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Synthesis, characterization and antimicrobial activity of di-nitro benzil by conventional and microwave irradiation methods

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ABSTRACT

Microwave induced organic reaction enhancement (MORE) is a simple, clean, fast, efficient & economical method for the synthesis of organic molecules and has emerged as a tool towards green chemistry. Dinitro Benzil was synthesized by both conventional and Microwave-assisted method. Comparative analysis of percentage yields and total reaction time for synthesized Dinitro Benzil by both conventional method and microwave-assisted method was carried out. It was found that there is improvement in percentage yield and also drastic reduction in total reaction time, by the microwave irradiation method.

Benzil is well known for their bacterial activity, so the anti-bacterial activity of synthesized Dinitro Benzil was studied by using disc diffusion method. The compound was screened for its antibacterial activity against gram positive bacteria such as *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* and *gram negative bacteria such as E. coli* and *Pseudomonas aeruginosa*. The Compound showed highest zone of inhibition against *Staphylococcus epidermitis* in case of gram positive bacterium and *E. coli* in case of gram negative bacterium compared to standard streptomycin. The activity of compound against various pathogens is mainly in a dose dependent manner that is by increasing the dose from 25, 50, 75 and 100 (µg) the activity also increases.

1. INTRODUCTION

Microwave induced organic reactions have emerged as a new 'Lead' in organic synthesis. The microwave enhanced chemical reactions are gaining importance due to the advantages and environmentally friendly processes they offer, as compared to conventional reaction[1,2]. Conventional methods of organic synthesis usually need longer heating time, elaborate and tedious procedures which result in higher cost of process and the excessive use of solvents, reagents leads to environmental pollution. Pharmaceutical chemistry laboratories use large quantities of toxic chemicals and solvents to perform reactions exposing laboratory persons including students and environment to related hazards. Review of literature states that in most cases, the cause for the observed rate increase is a purely thermal/kinetic effect. It is a result of the high reaction temperature that is quickly attained when irradiation of polar materials is done in a microwave field. The microwave energy reduces the heat-up and cool-down time for reactions. It uses 50% less power than electric furnaces of equivalent capacity.

Microwave-assisted synthesis is a branch of green chemistry. Microwave-assisted synthesis has gained much attention in recent years. Microwave irradiation-assisted chemical transformations are pollution free, eco-friendly and offer high yields together with simplicity in processing and handling[3-5].

Heating reactions with traditional equipment such as oil baths, sand baths and heating mantles, is not only slow but it creates a hot surface on the reaction vessel where products, substrates and reagents often decompose over time. Microwave energy, in contrast, is introduced into the chemical reactor remotely and passes through the walls of the reaction vessel, heating the reactants and solvents directly. Microwave dielectric heating drives chemical reactions by taking advantage of the ability of some liquids and solids to transform electromagnetic radiation into heat wherein chemical reactions are accelerated because of selective absorption of microwave energy by the polar molecules[6]. A properly designed vessel allows the

temperature increase to be uniform throughout the sample, leading to fewer by-products and/or product decomposition. The use of microwave energy instead of conventional heating often results in good yields in a short time as compared with reaction by classical synthetic methods[7-9].

Now a days, microwave-assisted organic synthesis is gaining widespread acceptance in drug discovery laboratories. The rapid acceptance of this technology parallels the rising cost of R&D and decrease in the number of Food and Drug Administration (FDA) approvals, which have led to what is termed as a productivity crisis. Reducing the cost of failure, either by failing candidates sooner or by improving the overall probability of success, is the most powerful solution to improving R&D productivity. Microwave technology, by accelerating chemical reactions from hours or days to minutes, provides quick results. From time to time, microwave heating enables chemistries that were not previously possible by classical methods, expanding the realm of structures accessible to the chemist[10].

Mechanism involved in microwave irradiate method: The probable mechanisms involved in microwave heating are as follows.

i. Dipolarization

ii. Ohmic heating

iii. Interfacial polarization

Dipolarization: In case of polar dielectric molecules such as water, methanol and ethanol, the molecules get aligned with the oscillating microwave field by the influence of microwave radiation. If this oscillating field is of high frequency, intermolecular interactions prevent any molecular movement before the field has reversed. In the same way, low frequency radiation causes uniform polarization and thus, there is no molecular motion. For intermediate frequencies, such as microwave frequencies, the applied field is of such character that polar molecules are not quite in position to keep itself up with the changing polarity of the same field. This results in random motion. Thus, heating through molecular collision takes place.

Ohmic heating: For materials to get conducted, the conductivity species, ions, electrons, etc., get movement through the material. This occurs under the influence of applied electric (microwave) field. This causes polarization. The induced currents cause heating through electric (ohmic) resistance.

Interfacial polarization: This type of polarization occurs in composites of insulating materials and conducting materials. An example of this kind is metallic particles dispersed in dielectric (insulating) silicone matrices. When microwave field is applied to the composite, the surfaces of the metallic particles become polarized. Since the surface of the metallic particle is in contact with the dielectric matrix, this results in reduction of the polarization of particles. Thus, polarization of the particles does not occur instantaneously (10- 18s in a 2.4 GHz field). It remains behind the polarity of the applied field. This procedure is seen for the dielectric heating regime. It causes dissipation of the applied field and results in heating[3,4].

Each time the products were isolated, the % yield and quality of the products was compared with the one obtained by conventional method. Each reaction was repeated for at least three times (different time intervals) and the products by studying their melting point and percentage yield the comparative results.

Benzil: Benzil (systematically known as 1,2-diphenylethane-1,2-dione) is the organic compound with the formula $(C_6H_5CO)_2$, generally abbreviated as $(PhCO)_2$. This yellow solid is one of the most common diketones. Its main use is as a photoinitiator in polymer chemistry[3]. The compound's most noteworthy structural feature is the long carbon-carbon bond of 1.54 Å, which indicates the absence of π-bonding between the two carbonyl centers. The PhCO centers are planar, but the pair of benzoyl groups are twisted with respect to the other with a dihedral angle of 117 degrees[4]. In less hindered analogues (glyoxal, biacetyl, oxalic acid derivatives), the $(RCO)_2$ group adopts a planar, anti-conformation.

Applications: Benzil is used in the free-radical curing of polymer networks. Ultraviolet radiation decomposes benzil, generating free-radical species within the material, promoting the formation of cross-links. Benzil is a potent inhibitor of human carboxylesterases, enzymes involved in the hydrolysis of carboxylesters and many clinically used drugs[5]. Benzil is a relatively poor photoinitiator, and is seldom used. It absorbs at 260 nm wavelength. It undergoes photo bleaching, which allows the curing light to reach deeper layers of the material on longer exposure[6].

2. MATERIALS AND METHODS

Para-nitro benzaldehyde, Thiamine hydrochloride, Ethanol, 10% sodium hydroxide, Con. Nitric acid, Activated Charcoal.

Chemicals: All reagents were of analytical-reagent grade.All chemicals used are of high purity. Para-nitro benzaldehyde was obtained from Fisher scientific, Mumbai. Thiamine hydrochloride was obtained from Hi media, Hyderabad. Ethanol, 10% sodium hydroxide, Con. Nitric acid, Activated Charcoal was obtained from National Scientific Product, Guntur. All chemicals were of laboratory grade.

Instruments: Purity of the compounds was checked by Thin layer Chromotography (TLC) using glass plates coated with Silica gel G and spots were detected by

iodine vapours and on Pre-coated silica gel aluminium plates procured from Merck, India. Melting points were detected in open capillaries on a tempo apparatus and are uncorrected. Synthesis was done with Reflux apparatus.^{1}H &^{13}C NMR were recorded on Bruker Avance 400 spectrophotometer operating at 400 MHZ and 100 MHZ respectively, $CDCl_3$ is used as a solvent. Tetramethylsilane (TMS) is used as internal standard. IR spectra were recorded on a Bruker optics (FT-IR) spectrophotometer using KBr-disk. Mass spectra were recorded on Shimadzu – GCMS 2010 spectrometer (equipped with a direct inlet probe) operating at 70ev.

Methods:

i. Conventional method

ii. Microwave assisted method

iii. Melting points were determined in open capillaries using melting point apparatus, expressed in °C and are uncorrected.

Step 1: Preparation of Dinitro benzoin by conventional method: A mixture of 0.35 gm of Thiamine hydrochloride, 3.5 ml of ethanol was taken in a beaker and cooled to $0^{O}C$. Then 1 ml of 10% sodium hydroxide was added. 2gm of para nitro benzaldehyde was taken in a 100 ml round bottomed flask and the above mixture was added to it. Reflux was carried out for 1.5 hours. Then, the mixture was cooled to room temperature and the solution was poured into 50 gms of crushed ice taken in a beaker. Mixed thoroughly until pale brown precipitate has been separated out. Then, the solution was filtered under suction and the precipitate was collected, dried, recrystallized with ethanol & carried out for Melting Point & Spectral studies.

Step 2: Synthesis of Dinitrobenzil from Dinitro benzoin: 1 gm of Dinitro Benzoin was taken in a 100ml RB flask. To this 2.5ml of concentrated nitric acid was added & dissolved. Reflux was carried out at 1.5 hrs until the fumes of nitrogen gas was ceased. Pour the reaction mixture into 50ml of ice cold water & stirred well until yellow colour oil globules were separated out. Then the solution was filtered under suction and the precipitate was collected, dried, recrystallized with ethanol & carried out for Melting Point & spectral studies.

Step 1: Preparation of dinitro benzoin by microwave assisted method: A mixture of 0.35gm of Thiamine hydrochloride, 3.5 ml of ethanol was taken in a beaker and cooled to $0^{O}C$. Then 1 ml of 10% sodium hydroxide was added. 2gm of para nitro benzaldehyde was taken in a 100 ml round bottomed flask & the above mixture was added to it. The reflux condensation was carried out at 210 watts for 4 minutes. Then, the mixture was cooled to room temperature and the solution was poured into 50 gms of crushed ice taken in a beaker.Mixed thoroughly until pale brown precipitate has been separated out. Then, the solution was filtered under suction and the precipitate was collected, dried, recrystallized with ethanol & carried out for melting point & spectral studies.

Step 2: Synthesis of Dinitrobenzil from Dinitro benzoin: 1 gm of Dinitro Benzoin was taken in a 50ml RB flask. To this 2.5ml of concentrated nitric acid was added & dissolved. The reflux condensation was carried out at 210 watts for 3 minutes.Pour the reaction mixture into 50ml of ice cold water & stirred well until yellow colour oil globules were separated out.Then, the solution was filtered under suction and the precipitate was collected, dried, recrystallized with ethanol & carried out for melting point & spectral studies.

Preparation of Antibacterial Solution: Dinitro Benzil was dissolved in dimethyl sulfoxide (DMSO). Proper drug controls were used. The zone of inhibition of the control and the Compound was taken at concentration of 25,50,75,100 µg/ml for testing antibacterial activity. The compound diffused into the medium produced a concentration gradient. After the incubation period, the zones of inhibition were measured in mm.

Antibacterial activity of the synthesized Dinitro Benzil compound was investigated by using disc diffusion method (Murray et al., 1995). Petri plates were prepared with 20 ml of sterile Nutrient Agar Medium (Hi-media, Mumbai). Four different concentrations of the compounds (25, 50,75 and 100 µg/disc) were loaded on a sterile disc and placed on the surface of the medium and left for 30 minutes at room temperature for compound diffusion. Streptomycin (10 µg/disc) was used as a positive control. These plates were incubated for 24 hrs at 37 °C. Zone of inhibition was recorded in millimetres (mm).

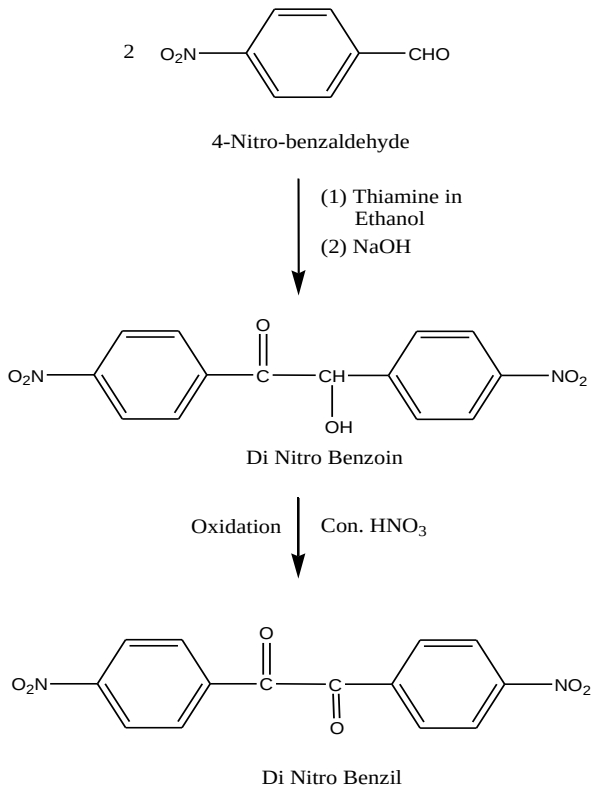
3. RESULTS AND DISCUSSION

In case of microwave-assisted synthesis gave better yields and was completed in much shorter period of time. Conventional synthesis of final Dinitro Benzil was completed within 3hrs. Microwave-assisted synthesis of Dinitro Benzil was completed within 3-4 mins. Comparative analysis of percentage yields and total reaction time for synthesized dinitro Benzil by both conventional method and microwave-assisted method was carried out to find out if microwave-assisted synthesis of Dinitro Benzil derivatives adds any advantage or not. It was found that there is improvement in percentage yield and also drastic reduction in total reaction time. By using microwave irradiation, reaction is possible within few minutes; and it also improves the yield. This would be highly advantageous for drug discovery laboratories where small amounts of different analogues have to be synthesized in shorter period of time. This is very useful for combinatorial synthesis of new libraries of compounds. Microwave-assisted

synthesis is quicker, high yielding, environment friendly and shows cleaner chemistry.

The results of the study suggested that microwave assisted synthesis led to higher yields within very short reaction times compared to the conventional methods.

Antimicrobial activity of Dinitro Benzil was tested against three gram-positive, two gram-negative bacteria. It was observed that the compound Dinitro benzil compound exhibits sufficient antimicrobial activity by showing maximum zone of inhibition (mm) at dose dependent manner. Compound Di-nitro Benzil showed highest zone of inhibition of 20.9 (mm) at 100 (µg), 4.6 (mm) at 25, 7 mm at 50 (µg) and 11.2 mm at 75µg against *staphylococcus epidermitis* compared to standard streptomycin. In the case of gram negative bacterium, *E. coli* compound showed highest zone of inhibition of 19.4 mm at 100 µg, 11.6 mm at 75 µg, 6.6 mm at 50µg compared to standard streptomycin. Dinitro Benzil showed zone of inhibition of 6 mm at 100 (µg) against *Bacillus subtilis.* In the case of gram negative bacterium *Pseudomanas aeruginosa* compound showed a zone of inhibition of 12.6 mm at 100 (µg). The pathogens *Staphylococcus epidermitis* and *E. coli* showed higher antimicrobial activity for the compound and the pathogen *Bacillus subtilis, Staphylococcus aureus* and *Pseudomanas aeruginosae* showed moderate activity for the compound Dinitro benzil. Thus the activity of compound against various pathogens is mainly in a dose dependent manner that is by increasing the dose from 25, 50, 75 and 100 (µg) the activity also increases.



Scheme: Synthesis of Dinitro Benzil from 4- Nitro benzaldehyde

Table.1. Physical data of comparative studies of conventional vs microwave method

Compound	Conventional method				Microwave method			
	M.P(^{0}C)	Yield	%Yield	Time	M.P(^{0}C)	Yield	%Yield	Time
Di nitro benzil	122	0.75g	75	1.5hr	120	0.87g	87	3 min
Di nitro benzoin	133	1.25g	62.5	1.5hr	131	1.45g	72.5	4 min

Interpretation of spectra:

IR data (cm^{-1}): The IR spectrum (KBr) showed absorption bands at 1696 cm^{-1} and 1603 cm^{-1} indicates the presence of C=O (stretching), 1535 cm^{-1} indicates the presence of aromatic NO_2 (stretching); 3072 and 3111 cm^{-1} shows that aromatic C-H stretch; 2851 and 2920 cm^{-1} indicates that aromatic methylene groups.

^{1}HNMR: ^{1}H NMR spectrum of the compound showed that the signals in the range of 7.26-8.41 ppm indicates the presence of aromatic protons of Di nitro Benzil.

^{13}C NMR: ^{13}C NMR spectrum of the compound showed that the aromatic carbon atoms appear in the range of 123 to 131 ppm. The signal at 190 ppm indicating the carbonyl carbon of aromatic 1,2-diketone.

MASS spectra: The molecular mass of the synthesized compound is identified by mass spectral analysis and it was found to be m/z value 300 (M^+). From the mass spectral data the existence of the compound could be confirmed. On careful comparison of physical and spectral data (NMR, Mass and IR) of synthesized compound was confirmed as Di Nitro Benzil.

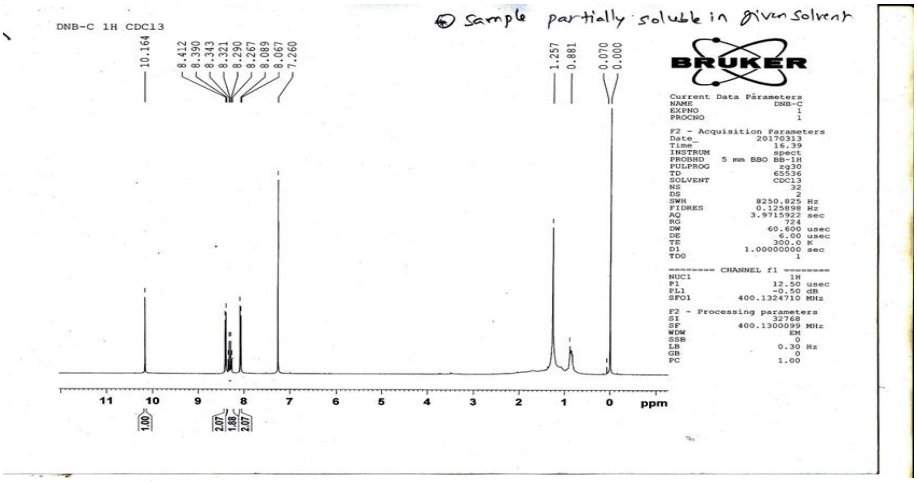


Figure.1.^{1}H NMR Spectra of DinitroBenzil by Conventional method

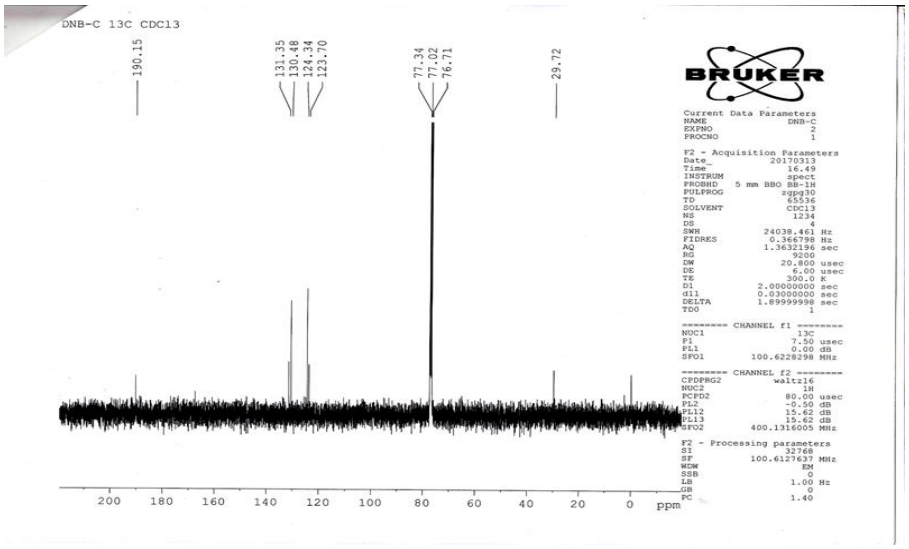


Figure.2. ^{13}C NMR Spectra of DinitroBenzil by Conventional method

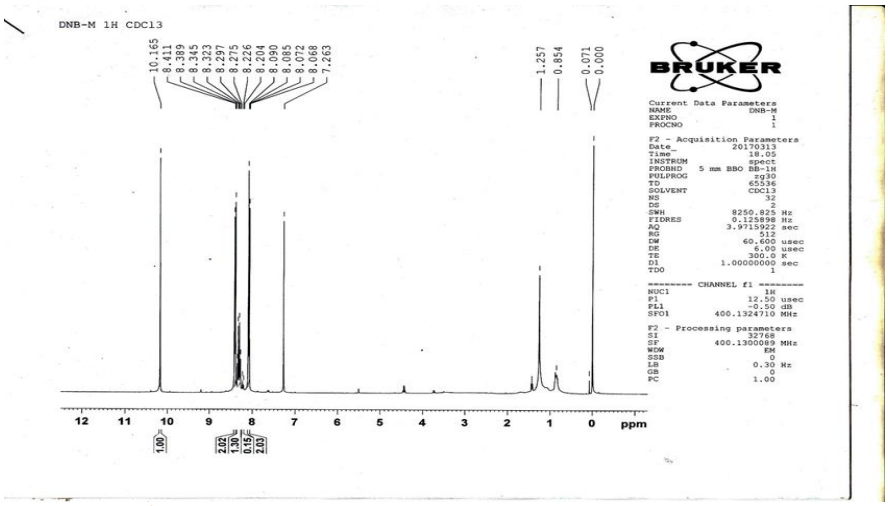


Figure.3.^{1}H NMR Spectra of DinitroBenzil by Microwave method

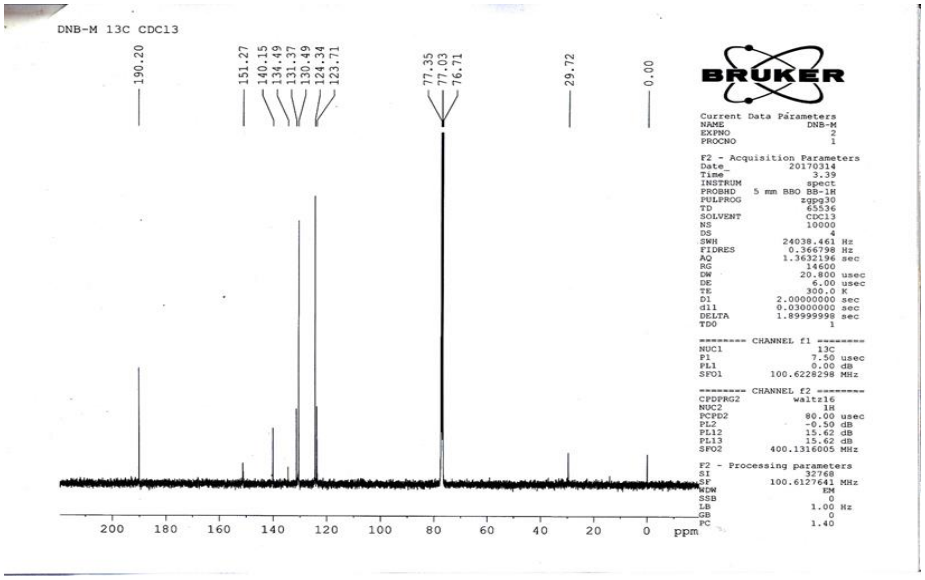


Figure.4. ^{13}C NMR Spectra of DinitroBenzil by Microwave method

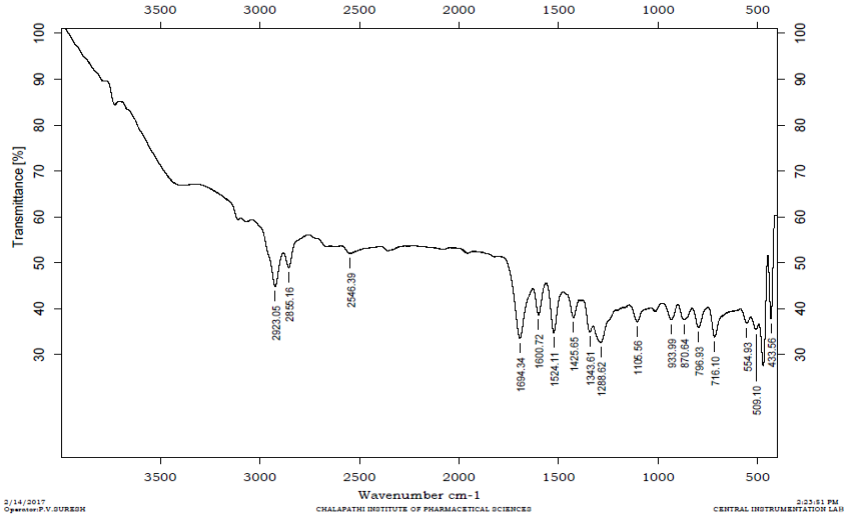


Figure.5.IR Spectra of DinitroBenzil (Conventional)

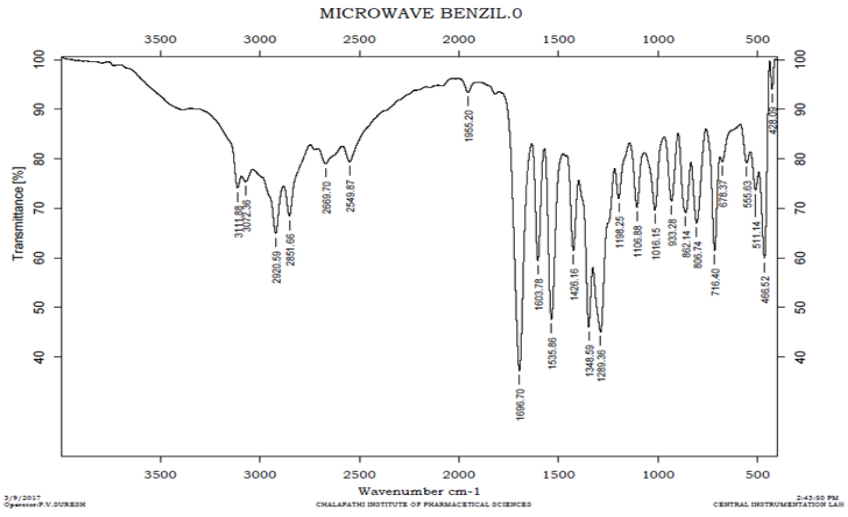


Figure.6.IR Spectra of DinitroBenzil (Microwave)

Table.2. Zone of inhibition of DinitroBenzil by disc diffusion method

Name of pathogen	Zone diameter (mm)				
	Concentration of dinitrobenzil(µg/ml)				Standard
	25	50	75	100	Streptomycin (10µg)/Disc
Bacillus subtilis	-	-	-	6	14
Staphylococcus aeureus	3	4.1	6.8	15.8	20
Staphylococcus epidermitis	4.6	7	11.2	20.9	20
E. Coli	-	6.6	11.6	19.4	19
Pseudomonas aeruginosa	-	-	8.2	12.6	20

4. CONCLUSION

From the above result, it would be concluded that the microwave assisted method is an efficient, fast, simple and environment friendly method for the synthesis of many organic synthetic molecules. In addition, the yield is also increased. Hence it is a viable and feasible method for performing the synthesis of drug, intermediates and chemicals.

Thus, it was also concluded that the compound Dinitro Benzil exhibits anti-bacterial activity for all pathogens *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, E. coli* and *Pseudomonas aeruginosa*. The activity of the compounds was found to be dose dependent *i.e*., 100 µg/mL showed greater inhibition. The susceptibility of the microbes to the compound was compared with standard antibiotic streptomycin. The thermal stability of the synthesized compounds is comparable to the standard. It can be concluded that this class of compounds certainly holds great promise towards good activity worth to be studied in medicinal chemistry. A further study to acquire more information concerning pharmacological activity is in progress.

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