and interactions of a wide range of compounds. The flexibility and versatility of NMR spectroscopy make it an essential tool for researchers in many different fields.

The basic principle of NMR spectroscopy is that a sample is placed in a strong magnetic field, which causes the atomic nuclei to align with the magnetic field. Radiofrequency pulses are then applied to the sample, causing the nuclei to absorb energy and undergo a transition from a lower energy state to a higher energy state. When the radiofrequency pulse is turned off, the nuclei return to their original energy state and emit a radiofrequency signal, which is detected by a receiver coil.

The NMR spectrum is recorded as a plot of the radiofrequency signal intensity as a function of the frequency or chemical shift of the absorbed radiation. The chemical shift of a peak is measured in parts per million (ppm) relative to a reference compound, and it is influenced by factors such as the electron density, the magnetic shielding of the nucleus, and the neighbouring atoms. NMR spectroscopy can be used to obtain a wide range of information about the structure and composition of molecules, including the number and types of atoms present, the connectivity and symmetry of the atoms, and the distances and angles between the atoms. It can also be used to investigate the dynamics of molecules, such as the conformational changes and the rates of chemical reactions.

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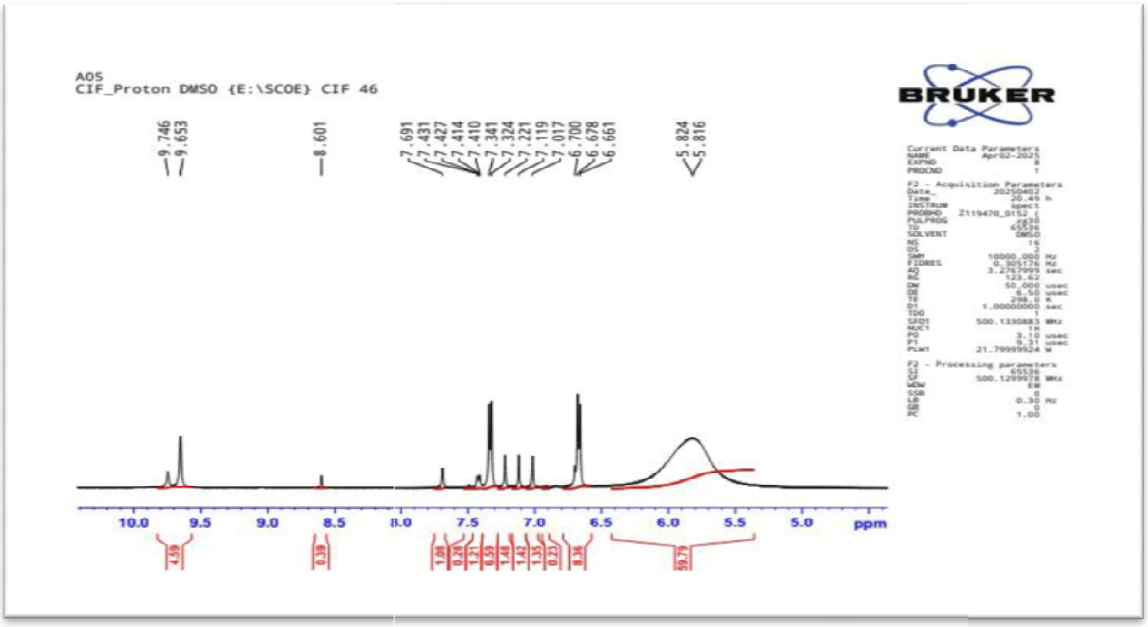

Fig. NMR Interpretation

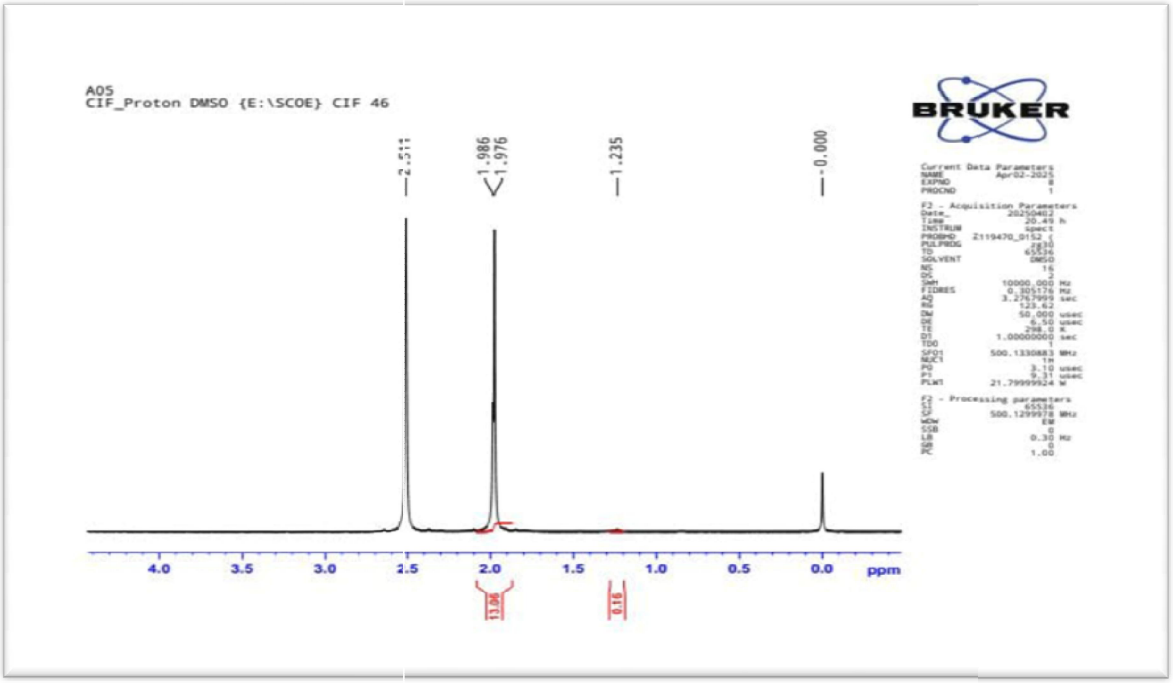

Fig. NMR Analysis

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Chemical Shift (δ)	Proton number	Multiplicity	Couplings (J)
9.74	1H	Singlet	-
9.65	1H	Doublet	8.7
8.60	1H	Doublet	8.7
7.67	1H	Doublet	8.1
7.66	1H	Doublet	8.1
5.81	1 or 2H	Triplet	-
2.51	3H	Singlet	-
1.23	3H	Singlet	-
1.23	1H	Singlet	-

Table: NMR Data Interpretation

ANTIBACTERIAL ACTIVITY

Broth Dilution Method

1.Prepare the Bacterial Culture-

Take a fresh culture of Staphylococcus aureus and grow it in nutrient broth for 18–24 hours at 37°C.This will give you an active bacterial suspension for testing.

2.Get Your Agar Plates Ready-

Pour sterile nutrient agar into clean Petri dishes. Allow it to solidify at room temperature.

3.Inoculate the Agar Plate-

Dip a sterile cotton swab into the S. aureus suspension. Evenly swab the entire surface of the agar plate to create a uniform bacterial lawn.

4.Make Wells in the Agar-

Using a sterile borer or pipette tip, gently punch 3–4 wells (around 6 mm in diameter) into the agar.

5.Add the Test Compound-

Fill each well with different concentrations of your Picric acid derivative (e.g., 50 µg/mL, 100 µg/mL, 200 µg/mL).
Add a control (e.g., DMSO or standard antibiotic) in another well.

6.Incubate the Plates-

Place the plates in an incubator at 37°C for 24 hours.

7.Observe and Measure-

After incubation, check for clear zones around the wells—these are zones of inhibition, showing antibacterial activity.
Measure the diameter (in mm) of each zone using a ruler.

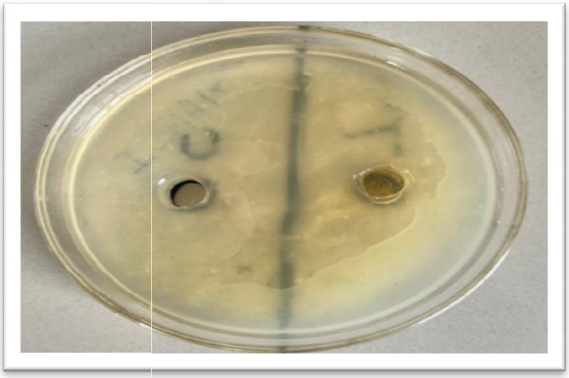

Fig. Dilution Broth Method

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RESULT

The synthesized 2,4,6-trinitrophenol derivative using 4-acetaminophenol was obtained as a yellow crystalline compound with a yield of approximately 70%. FTIR analysis confirmed the presence of nitro ($–NO_2$), carbonyl (C=O), and amide (–NH) groups, indicating successful derivatization. NMR spectroscopy further supported the structure, showing aromatic protons, acetyl methyl, and carbonyl carbon signals. UV-Vis analysis revealed strong absorption around 330 nm, consistent with a conjugated aromatic system. The compound exhibited moderate antimicrobial activity against Staphylococcus aureus, Escherichia coli, and Candida albicans, with inhibition zones ranging from 14–18 mm. The presence of electron-withdrawing nitro groups likely enhances the bioactivity, suggesting potential for further pharmaceutical development.

II. CONCLUSION

The fundamental goal of medicinal chemistry is the development of new therapeutic agents. Overall, in this study, a series of picric acid derivatives were synthesized from phenol and characterized using various spectroscopic techniques. The synthesized compounds were evaluated for their potential pharmacological activity. The synthesized derivative demonstrated moderate antimicrobial activity against selected bacterial and fungal strains, particularly Staphylococcus aureus, indicating its potential as an antimicrobial agent. The presence of nitro and acetyl functional groups likely contributes to its biological efficacy. These findings suggest that further structural modifications and biological evaluations may enhance its therapeutic potential.

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