CHAPTER 1
CHAPTER 1

Synthesis and Characterization of Ligand with Bioactivity evaluation

Section I

Synthesis and Characterization of 4-hydroxy – 3-nitro-acetophenone – Compound 1

Section II

Thermal and Electrochemical study with bioactivity evaluation

Publications

- Antioxidant potential of para - Hydroxy - meta - nitro acetophenone and it’s Metal Complexes by DPPH assay, Communicated to International Journal of Chemical and Analytical Research.

- Antioxidant potential of para - Hydroxy - meta - nitro acetophenone with it’s Metal Complexes by Nitric Oxide assay, Communicated to International Journal of Pharmaceutical, Chemical & Biological Research.

- Synthesis, characterization and electrochemical study of bioactive ligand, communicated to Transition metal chemistry
Section 1
Synthesis and Characterization of 4- hydroxy - 3-nitro-acetophenone – A ligand

1. Introduction
A precursor, acetophenone has industrial applications in variety of resins. It is also used in perfumery, as a flavouring agent in food industry etc. It is utilized in polymer industry.

Acetophenone
Acetophenone is the organic compound with the formula C₆H₅COCH₃. It is the simplest aromatic ketone. This colourless, viscous liquid is a precursor to useful resins and fragrances¹,³,⁴ and as an industrial solvent². Acetophenone is serving as a novel group of useful therapeutics against the mycobacteria⁴. It is used in perfumery as a fragrance ingredient in soaps, detergents, creams, lotions, and perfumes. It is also employed as a flavoring agent in foods, nonalcoholic beverages and tobacco. It has commercial applications in diverse fields such as plastics, a specialty solvent for resins, as a catalyst for the polymerization of olefins and in organic syntheses as a photosensitizer⁵-⁷. Acetophenones are widely found in the environment as degradation products of industrial chemicals. In the late 19th and early 20th centuries, acetophenone was used in medicines⁸. It is marketed as a hypnotic and anticonvulsant under brand name Hypnone⁹. It was considered to have superior sedative effects to both paraldehyde and chloral hydrate¹⁰. In humans, acetophenone is metabolized to benzoic acid, carbonic acid and acetone¹¹. It is exploited in laboratories for asymmetric transfer hydrogenation. Mannich bases of acetophenones have been disclosed to have antitumour and cytotoxic activities⁴. It is also used to synthesize various drug intermediates due to carbonyl group attached to aromatic ring. Many condensation reactions leading to bio active molecules have been synthesized by taking the help of active methyl group. Its para hydroxy derivative is found to be potent against microbes. Many acetophenones are also natural products in plants and fungi¹.
**para – Hydroxyacetophenone – Piceol**

Piceol is a phenolic compound found in mycorrhizal roots of Norway spruces (*Picea abies*)¹². Picein is the glycoside of piceol¹³. Capillary electrophoresis with electrochemical detection has been employed for the determination of *p*-hydroxyacetophenone, chlorogenic acid, and caffeic acid in Herba *Artemisiae Scopariae* (the dried sprout of *Artemisia scoparia* Waldst. et Kit.). The proposed method has been successfully applied to monitor the three bioactive constituents in real plant samples and to differentiate between different herbal drugs with satisfactory assay results¹⁴. Aryl-alkyl ketones are important starting materials for the synthesis of naturally occurring polyphenolics, i.e. chalcones, flavones, flavanones, chromones, etc. For the total synthesis of these natural products, proper protection/deprotection of acetophenones is always required which affect the yield¹⁵. The yeast lipase *Candida cylindracea* (CCL) and *porcine pancreatic* lipase (PPL) have been used for regioselective deacylation of para acetylated benzopyrones, diphenylpropenones and acetophenones, which provide structural leads to anti AIDS and anticancer agents¹⁶. Several aerobic microorganisms are capable of utilizing acetophenones for their growth. Reports depicted elucidation of the 4-Hydroxyacetophenone by Catabolic Pathway in *Pseudomonas fluorescens*¹⁷.

**meta – Nitro Acetophenone**

Nitro aromatic compounds are extensively used as chemical feed stocks for a wide range of materials such as dyes, pharmaceuticals, perfumes and plastics. Therefore, nitration of organic compounds has been a long, very active and rewarding area of research and subject of a large body of literature¹⁸-²¹. More specifically the nitration of benzene and toluene is some of the most important routs to substituted aromatics in the production of chemical inter-mediate. The introduction of a nitro group into an aromatic ring is commonly performed in strongly acidic polar media²²-²⁹. *meta – Nitro acetophenone* has been managed by the nitrination of acetophenone³⁰-³⁵. It has been made also by the hydrolysis of *m*-nitro benzoil acetoacetic ester³⁶. Electron impact studies showed release of kinetic energy during negative-ion fragmentations. The loss of NO⁺ from *meta* nitro acetophenone molecular
anions. They are used as a solvent and for the synthesis of dyestuffs, urethane polymers, and other plastics as well as of anilines, and among derivative products are also insecticides, herbicides, and pharmaceuticals. The latter is of ongoing interest also in an effort to better understand the mutagenicity of ambient air, pointing to an important aspect of the toxicological profile of this class of compound.

**para - Hydroxy - meta - nitro acetophenone**

2-Amino-4-ethynylphenol is prepared by a four-step synthetic sequence in which the key reaction is the treatment of 4-acetoxy-3-nitroacetophenone with a Vilsmeier reagent derived from N,N-dimethyl formamide and phosphorus oxychloride. Literature reviewed the compound is useful as an end capping agent in the synthesis of fluorocarbon ether bi-benzoxazole oligomers which, because of the presence of acetylenic terminal groups, can be cured by thermal means to provide broad-use such as temperature, fuel and fluid resistant vulcanizates. 4-nitro-acetophenone-reductase is used in human erythrocytes. It has application in adrenal glands of animals. Intermediate containing the pharmacophore of the β-2-adrenoceptor agonist was synthesized from the commercially available para-hydroxy-meta-nitro-acetophenone. Further it was synthesized a novel class of dual pharmacology bronchodilators targeting both β₂-adrenoceptor and PDE4 by applying a multivalent approach. It is also used in the preparation of guamdinoajcyl derivatives of substituted anilides. It is also employed in reaction between peroxynitrite and boronates having biological implications of the minor free radical pathway.

**1.1.1 Present work**

Bio-activity of various nitro aromatic compounds has been reported. It is having molecular formula C₈H₇NO₄ and was obtained by nitration reaction. Its purification was tried in various solvent systems to get pale yellow compound in pure form for its physical, analytical parameters, redox potentials, antioxidant activity and coordination study with transition metals. The said **compound 1** has been synthesized and compared with M.P IR, UV & LC-MS.
with the reported. The structure was confirmed by modern spectral data obtained from UV- Vis, IR, LC-MS, CV and TGA etc. Antioxidant potential of various concentrations is determined using DPPH and Nitric Oxide assays for the first time.

\[
\text{HO} \quad [\text{NO}_2] \quad \text{HO} \\
\text{Compounds} 1
\]

4- Hydroxy - 3- nitro-acetophenone – compound 1

Pale yellow crystalline solid
Melting point: \(133^\circ\text{C}\) (Observed), \(133-135^\circ\text{C}\) (reported)
Molecular ion peak: 181 amu
Molecular formula: \(\text{C}_8\text{H}_7\text{N O}_4\)
IR: (KBr) cm\(^{-1}\): 3300, 1684, 1543, 1497, 1429, 1320, 764 and 698 cm\(^{-1}\).

### 1.1.2 Results and Discussion

The synthesized compound is pale yellow crystalline solid. The LC-MS spectrum (Fig 1) of the compound exhibits a molecular ion peak at 181 amu which suggests the molecular formula is \(\text{C}_8\text{H}_7\text{NO}_4\).

**IR spectrum (Fig 2)** displays a characteristic absorption frequency at 3300 cm\(^{-1}\) for associated OH stretching. Other specific frequencies are perceived at 1543 cm\(^{-1}\), 1497 cm\(^{-1}\) and 1429 cm\(^{-1}\) for presence of aromatic ring. Aromatic methyl ketone frequency is depicted at 1684 cm\(^{-1}\). Peaks at 1543 cm\(^{-1}\) and
1320 cm$^{-1}$ are detected for asymmetric and symmetric stretching of nitro group respectively. The bands at 764 cm$^{-1}$ and 698 cm$^{-1}$ relate to the monosubstituted aromatic ring. Structure has been confirmed by melting point, IR and UV Visible Spectrum (Fig 3).

**Compound 1**

**Fig 1 LC-MS Spectrum**

**Fig 2 IR Spectrum**

**Fig 3 UV Visible Spectrum**
Section II

Thermal and Electrochemical study with bioactivity evaluation

1.2 Introduction

Studies on thermal properties of substances have a great importance from both scientific and practical point of view. Some compounds/complexes/substances get deform at certain temperature. In industries stability of row materials/intermediates/final product have become an essential criteria. In pharmaceutical industries as per the requirement of the drug in body at receptor site stability counts a lot, thus thermal properties play a very significant role in day to day life.

Scientific and technological achievements together with demands based on industrial requirement have permitted the development of various types of materials that can withstand at much higher temperatures to avoid corrosive environments by balancing pollutant and effluent.

These thermal properties can be studied by various thermal techniques which are among the most powerful experimental tools developed during the last century. These techniques are able to characterize a wide range of materials and material properties. In these techniques, the change in properties of material are followed as a function of temperature when it is heated at constant predetermined rate under specified ambient atmospheric conditions.

Some of the most commonly used techniques are Differential Scanning Calorimetry (DSC), Differential Thermal Analysis (DTA), Thermo Gravimetric Analysis (TGA), Evolved Gas Detection (EGD), Evolved Gas Analysis (EGA) etc. are essential for industries as per their rules. In TGA, the mass of sample is recorded as a function of temperature or time, when it is subjected to a programmed temperature change in a specified atmosphere. The plot of mass change versus temperature is known as thermogram or TG curve. TG curves are characteristic for a given compound because of unique sequence of physicochemical reactions which occur over definite temperature ranges and at rates that are a function of molecular structure. The changes in weight are due to various physical and chemical changes which lead to the evolution of volatile products or the formation of heavier reaction products.
1.2.1 Review of Literature

Literature survey shows that thermal analysis of various types of compounds such as drugs\textsuperscript{49-52}, Polymers\textsuperscript{53-56}, catalyst\textsuperscript{57-58} nuclear fuel\textsuperscript{59}, pharmacy materials\textsuperscript{60-64} dyes\textsuperscript{65-66}, fertilizers\textsuperscript{67}, inorganic\textsuperscript{68-70} and organic compounds\textsuperscript{71-73} have been reported. Recently, a number of investigators\textsuperscript{74-83} has studied the thermal properties of various materials. Thermo gravimetric Analysis (TGA) is finding increasing utility in investigations of the pyrolysis and combustion behavior of materials. Consequently, a fairly large number of analytical methods have been proposed for obtaining kinetic parameters from TGA curves\textsuperscript{84}. Schiff bases have often been used as chelating ligands in the field of coordination chemistry and their metal complexes are of great interest for many years. It is well known that N and S atoms play a key role in the coordination of metals at the active sites of numerous metallo bio-molecules\textsuperscript{85}. Ligand 2-hydroxy-5-methyl-3-nitroacetophenone 4-phenyl-2 imino thiazole and their metal complexes with concluded water loss in metal complexes and ligand. Also concluded half decomposition temperature and parameter of activation energy\textsuperscript{86}. Schiff base ligand originated from 2-hydroxy-5-methyl-acetophenone, 2-hydroxy-5-methyl-3-nitroacetophenone and carbohydrazide have been prepared. The complexes were found to be quite stable and decomposition of the complexes ended with respective metal oxides as an end product\textsuperscript{87}. The use of thermo gravimetric data to evaluate kinetic parameters of solid state reactions involving weight loss (or gain) has been investigated by a number of workers\textsuperscript{88}.

1.2.2 Present work

Evaluation of kinetic parameters by TGA

TGA measures changes in weight in relation to changes in temperature. The measured weight loss curve gives information on changes in sample composition, thermal stability and kinetic parameters for chemical reactions in the sample. A derivative weight loss curve can be used to tell the point at which weight loss is most apparent. In the present study, thermal properties of synthesized \textit{para} - Hydroxy - \textit{meta} - nitro acetophenone have been studied.
by TGA techniques. Using thermograms, various kinetic parameters such as activation of energy, co relation factor etc. have also been evaluated.

**Cyclic voltammetry – CV**

Cyclic voltammetry is the most widely used technique for acquiring qualitative information about electrochemical reactions. It provides qualitative information about the number of oxidation states and their stability, as well as the rate of heterogeneous electron transfer reactions. It offers a rapid location of redox potentials of the electro active species. CV has the further attraction of providing information not only on the thermodynamics of redox processes but also on the kinetics of heterogeneous electron-transfer reactions and coupled chemical reactions.

The characteristic shapes of the voltammetric waves and their unequivocal position on the potential scale virtually fingerprint the individual electrochemical properties of redox systems. The real power of this technique lies in its ability to investigate mechanisms and potentials of electrode reactions. Usually the conditions are used, where capacitive current is smaller than the current from electron transfer. Cyclic voltammetry (CV) is the most commonly used method in electro catalysis. The technique offers a first view on electrode/electrolyte systems in the potential window of interest. For most metals and alloys, reproducible results can be obtained after cycling the surface in a base electrolyte and over suitable potential ranges. Cyclic voltammograms of some metal/electrolyte interfaces and some selected charge transfer reactions, of interest in fuel cell electro catalysis, are presented. The use of CV for investigations, e.g., with ultra micro-electrodes, rotating electrodes (disc and ring/disc), as well as in combination with online mass spectroscopy and FTIR spectroscopy, is outlined.

Variation of scan rate is an important element of mechanistic investigations using cyclic voltammetry. The electrochemical behavior of the ligand is studied in aprotic solvent CH$_3$CN. To get accurate measurement of current CVs of solvents are tested for residual current prior to use, to avoid errors due to dissolve oxygen or other redox impurities. The CV waves are corrected for solvent background employing Guttmann’s correlation. CV experiments demonstrates the determination of the formal reduction potential ($\lambda$), the number of electrons transferred in redox process ($n$), the diffusion coefficient.
(D), electrochemical reversibility and the effects of varying concentration and scan rate. The CV is characterized by several important parameters such as cathodic ($E_{pc}$) and anodic ($E_{pa}$) peak potentials, the cathodic ($i_{pc}$) and anodic ($i_{pa}$) peak currents, the cathodic half peak potential ($E_{p/2}$) and half-wave potential ($E_{1/2}$). Rate constants for reversible quasi-reversible and irreversible electron transfer reactions can be calculated.

1.2.3 Results and Discussions
The TG of compound 1 (Fig 4) demonstrates the mass loss as a function of temperature which is seen in one step. The decomposition of the molecule starts at nearly at 100°C and completed at 511°C with removal of total organic molecule to get nearly 100% weight loss. Kinetic parameters are presented (Table 1). Kinetic parameters are calculated by Coats-Redfern method where correlation coefficient ‘r’ may be positive or negative which meets to 0.999. The kinetic data such as order of reaction and activation energy for the molecule have been reported (Table 2). Kinetic plots are depicted (Fig 5).
Cyclic voltammograms of compound 1, (Fig 6) proves cathodic peak potential at +0.95 V and anodic peak potential at -0.3 V at 100mV/sec scan rate. As $\Delta E_p$ is greater than 0.059/n it is a quasi-reversible electron transfer with electron transfer rate const $K_o = 2.8 \times 10^{-8}$ cm/s. The plot of $I_{pa}/I_{pc}$ vs scan rate (Fig 7) variation shows C type of mechanism, ErCir means irreversible electron transfer followed by irreversible chemical reaction. The results have been reported (Table 3-6)

1.2.3 Conclusion
Compound 1 is synthesized for their electrochemical and thermal studies. The results indicate the stability of Compound 1. The energy values associated with this molecule and its redox potentials are the important clues for their biological activity evaluation.

1.2.4 Experimental
Reagents and solvents used were of commercially available analytical grade. Compound 1 is synthesized by nitration of para hydroxy acetophenone. This reaction mixture is refluxed on oil bath at 50°C for five minutes. Yellow solid
with gummy mass poured into boiling water bath. Fine yellow product was obtained. The product was recrystallized from mix solvents like ethyl acetate and hexane. Sharp melting nature indicates purity and formation of product is confirmed by reported values. Melting point and TLC was recorded. Structure was confirmed by modern analytical spectral techniques. The IR and UV absorption spectra of compound 1 was obtained by using ‘Schimadzu FTIR 3600 spectrophotometer’ and ‘UV-Vis 1700 spectrophotometer’. LC-MS spectra were recorded on LC-MS-MS Perkin Elmer Applied Bio-systems SCIEX-2000 at room temperature. Cyclic voltammograms of the compound 1 was recorded in acetonitrile solution used as solvent at 300K on ‘CHH Instrument Element Analyzer’ which is composed of three – electrode cell. Here internal standard comprises as a reference electrode Ag/AgCl and auxillary Pt with working Pt electrodes. Electrochemical behavior of compound 1 was studied in aprotic solvent Acetonitrile at room temperature. Voltamograms of acetonitrile were recorded to eliminate the effect of solvent. Molar conductivity was measured on a ‘Systronic conductivity bridge’ with a dip type cell, using 10⁻³ M solution of compound in acetonitrile. Decomposition experiments were carried out by non- isothermal thermogravimerty using Shimadzu TGA- 50 analyzer. Thermogravimetric experiments were carried out from room temperature to 700°C in air at heating rate of 3°C/min. The thermal kinetic parameters such as order of reaction ‘n’ and energy of activation ‘Ea’ were determined from computer programme of rising temperature expression of Coats and Redfern. Coats and Redfern developed an integral method, which can be applied to presume to lead to the best linear plot, from which the activation energy is determined. The final form of the equation, which is used for analysis, taken the form:

\[
\ln \left( 1 - \frac{1}{1-(1-\alpha)^{1-n}} \right) = \frac{1}{(1-n)T^2} V\alpha - \frac{1}{T}
\]

The criterion for straight line is closeness of correlation coefficient ‘r’ to positive or negative 0.999. Slope of the line gives energy of activation as,

\[Ea = \text{Slope} \times R, \text{Where } R \text{ is Gas constant (8.314 J/deg/mol).}\]
Table 1  Data from Dynamic TGA for compound 1 (Air atmosphere)

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp. range (°C)</th>
<th>% wt. Loss Observed</th>
<th>% Wt. Loss calculated</th>
<th>Loss of probable moiety</th>
<th>Order of reaction (n)</th>
<th>Ea (KJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>100-511</td>
<td>100</td>
<td>100</td>
<td>C₆H₇ N O₄</td>
<td>2.8</td>
<td>64.06</td>
</tr>
</tbody>
</table>

Table 2  Kinetic data from TG of compound 1 in air atmosphere

<table>
<thead>
<tr>
<th></th>
<th>Initial % Wt loss = 0</th>
<th>Final % Wt loss = 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (Kelvin)</td>
<td>Wt</td>
<td>1/T</td>
</tr>
<tr>
<td>312</td>
<td>9.44</td>
<td>0.00225</td>
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<tr>
<td>350</td>
<td>15.42</td>
<td>0.00219</td>
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<tr>
<td>390</td>
<td>22.25</td>
<td>0.00214</td>
</tr>
<tr>
<td>412</td>
<td>29.09</td>
<td>0.00211</td>
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<tr>
<td>460</td>
<td>35.92</td>
<td>0.00209</td>
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<tr>
<td>484</td>
<td>41.89</td>
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<td>530</td>
<td>49.56</td>
<td>0.00204</td>
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Table 3  Electrochemical parameters with scan variation of (Compound 1) in aprotic solvent (CH₃CN)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Scan rate (Volts/Sec)</th>
<th>Eₚc (Volts)</th>
<th>Eₚa (Volts)</th>
</tr>
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<tr>
<td>1</td>
<td>0.05</td>
<td>0.95</td>
<td>-0.35</td>
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<tr>
<td>2</td>
<td>0.10</td>
<td>0.95</td>
<td>-0.30</td>
</tr>
<tr>
<td>3</td>
<td>0.15</td>
<td>0.95</td>
<td>-0.25</td>
</tr>
<tr>
<td>4</td>
<td>0.20</td>
<td>0.95</td>
<td>-0.20</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>0.95</td>
<td>-0.2</td>
</tr>
<tr>
<td>6</td>
<td>0.30</td>
<td>0.85</td>
<td>-0.2</td>
</tr>
</tbody>
</table>
### Table 4  Electro Chemical data for quasi reversible charge transfer with scan rate variations of compound1 in aprotic solvent (CH$_3$CN)

<table>
<thead>
<tr>
<th>$\nu$ (v/sec)</th>
<th>$\nu^{1/2}$</th>
<th>$i_{pa}$ (A)</th>
<th>$i_{pc}$ (A)</th>
<th>$i_{pa}/i_{pc}$</th>
<th>$i_{pc}/\nu^{1/2}$</th>
<th>$\lambda$ (V)</th>
<th>$E_{pc}$ (V)</th>
<th>$E_{pa}$ (V)</th>
<th>$\Delta E_p$ (V)</th>
<th>$E_{1/2}$ (V)</th>
<th>$\tau$ (Sec)</th>
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<tr>
<td>0.05</td>
<td>0.2236</td>
<td>0.025</td>
<td>-</td>
<td>0.025 -1</td>
<td>0.1118 -1.35</td>
<td>0.95</td>
<td>0.35</td>
<td>-1.3</td>
<td>0.3</td>
<td>33</td>
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<td>0.10</td>
<td>0.3162</td>
<td>0.025</td>
<td>-</td>
<td>0.050 -0.5</td>
<td>0.1581 -1.4</td>
<td>0.95</td>
<td>-0.3</td>
<td>1.25</td>
<td>0.325</td>
<td>17.25</td>
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<td>0.15</td>
<td>0.3872</td>
<td>0</td>
<td>0.075</td>
<td>0.075 -0.75</td>
<td>0.1936 -1.4</td>
<td>0.95</td>
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<td>0.35</td>
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<td>0.20</td>
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<td>0.25</td>
<td>0.5000</td>
<td>0</td>
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<td>-0.05 -0.1</td>
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<td>0.3</td>
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<td>0.025</td>
<td>0</td>
<td>0.0456 -1.4</td>
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<td>1.05</td>
<td>0.725</td>
<td>4.66</td>
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### Table 5  Electrochemical and kinetic data for compound 1 in CH$_3$CN

<table>
<thead>
<tr>
<th>$V$ (v/s)</th>
<th>$i_{pc}$ (A)</th>
<th>$E_{pc}$ (v)</th>
<th>$E_{pa}$ (v)</th>
<th>$\Delta E_p$ (v)</th>
<th>$\alpha$ na</th>
<th>$D \times 10^{-10}$ (cm$^2$/s)</th>
<th>$K_o \times 10^{-9}$ (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>-0.025</td>
<td>0.95</td>
<td>-0.35</td>
<td>-1.30</td>
<td>0.04</td>
<td>0.02</td>
<td>1.56</td>
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<tr>
<td>0.10</td>
<td>-0.050</td>
<td>0.95</td>
<td>-0.30</td>
<td>-1.25</td>
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<td>0.04</td>
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</tr>
<tr>
<td>0.15</td>
<td>-0.075</td>
<td>0.95</td>
<td>-0.25</td>
<td>-1.20</td>
<td>0.05</td>
<td>0.06</td>
<td>4.73</td>
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<tr>
<td>0.20</td>
<td>-0.050</td>
<td>0.95</td>
<td>-0.20</td>
<td>-1.15</td>
<td>0.05</td>
<td>0.02</td>
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<td>0.25</td>
<td>-0.050</td>
<td>0.95</td>
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<tr>
<td>0.30</td>
<td>-0.025</td>
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<td>-0.20</td>
<td>-1.05</td>
<td>0.06</td>
<td>0.03</td>
<td>1.58</td>
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Average rate constant ($K_o$) = $2.8745 \times 10^{-9}$ cm/s, $C_o = 0.001$ M

$A = 0.2826$ cm$^2$, Quasireversible charge transfer
Table 6 Type of reaction mechanism and redox potential (compound 1)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Type of Mechanism</th>
<th>Rate constant (K_o) cm/sec</th>
</tr>
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<tr>
<td>1</td>
<td>ErCir</td>
<td>2.8 x 10^{-8} cm/s</td>
</tr>
<tr>
<td></td>
<td>Er : O + e → R → Z</td>
<td></td>
</tr>
</tbody>
</table>

Fig 4 Dynamic TGA in Air atmosphere compound 1

Fig 5 Kinetic plot of 1/T Vs f(α) from TG of compound 1
Fig 6  Cyclic voltammograms in CH$_3$CN at various scan rates

(Compound 1)

(50 mV/s)  (100 mV/s)

(150 mV/s)  (200 mV/s)

(250 mV/s)  (300 mV/s)

Fig 7  Plot of Reaction Mechanism for compound 1
1.2.5 A: Antioxidant potential

Recent years have witnessed an unprecedented progress in biological applications of metal coordination compounds of biologically active ligands because of their key role in clinical therapy.

A - 1 Introduction

A methodological consideration for characterizing potential antioxidant actions of bioactive components in plant parts has become challengeable scenario. The study of free radicals and antioxidants in biology is producing medical revolution that promises a new age of health and disease management. From prevention of the oxidative reactions in foods, pharmaceuticals and cosmetics to the role of reactive oxygen species (ROS) in chronic degenerative diseases including cancer, autoimmune, inflammatory, cardiovascular and neurodegenerative (e.g. Alzheimer's disease, Parkinson's disease, multiple sclerosis, Downs syndrome) and aging challenges continue to emerge from difficulties associated with methods used in evaluating antioxidant actions in vivo. Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. The various antioxidants exert their effect by scavenging superoxide or by activating a battery of de-toxifying / defensive proteins. The prevention of oxidation is an essential process in all the aerobic organisms. The decreased antioxidant protection may lead to cytotoxicity, mutagenicity / carcinogenicity. Many activities of metal ions in biology have stimulated the development of metal-based therapeutics. Metal-based chemotherapeutic compounds have been investigated for potential medicinal applications, including superoxide dismutase mimics and metal-based NO donors/scavengers. These compounds have the potential to modulate the biological properties of superoxide anion and nitric oxide. Thus metal complexes become as potential therapeutics. Antioxidants are the stress reducing compounds. They help in preventing the health ailments like hypertension, aging etc. caused due to accretion of radical species in the body. The 1, 1-diphenyl -2-picryl-hydrazyl (DPPH) radical and Nitric oxide (NO) assays presents an easy and rapid way to evaluate the antioxidant potential.
A - 2 Review of Literature

It appears from existing literature that nitro aromatic and its related compounds have been extensively used as biologically active complexing agents. Substituted 2-Hydroxy-4-methoxyacetophenone was exhibited good antioxidant potential\(^99\). 2′, 4′-dihydroxy-5′-nitrochalcone and their derivatives were reported antioxidant potential\(^{100-102}\). A series of chalcones was synthesized by 2-hydroxyacetophenone and substituted aldehyde showed the most potent anti-oxidant activity\(^{103}\). Synthesised chalcones from the 2-hydroxy acetophenone and 5-nitro thiophene carboxyaldehyde were evaluated for their antioxidant potential\(^{104}\). 2′-hydroxy 4-methoxy acetophenone was screened by anti-oxidant potential\(^{105}\). However there is no information available about the antioxidant potential of para - Hydroxy - meta - nitro acetophenone against DPPH and NO assays.

A - 3 Present Work

Acetophenone derivatives have immense biotechnological applications\(^{106}\). Keeping this into mind antioxidant potential of various concentrations is determined using DPPH and Nitric Oxide assays for the first time. 1, 1-diphenyl -2-picryl-hydrayl (DPPH) radical scavenging activity of ethanol extract of para - Hydroxy - meta - nitro acetophenone was investigated spectrophotometrically. Ascorbic acid was used as standard.

A- 4 Results and Discussion

Freshly prepared extracts of the compound 1 were subjected to screening for their possible antioxidant potential. For this purpose, DPPH free radical scavenging activity and Nitric Oxide scavenging methods using UV- VIS spectrophotometer were employed. DPPH radical scavenging test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to form a stable diamagnetic molecule. DPPH radical is reduced to the corresponding hydrazine, a color change of the solution from violet to yellow is observed and that is monitored
spectrophotometrically. More reduction of DPPH radical is related to the high scavenging activity of the particular extract.

A significant decrease in the NO radical is due to the scavenging activity of the extracts. At the range of concentration under study, ascorbic acid exhibited 90.16% inhibition; Compound 1 exhibited higher radical scavenging activity than ascorbic acid by DPPH assay and by Nitric oxide method but it is higher than ascorbic acid. Results are reported (Table 7 & 8). The IC$_{50}$ values for each standard and extract is explained by graph (Fig 8 & 9).

![Fig 8 IC$_{50}$ value for DPPH assay](image)

![Fig 9 IC$_{50}$ value for NO assay](image)

**A- 5 Conclusion**

It will focus on a novel approach to design synthetic antioxidant metal-based compounds and to study their activities in the oxidation processes. This work underlines some important features for the research on biologically active ligand as antioxidants and supports future evaluation of some of these compounds as possible therapeutic agents.

**Table 7 DPPH radical scavenging activity**$_{107}$

<table>
<thead>
<tr>
<th>Extracts/ Standard</th>
<th>IC$_{50}$ (µg/ml)</th>
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</thead>
<tbody>
<tr>
<td>Compound 1</td>
<td>20.33</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1.43</td>
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</table>

**Table 8 Nitric Oxide scavenging activity**$_{108}$

<table>
<thead>
<tr>
<th>Extracts/ Standard</th>
<th>IC$_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 1</td>
<td>18.83</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>13.11</td>
</tr>
</tbody>
</table>
**A- 6 Experimental**

The ligand, para hydroxy *meta* nitro acetophenone, was purified for its antioxidant potential evaluation. Its purity was confirmed by TLC and its sharp melting nature. Various aliquots of it were prepared by weighing exactly amounts of it. The solutions were prepared by dissolving in known volume of ethanol to acquire 50-350 µg/ml. Each aliquot of the concentration was employed for the DPPH and Nitric Oxide assays.

**DPPH radical scavenging activity**

1, 1-diphenyl -2-picryl-hydrazyl (DPPH) is converted to 1, 1-diphenyl -2-picryl hydrazine when it reacts with antioxidants. A change in color from purple to yellow is observed. Aliquots of extract solutions were taken and a total volume of 3ml was made using methanol. 0.15ml of freshly prepared DPPH solution (98µg/ml) was added, stirred and left to stand at room temperature for 30 minutes in dark. The control contains only DPPH solution in methanol while methanol served as the blank. The reduction capability of DPPH radicals was determined by the decrease in its absorbance. Absorbance was noted at 517nm by using UV–VIS (Fig 8).

**Nitric Oxide scavenging activity**

In this spectrophotometric method the absorbance of chromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling with naphthyethylene diamine dihydrochloride was measured. Sodium nitroprusside in phosphate-buffer saline was mixed with an equivalent amount of methanol to get the control. Methanol served as blank. Methanol was added to test solutions at different concentrations to make up a volume of 3ml and incubated at room temperature for 90 minutes. This incubated solution (1.5 ml) was added to 1.5 ml of Greiss Reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyethylenediamine dihydrochloride). Absorbance at 546nm was noted using UV–VIS (Fig 8). In both methods the capacity of scavenging free radicals was calculated as:

\[
\text{Scavenging activity (\%)} = \frac{(\text{Control Abs.} - \text{Sample Abs.})}{\text{Control Abs.}} \times 100
\]

Ascorbic acid was used as the reference compound (positive control) with concentrations 20 to 500µg/ml for both the above spectroscopic methods of evaluating the radical scavenging activity.
References

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