CERIC(IV) AMMONIUM NITRATE CATALYSED HUNTZSCH SYNTHESIS,CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF 1,4-DIHYDROPYRIDINE

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ABSTRACT

1,4-Dihydropyridine (1,4-DHP) is among the most beneficial scaffolds that have revolutionized pharmaceutical research with unprecedented biological properties. 1,4-dihydropyridines are generally synthesized by Huntzsch reaction which involve the condensation of aldehyde, betaketoester, and ammonia or ammonium acetate. A number of improved methods have been reported in the literature for this condensation which involves the use of microwave, ionic liquids, TMSI etc. This work involves the synthesis of Diethyl4-(phenyl)'2,6-dimethyl-1,4-Dihydropyridine-3,5-dicarboxylate by the condensation of ethyl acetoacetate, benzaldehyde and ammonium acetate in presence of an effective catalyst Ceric(IV) Ammonium Nitrate in methanol, characterization of the sample by TLC, column chromatography and NMR. The work involve also the antibacterial activity of 1, 4-dihydropyridine. Huntzsch 1, 4-dihydropyridines and their derivatives have gained great importance in the field of medicinal chemistry since they display a fascinating array of pharmacological properties. 1, 4-dihydropyridines are well known as calcium channel blockers and important classes of drugs for the treatment of several cardiovascular.

Key Words: Aroyl Hydrazones IR,H^1 NMR, C^{13} NMR

INTRODUCTION

Ceric (IV) Ammonium Nitrate (CAN)

Cerium (IV) ammonium nitrate (CAN) is used as one electron oxidant and Lewis acid catalyst among the Lanthanide (IV) complexes in organic synthesis [1]. The reason for its general acceptance as a one-electron oxidant and Lewis acid is due to its large reduction potential valve of +1.61V*vs* NHE (Normal Hydrogen Electrode) and low affinity for oxygen as compared to other Lewis acids [2]. CAN have been found chemically superior in many aspects to the widely employed manganese triacetate for the generation of radicals [3]. Also, CAN offers several advantages in terms of low toxicity, inexpensive, reasonably soluble in many organic media, air stable, easily handled and allows a considerable degree of experimental simplicity. This is evident from the vast number of research papers and reviews that have been published for CAN mediate reactions.

Cerium belongs to the family of lanthanides, which constitute the so called rare earth elements. Cerium is most abundant of these elements and has been estimated to

constitute about 0.0046% of the Earth's crust by weight. In fact cerium is cannot be considered as rare at all, because its abundance is similar to or higher than that of better known elements such as copper, cobalt, zinc and tin.

Cerium has a property, unique among the lanthanides, which explain its ability to participate in one- electron transfer reactions that is its ability to exist in two stable adjacent oxidation states 3 and 4. Cerium in its ground state has an electronic configuration of [Xe] $4f^2$, $6s^2$, where Xe represents the xenon configuration. The electronic configuration of the Ce^{+3}ions is [Xe] $4f^1$, while that of Ce^{+4} ion is [Xe] $4f^0$. The enhanced stability of the vacant f shell in Ce^{+4} accounts for the ability of cerium to exist in the +4 oxidation state. The large reduction potential value of 1.61V (*vs*NHE) makes Ce(IV) every efficient oxidizing reagents compared to other metal ions. This salt consists of the anion $[Ce(NO_3)_6]^{2-}$ and a pair of NH^{4+} counter ions. The ammonium ions are not involved in the oxidizing reactions of this salt. In the anion each nitrato group is chelated to the cerium atom in a bidentate

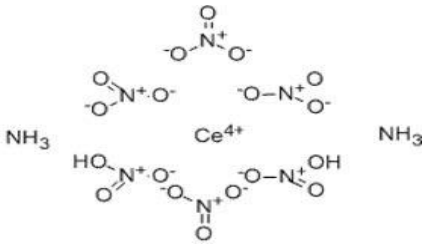

Fig.1.1 Ceric ammonium nitrate

Cerium (IV) ammonium nitrate is used as a catalyst for several type of transformation. They are classified into the following categories:

1. Reactions involving the formation of carbon-carbon bond.
2. Reactions involving the formation of carbon-heteroatom bond.
3. Miscellaneous transformations

Ceric Ammonium Nitrate Catalysed Multicomponent Reactions

Organic synthesis generally involves the use of various solvents, which may be toxic, hazardous, corrosive and inflammable. These solvents are highly harmful to the environment and human being. Nowadays it has become one of the major issues that have been brought forward at the global level. Furthermore, multistep reactions for the synthesis of various organic compounds also consumed various solvents, reagents and energy. According to the green chemistry principle[4], modern organic synthesis should be

carried out using processes that maximize the conversion of the reactant into products, generate low amount of waste or by products as far as possible, utilize minimum energy requirements, and reduce the use of toxic, hazardous, corrosive solvents and catalyst[5-7]. In view of the seriousness of chemical pollution, the use of a wide range of volatile, toxic, corrosive and inflammable chemicals are being re-examined, leading to a search for the design and development of environmentally benign multi component green organic transformations for synthesis of various chemical compounds.The length of a synthesis depends upon the average molecular complexity produced per operation, which depends in turn on the number of chemical bond being formed. Therefore, 7devising reactions that achieve multi bond formation in one operation is becoming one of the major challenges for step economic synthesis as well as green synthesis. Multi component reactions (MCR) s processes in which three or more reactant are combined in a single chemical step to produce product and incorporate substantial portions of all the components, naturally comply with many of the stringent requirements for ideal organic synthesis[8]. In fact, there have been various classification systems for MCRs.

In recent years, Ceric (IV)Ammonium Nitrate has been used for various organic transformations including Lewis acid catalyst. Owing to numerous advantages associated with this eco-friendly catalyst, CAN has been explored as a powerful catalyst for MCRs.

CAN has emerged as an important reagent for the construction of carbon-carbon bonds and carbon- heteroatom bonds [9-10]. In addition many advantages such as excellent solubility in water, cost- effectiveness, eco- friendly nature, easy handling, high reactivity, and easy work- up procedure make CAN a potent catalyst in organic synthesis. Besides, CAN is able to catalysed various organic transformations not only based on its electron transfer capacity, but also with its Lewis acidic property. In short the multi components reactions consist of two or more synthetic steps which are carried out without isolation of any intermediate thus reducing time, saving money, energy and raw materials. The development of MCRs in the presence of ceric ammonium nitrate is an efficient approach that in meets with the requirements of sustainable chemistry

Huntzsch Synthesis of 1,4- Dihydropyridines

1, 4-Dihydropyridine (DHP)[11]scaffold represents the heterocyclic unit of remarkable pharmacological efficiency. They are widely used clinically as calcium channel blockers for the treatment of cardiovascular diseases, such as, nifedipine and, nitrendipine are used for the treatment of hypertension and angina pectoris, nisoldipineis a potent vasodilator and nimodipine exhibits selectivity for cerebral vasculature [12]. A number of DHP derivatives are employed as potential drug candidates for the treatment

of congestive heart failure [13].Moreover DHPs also act as NADH mimics for the reduction of carbonyl compounds and their derivative[14]. In human body the main metabolic route of dihydropyridinedrugs involve their oxidation to pyridines catalysed by cytochrome-450 in liver [15]. Additionally, the synthesis of heteroatomatics by oxidative dehydrogenation is of fundamental importance in organic chemistry. These ubiquitous features always encourage synthetic chemist to explore improved protocols for the synthesis as well as the oxidation of 1, 4-DHPs.

1,4-Dihydropyridines are generally synthesized byHantzsch reaction which involvesthe condensation of aldehyde, beta-ketoester and ammonia or ammonium acetate. Arthur Huntzsch described preparation of 1, 4-Dihydropyridine more than a century ago [16-17]. Exploration of pyridine initially were quite slow, later it picked up very fast because of their structural resemblance to reduced nicotinamide adenine dinucleotide (NADH) which is an established hydrogen transforing agent in biological process[18]. Huntzsch pyridines are a subset of the co-enzymes. These pyridines are called Huntzsch pyridine (figure 1.2) and reaction as Huntzsch reaction.

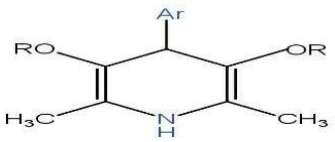

Fig.1.2 Huntzschpyridine

Original synthesis reported by Huntzsch is three components (acetoacetic ester, benzaldehyde and ammonia or ammonium salts) coupling reaction in refluxing ethanol.

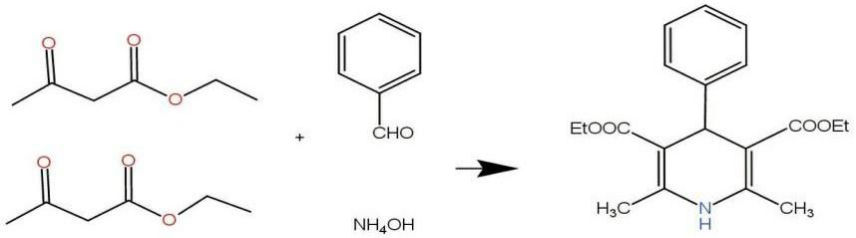

Fig.1.3 Huntzsch synthesis

A number of improved methods have been reported in the literature for this condensation which involve the use of microwave, ionic liquids, reflux at high temperature, TMSI , Yb(OTf)3, CAN [19], silica gel/NaHSO4 [20]and $Sc(OTf)^3$ [21]. On the other hand, a plethora of reagents have been employed for the oxidation of 1,4-DHPs [22-29]. In spite of potential utility of these reagents, most of the existing methods for the synthesis of 1,4-DHPs as well as their aromatization suffer from drawbacks such as low yields, long reaction times, occurrence of several side products, use of stoichiometric amount of reagents, use of strong oxidants, high temperature and the use of expensive and toxic transition metallic reagents. Therefore, exploring the new catalytic system

preferably in an environmentally benign method to overcome these drawbacks is a challenging task to the organic chemists.

Applications In Medicinal and Pharmacology

Dihydropyridine (DHP) is among the most beneficial scaffolds that have revolutionised pharmaceutical research with unprecedented biological properties. Over the years, metamorphosis of easily accessible1,2- and 1,4-dihydropyridine (1,4-DHP) intermediates by synthetic chemists has generated several drug molecules and natural products such as alkaloids.The1,4dihydropyridine (1,4-DHP) moiety itself is the main fulcrum of several approved drug The 1,4-DHP scaffold has served as a nucleus for several blockbuster drugs such as nifedipine and amlodipine.1Close resemblance to nicotinamine adenine dinucleotide(NADH) coenzyme, which has an important role in biological oxidation–reduction reactions, has made the 1,4-DHP core even more lucrative.

Despite the recent advances in medicine, antimicrobial chemotherapy still remains a problem in most developing and even developed countries. The narrow spectrum of activity of some antimicrobial drugs on the market and the many serious adverse effects reported explain the reasons for the failure of antimicrobial chemotherapy and why there is a search for more acceptable compounds. The inevitable emergence of resistant strains of pathological microorganisms and of the growing list of multi-drug resistant strains is another one of the very serious and major problem exist in the clinical industrte[30]. Among the many different chemical scaffolds screened,1,4 dihydropyridine compounds have attracted attention both as antimicrobial agents and MDR-reversing entities because of their ability to exert synergistic antibacterial effects in combination with known antibiotics[31-35]. It has been demonstrated that the type of C- 3, C-4 and C-5 substituent and the lipophilicity of the molecule have important effects on the antimicrobial activity of 1, 4-dihydropyridine compounds.

Hantzsch 1, 4-dihydropyridines (1,4-DHPs) and their derivatives have gained great importance in the field of medicinal chemistry since they display a fascinating array of pharmacological properties. 1,4-DHPs are well known as calcium channel blockers, and have emerged as one of the most important classes of drugs for the treatment of several cardiovascular diseases, including anginapectoris and hypertension. Interestingly, this heterocyclic system has been found to be the structural feature of several compounds belonging to different bio-active classes such as vasodilator, bronchodilator, analgesic, anti-inflammatory, antithrombotic, anticonvulsant, hepato-protective,radio

protective,antiatherosclerotic, and antidiabetic agents.

They were also reported to exhibit anticancer, antitubercular, antibacterial, and antiviral activities. 1,4-DHPs are also known to act as chemosensitizers in MDR tumour therapy. Aromatization of 1,4-DHP has also attracted considerable attention in recent years as Böcker has demonstrated that metabolism of those drugs involves a cytochrome P-450 catalysed oxidation in the liver. The so-obtained pyridines are devoid of the pharmacological activity of the parent heterocycles and are further transformed by additional chemical modifications. Due to the biological importance of the oxidation step of 1,4-DHP, that reaction has been the subject of a large number of studies and a plethora of reagents has been utilized to mimic the in vivo transformation. In that field, surprising results have been collected when the reactions are performed under microwave.

Dihydropyridines represent a group of small organic compounds based on pyridine core. Theoretically, five isomeric DHPs can exist, but actually, most of the DHPs have either the 1,2-dihydro or the 1,4-dihydro structure[36]. DHPs have a broad range of pharmacological actions as agents in vasodilation, bronchodilation, hepatoprotection, and geroprotection and as antiatherosclerosis, antidiabetics, antitumor, antimutagenic, antioxidant, anticonvolusant, and anti radical agents.

Although DHPs were primarily developed as cardiovascular agents, several have been used for other medical applications. For example, nimodipine is used as an anti-ischemic agent in the treatment of Alzheimer's disease and other dementias, migraine and post hemorrhagic vasospasm [37].Nifedipine is also used in the treatment of migraine, hypertrophic cardio myopathy and Raynauds phenomenon. It could also be used in the treatment of diabetic neuropathy. The platelet antiaggregatory DHP series, including trombodipine, have protective effects against Listeria monocytogenes. Dexniguldipine is a chemo sensitizer with low hypotensive properties. It is clear that the new generation of DHP derivative are a potential source of valuable drug candidates with remarkable potential and ongoing interest.

Since the introduction of DHPs into the clinical medicine, they have become almost indispensible in the treatment of cardiovascular disease. More than 30 years after the introduction of nifedipine, many DHP analogues has been synthesized and numerous second generation commercial products have appeared on the market with superior bioavailability and a slower onset and longer duration of action. For example, amlodipine is a DHP with slow absorption and prolonged effect. There is less reflecting tachycardia with amlodipine, possibly because the long half life reduces the plasma concentration peaks and troughs. Felodipine seems to have even greater vascular specificity than either nifedipine or amlodipine.

EXPERIMENTAL METHOD

The preparation of the sample and characterisation techniques is discussed in this chapter.

Characterization techniques

Some physical methods were used to understand the progress of the reaction and structure of the synthesized product and to confirm the expected properties. The methods used are TLC, column chromatography and spectral techniques such as NMR.

Thin Layer Chromatography

Thin layer chromatography (TLC) is a quick, sensitive, and inexpensive technique used to determine the number of components in a mixture, verify the identity and purity of a compound monitor the progress of a reaction, determine the solvent composition for preparative separations, and analyse the fractions obtained from column chromatography.

TLC is a method for analysing mixtures by separating the compounds in the mixture. TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. By observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction. TLC is a sensitive technique - microgram (0.000001 g) quantities can be analysed by TLC - and it takes little time for an analysis (about 5-10 minutes).TLC consists of three steps

- spotting, development, and visualization.

Principle of TLC

Thin layer chromatography uses a thin glass plate coated with either aluminium oxide or silica gel as the solid phase. The mobile phase is a solvent chosen according to the properties of the components in the mixture. The principle of TLC is the distribution of a compound between a solid fixed phase applied to a glass or plastic plate and a liquid mobile phase, which is moving over the solid phase. A small amount of a compound or mixture is applied to a starting point just above the bottom of TLC plate. The plate is then developed in the developing chamber that has a shallow pool of solvent just below the level at which the sample was applied. The solvent is drawn up through the particles on the plate through the capillary action, and as the solvent moves over the mixture each compound will either remain with the solid phase or dissolve in the solvent and move up the plate. Whether the compound moves up the plate or stays behind depend on the physical properties of that individual compound and thus depend on its molecular structure, especially functional groups. The solubility rule “Like Dissolves Like” is

followed. The more similar the physical properties of the compound to the mobile phase, the longer it will stay in the mobile phase. The mobile phase will carry the most soluble compounds the furthest up the TLCplate. The compounds that are less soluble in the mobile phase and have a higher affinity to the particles on the TLC plate will stay behind**.**

Procedure

- **Preparation of TLC plate**

6 g of silica gel, 1g of calcium sulphate and 10 ml of water were stirred well until fine slurry without clumps was obtained. The slurry was poured on the TLC plate and spread to form a uniform layer. Itwas then allowed to dry at room temperature for few minutes. Then the plate was dried in hot oven at 110^0c for 30 minutes.

The plate was then removed from the oven and kept at room temperature for 15 minutes.Once the temperature of the plate was reduced to room temperature, it was ready for spotting.

- **Spotting**

A minimum amount of the sample was taken in a capillary tube and dipped in 1ml of the solvent (ethyl acetate) in a test tube. Then the diluted sample was taken in another capillary tube and spotted on the TLC plate. In order to spot the sample the capillary end was touched to the plate with several repetitions. The spotting of the sample must be enough above such that the spotted sample should not get wet in the running solvent or mobile phase of solvent chamber. The sample was spotted above 1cm from baseline of the TLC plate.

- **Preparation of tank**

A mixture of ethyl acetate and hexane in the ratio 2.5:7.5 was used as solvent (mobile phase). The mixture was poured in the TLC tank in the depth of 1cm and closed it with a lid. Then it was allowed to stand for equilibrating the solvent. After the equilibrium is attained the lid was removed and the spotted TLC plate was placed vertically in the tank so that it stands in the solvent. The mobile phase was allowed to migrate across the spots towards the far end of the plate. Once the solvent front reaches the maximum the TLC plate was removed from the tank and the plates were dried.

- **Visualizing**

After plate was removed from the tank the spots can be immediately visualized by using spraying colouring agent $KMnO_4$.The compound was detected by spraying $KMnO_4$ on the plate, spots were visible as brown.

R_f value

The R_f value is defined as the ratio of the distance travelled by a particular

component to the distance travelled by the solvent front during the same time

i.e R_f = Distance travelled by the component from the origin line
Distance travelled by the solvent from the origin line at the same time The R_f value depends upon

- The solvent employed
- The quality of the medium used for the separation
- The temperature
- The nature of the substance

COLUMN CHROMATOGRAPHY Principle and theory

Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids when carrying out small-scale experiments. The separation can be liquid/solid (adsorption) or liquid/liquid (partition) in column chromatography. The stationary phase, a solid adsorbent, is usually placed in a vertical glass column and the mobile phase, is added from the top and let flow down through the column by either gravity or external pressure. Column chromatography is advantageous over most other chromatographic techniques because it can be used in both *analytical* and *preparative* applications. It can be used to determine the number of components of a mixture and as well as the separation and purification.

Column chromatography isolates desired compounds from a mixture in such a way that the mixture is applied from the top of the column. The columns are usually glass or plastic with sinter frits to hold the packing. The liquid solvent (eluent) is passed through the column by gravity or by the application of air pressure. The eluent, instead of rising by capillary action up a thin layer, flows down through the column filled with the adsorbent. Equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Stationary phases are almost always adsorbents. Adsorbent is a substance that causes passing molecules or ions to adhere to the surface of its particles. The mobile phase is a solvent that flows past the stationary phase, dissolving the molecules of the compounds to be separated some of the time.

Because the different components in the mixture have different interactions with

the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. The individual components, or elutants, are collected as solvent drips from the bottom of the column.

Packing of the column

- First the column was filled with a small amount of the solvent hexane.
- A ball of cotton was taken and inserted into thenarrowest part of the column using a long glass rod.
- The column was clamped securely and the tap was closed.
- 30g of silica gel was taken in a beaker and required amount of hexane was added to it, to make the slurry and mixedit thoroughly using a glass rod.
- The slurry was poured into the column, a little at a time.
- The column was gently tapped to encourage bubbles to rise and the silica to settle. This process is continuedtill all the silica gel was added.

- The solvent was drained to thelevel of the surface of the stationary phase.
- Small amount of sand was added on the top of the silica gel to prevent it from moving while adding the solvent.

Loading of the sample

- The sample was dissolved in the mixture of the solvents ethylacetate and hexane.
- The mixture was transferred into the column using a clean dropper.
- After the loading of the sample a small piece of cotton was again added to the top of the sand.
- Then the solvent was allowed to run on the top of the silica gel or stationary phase.
- First 200ml of 10% ethyl acetate was used to dilute the column and the fractions were collected in test tubes that were fitted in a rack.
- Next 200ml of 15% ethyl acetate was added and the fractions were collected. TLC was carried out using the fractions.
- Next 200ml of 20% ethyl acetate was added and the fractions are collected and TLC was carried out.
- The procedure was repeated until the compound separates out using solvent having different polarity and until the TLC shows no more products is coming from the column.
- The fractions in the test tubes were combined that shows the product spots.

Nuclear Magnetic Resonance spectroscopy (^{1H} NMR)

NMR is a study of transitions between the magnetically inducted spin states. It is concerned with the magnetic properties of atomic nuclei with an integral value I. This technique consists of exposing the protons in an organic molecule to a powerful field. The protons will process at different frequencies. Now, these processing protons are irradiating with steady changing frequencies and observe the frequencies at which absorptions occur. The signals obtained corresponding to the absorption is known as NMR Spectrum.

Studying a molecule by NMR spectroscopy enables us to record differences in the magnetic properties of various magnetic nuclei present and to deduce the positions of this nucleus within the molecule. One can deduce how many different kinds of environments there are in the molecule and also which atoms are present in neighbouring groups. Usually, the number of atoms present in each of these environments is measured. Therefore, the diagnostic features of the NMR Spectra are the number of signals, position of signals, splitting pattern of signals and area of signals.

synthesis of 1,4 dihydropyridine

chemicals required

Ethyl acetoacetate =

500 mg Benzaldehyde =

413.868 mg

Ammonium acetate = 300.612 mg

Ceric ammonium nitrate = 210.516 mg

Methanol = 7.7 ml

Procedure

- 500mg ethyl acetoacetate, 413mg benzaldehyde and 300mg ammonium acetate were taken in a RB flask.
- To this 3.84ml of methanol was added. The reaction mixture was stirred for a while. To the stirring mixture 210.516mg ceric ammonium nitrate dissolved in 3.84ml methanol was added drop wise.
- The reaction mixture was allowed to stir over night or until the reaction completed at room temperature (monitored by TLC).
- A small amount of water was added to the mixture followed by brine solution and the organic layer was extracted with ethyl acetate.
- The organic layer was mixed with anhydrous sodium sulfate and evaporated to dryness. The pure product can be separated using column chromatography and crystallization.

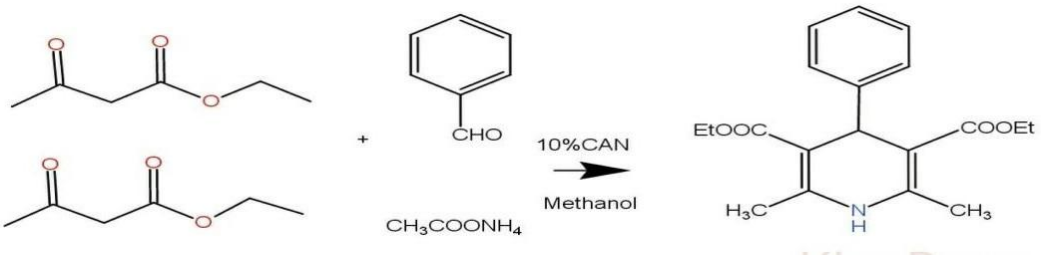

Figure 4.1: Preparation of Diethyl4-(phenyl)-2, 6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate

Antibacterial Activity

Disc diffusion method was used for testing antibacterial activity. Escherichia coli (E.coli) were used in this study. A stock solution of extract was prepared by dissolving 10mg/ML of Dimethyl Sulfoxide (DMSO) to produce a final concentration of 1mg/mL. The

stock solution was then loaded to sterile disc at desired concentration. Cephalosporin was used as positive control and DMSO served as negative control. The disc was allowed to air dry and place over the agar plate previously swabbed with test pathogen and incubated at 37^0C and zone of incubation was measured.

RESULT AND DISCUSSION

1,4-Dihydropyridines are among the most widely used drugs for the management of cardiovascular disease and have a broad range of other pharmacological activities and play a crucial role in CNS-targeted chemical delivery systems.Also, in spite of initial, largely unfounded, misgivings about their stability, 1, 4- dihydropyridines have also proven to be very important synthetic intermediates, finding application in the preparation of a large number of nitrogen alkaloids. The synthesis of 1, 4-dihydropyridines is often achieved by nucleophilic addition to pyridinium salts, available from the corresponding pyridine derivatives. The best known procedure for the preparation of 1,4-dihydropyridinesis the classical Hantzsch synthesis, a multicomponent condensation involving, in its original version, two molecules of a beta-ketoester, one molecule of an aldehyde, and one molecule of ammonia.

When the reaction was carried out using ethyl acetoacetate, benzaldehyde and ammonium acetate in the presence of catalyst ceric ammonium nitrate in methanol the best results was obtained. The catalyst have the advantages of eco- friendly, good to excellent yield, less time consuming required less amount, water solubility etc. The catalyst CAN attack the methylene group in the active methylene compound (ethyl acetoacetate) to form an enolate anion.

Thin Layer Chromatogaphy

Analytical Thin layer chromatography of reactants and product were performed on aluminium sheets of silica and visualized using KMnO4 as colouring agent and brown spots were obtained. Using the spots Rf value was calculated.

Rf value of

- Ethyl acetoacetate = 3.2/3.9
  = $0.82cm^{-1}$
- Benzaldehyde = 3.4/3.9
  = $0.87cm^{-1}$
- Sample = 1.8/3.9
  = $0.46\ cm^{-1}$

COLUMN CHROMATOGRAPHY

Column chromatography is the one of the efficient method for the purification of compounds**.** Column chromatography was done using silica gel as stationary phase and mixture of ethyl acetate and hexane as mobile phase. The sample was separated and purified by column chromatography. The sample fractions were collected in test tubes and the fractions containing single spots were concentrated together in a RB flask and fractions containing slight impurities and other components were concentrated in another RB flask. The concentrated fractions were allowed to stand overnight for crystallization (slow crystallization).

The crystals were collected and the left mother liquor was crystallized by again. In order to ensure the purity, the crystallized product was washed with hot hexane until the yellow color of the sample was disappeared and dried by applying vacuum at 35ºC. The purity of the crystal was again checked by TLC. A single spot on TLC silica gel glass plate with mixture of ethyl acetate and hexane as raising solvent confirmed the purity of the synthesized sample.

^{1}Hnmr spectra of Diethyl4-(phenyl)-2, 6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate is given below:

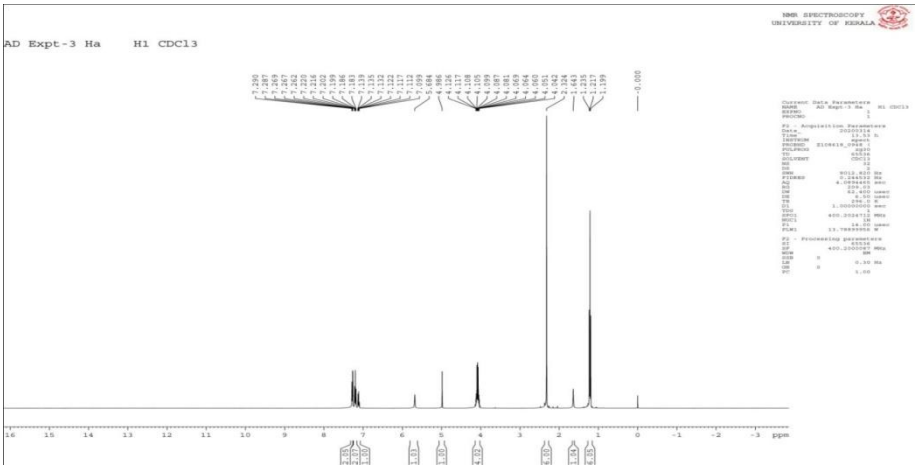

Fig.5.5[1]Hnmr spectra of Diethyl4-(phenyl)-2, 6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.

The [1]Hnmr spectra of the compound were recorded in CDCl3 (400 MHz). The figure 5.5 and 5.6 shows the proton nmr spectra of the compound. A singlet at 5.68 ppm shows the presence of N-H bond which conform the presence of pyridine. A complex multiplet between 7.09-7.29 ppm shows the presence of 5H of the Ar group. The tripletbetween 1.19-1.23 ppm indicates the H at the C-2 and C-6.

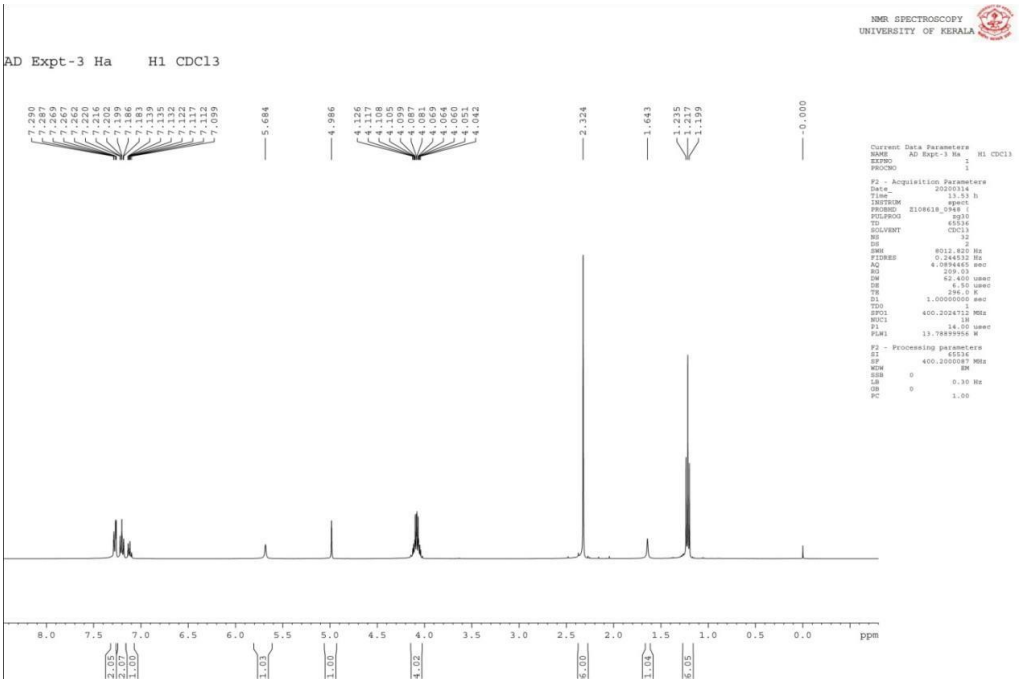

Fig.5.6 Expanded[1]Hnmr spectra of Diethyl4 4-(phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.

Antibacterial Activity

According to the result of disc diffusion assay this compound has active compounds that are effective for the prevention of infections caused by E.coli. The zone formed by the compound against E.coli was 18mm. The inhibition showed by positive control was 30mm diameters. It shows that 1, 4-dihydropyridine has antibacterial activity against gram negative bacteria like E.coli. Antibacterial activity of 1,4-dihydropridine depends upon the substituent present in the compound.

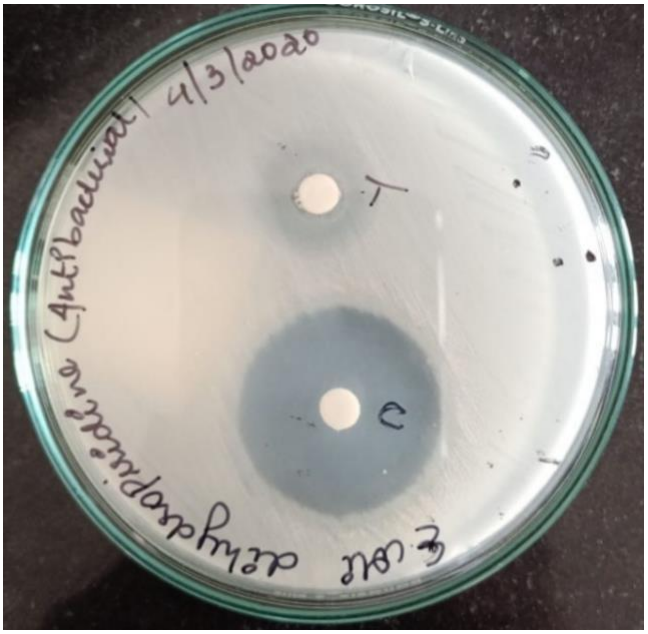

Fig.5.7 Antibacterial activity of synthesized compound against E.coli

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