ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

SCHOOL OF APPLIED NATURAL SCIENCE

APPLIED CHEMISTRY MSc PROGRAM (SYNTHESIS ORGANIC CHEMISTRY)

ZnO Nanoparticle Catalyzed Synthesis of Chalcone and Flavanone Derivatives and their Antibacterial Activity

A MASTER THESIS SUBMITTED TO THE OFFICE OF GRADUATE STUDIES OF ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY, SCHOLL OFAPPLIED NATURAL SCIENCE DEPARTMENT OF APPLIED CHEMISTRY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN APPLIED CHEMISTRY (SYNTHESIS ORGANIC CHEMISTRY)

By

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Co-Advisor: Bedasa Abdisa, PhD

June, 2018

DECLARATION

I, undersigned, declare that this thesis is my original work and has not been presented for a degree or diploma in any other University. All sources or materials used for this thesis have been duly acknowledged.

Dinka Mulugeta

Signature: _____

This thesis has been submitted for examination with my approval as University advisor and co-advisor.

Advisor: Milkyas Endale , PhD

Signature: _____

Co-advisor: Bedasa Abdisa, PhD

Signature: _____

APPROVAL SHEET

This is to certify that the thesis entitled "**ZnO Nanoparticle Catalyzed Synthesis of Chalcone and Flavanone Derivatives and their Antibacterial Activity"** submitted in partial fulfillment of the requirements for the degree of Master of Science in Applied Chemistry with specialization in Synthesis Organic Chemistry of School of Applied Natural Science MSc program of Department of Applied Chemistry, Adama Science and Technology University and is a record of original research carried out by **Dinka Mulugeta** (ID. No GSR/0178/09), under my supervision, and no part of the thesis has been submitted for any other degree or diploma in any other University.

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ACKNOWLEDGEMENT

Above all things I would like to thank almighty God, the most gracious and merciful who gave me the strength, health and patience with his handful blessing to complete this study. It is my pleasure to express my deepest sense of gratitude, sincere appreciation and profound regards to my advisor Dr. Milkyas Endale for his guidance and kindness, allowing me to think freely and thanks for your excellent communication, continuous technical, practical, moral support from the starting to the end of my work.

I wish to express my deepest appreciation to my Co-advisor Dr. Bedasa Abdisa for his continuous technical, practical and moral support from the starting to the end of my work. My sincere gratitude is given to Ethiopian Institute of Agricultural Research for financial support. My special thanks to my parents for their support, endless love and blessing that shape my life. I am also very much grateful to Legesse Shiferaw (for his support in providing some chemicals).Finally I would like to thank all who supported me by sharing ideas or offering material support which is an input for this work.

Abstract

Chalcones are a class of open chain flavonoids that are linked by a three carbon spacer between two aromatic rings. Chalcones and their heterocyclic analogues enjoy a range of biological activities such as antimicrobial, antioxidant, cytotoxic, anticancer, antiprotozoal, antihistaminic, antiulcer and anti-inflammatory activities. In this project, a series of chalcones derivatives and flavonoids were synthesized by green synthesis protocol using zinc oxide (ZnO) nanoparticles as catalyst. The structures were characterized by spectroscopic techniques (FT-IR spectra, ^{1}H, ^{13}C and DEPT-135 NMR spectra). The synthesized chalcones has been screened for their antibacterial activities against (*E coli S.aureus , P.aeroginosa* and *K. Pneumonia*) and the result revealed that **chalcones *42* and *43* are active on against** *S. aureus* (13 and 11mm zone of inhibition) whereas flavonones **44** and **45** showed promising activity **against** *E. coli* and *S. aureus*, respectively, with zone of inhibition of 10 mm for respective strains.

Keywords: Antibacterial activity, Chalcones, Flavonoids, Green synthesis, ZnO nanoparticles

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LIST OF ABBREVATIONS

$CDCl_3$	Deuterated Chloroform
DMSO	Dimethyl sulfoxide
IR	Infrared
KOH	Potassium Hydroxide
NMR	Nuclear Magnetic Resonance
TLC	Thin layer chromatography
NPS	Nano particles
s	Singlet
d	Doublet
dd	Double of doublet
t	Triplet
m	Multiplet
Hz	Hertz
nm	Nanometer
MHz	Megahertz
ZnO	Zinc Oxide

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1. INTRODUCTION

1.1. Background

Chalcones (1,3-diphenyl-2-propen-1-one) derivatives are open chain unsaturated carbonyl system in which two aromatic rings are joined by three carbons having α,β-unsaturated system [1] (Fig 1). Chalcones are called "anthochlor pigments", coined to identify a group of yellow pigments that turn red in the presence of alkali [2]. Chalcones are considered as the precursors of flavonoids and isoflavonoids [3] and are secondary metabolites of terrestrial plants that exhibit various biological activities [4] Chalcones are popular intermediates for synthesizing various heterocyclic compounds [5] like flavones, isoxazoles, pyrazoles and tetrahydro-2-chromens [6].

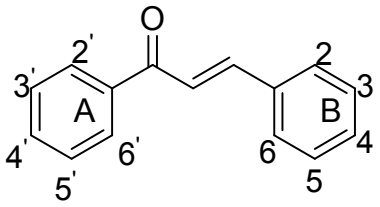
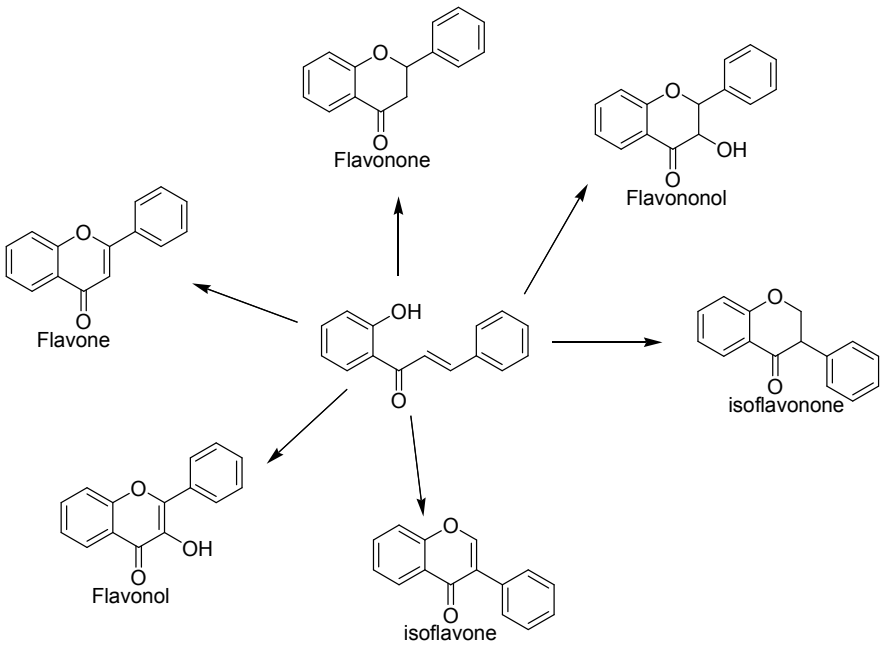


Figure 1. Molecular scaffold of chalcone.

Chalcones either natural or synthetic are known to exhibit various biological activities. Kostanecki [7] was the first to give the term chalcone and did pioneer work in the synthesis of naturally occurring compounds. The chalcones have been reported to possess various biological activities such as antimalarial [8], antibacterial [9], anti-cancer[10], antileishmanial [11], antifibrogenic [12] and anti-inflammatory[13]activities. Some chalcone derivatives show herbicidal activity [14] and substituted chalcones have exhibited fungi static and fungicidal activity.

Application of chalcones in the synthesis of many heterocycles [15] and as intermediate in the synthesis of many pharmaceuticals [16] has been well 2 ′ -hydroxychalcone is the main synthon in the synthesis of different flavonoids (Scheme 1). Having such a varied pharmacological activity and synthetic utility, chalcones have attracted chemists to develop a large number of synthetic methodologies for their synthesis around the world.



Scheme 1: Conversion 2′-hydroxychalcone to different flavonoids.

1.2. Statement of the problem

There were several basic catalysts that have been applied in the synthesis of chalcone, such as NaOH, KOH, $Ba(OH)_2$, $Ca(OH)_2$, LiOH, magnesium t-butoxide, potassium carbonate, alumina, MgO, calcinated hydrotalcites. Also this reaction is carried using acids like $AlCl_3$, dry HCl, $Zn(bpy)(OAC)_2$, $TiCl_4$, and $RuCl_3$. Apart from this method chalcones are synthesized from other methods like using $BF_3.OEt_2$, Suzuki coupling, Juliae Kocienski olefination. However these methods are suffer from drawbacks like expensive catalyst, drastic reaction condition, use of toxic and hazardous solvent, longer reaction time, low yield, not applicable to acid and base sensitive functional group, addition of reactant and catalyst in cooling condition, isolation and purification of product requires lot of solvent. Recently, bulk Zinc oxide has been employed as a heterogeneous catalyst for various organic transformations.

Nano ZnO as heterogeneous catalyst has received considerable attention because of its inexpensive, non-toxic catalyst and has environmental advantages i.e., minimum execution time, low corrosion, waste minimization, recycling of the catalyst, easy transport and disposal of the catalyst. Hence, it is among the suitable candidate to be used as support in chalcone synthesis in respect to its unique properties.

1.3. Significance of the study

The study was follow green synthesis protocols towards synthesis of bioactive chalcone derivatives. It was adopt inexpensive and easily available solid base catalyst, shorter reaction time, high yield compared to other reported methods, easy work up procedure, no side product,avoid and use of toxic regents. Thus, environmentally sound and ecofriendly method was developed by the project to synthesize related bioactive chalcone derivatives.

1.4. Objectives of the study

1.4.1. General objective

To synthesize chalcone and flavanone derivative compounds by Claisen-Schmidth reaction using ZnO nanoparticles as catalyst and evaluate their antibacterial activities

1.4.2. Specific objectives

- To synthesize chalcone derivatives of vanillin and acetophenone, *P*-hydroxyacetophenone, P-nitroacetophenone, *P*-phenylacetophenone and *O*-hydroxylacetophenone using ZnO nanoparticles as a catalyst.
- To synthesize flavanone derivatives from *O*-hydroxylchalcones and O-hydroxy *P*-nitro chalcones using ZnO nanoparticles as a catalyst.
- To characterize the synthesized chalcones and flavanone derivatives using FT-IR, ^{1}H,^{13}C and DEPT-135 NMR spectra.
- To screen the synthesized chalcones and flavanone derivatives for their antibacterial activities.

2. LITERATURE REVIEW

2.1. ZnO nanoparticles

The synthesis of nanoparticles with specific morphologies and properties is one of the most important aspects of nanoscience which studies materials whose size lies within the nanometer range (1nm - 100 nm) [17]. Applications of these nanostructures are seen in catalysis, sensors, and nano electronics.

Zinc oxide is a semiconductor with wide band gap (3.37), high excitation binding energy (60 meV) at room temperature [17,18] and has unique optical and as well as excellent thermal and chemical stability [19]. ZnO nanoparticles have gathered the increasing interest in the scientific and industrial community due to diverse application in solar energy conversion, sensors, catalysis, cosmetics, paints, fibers, drug-delivery and luminescence properties. Zinc oxide nanoparticles typically have neutral hydroxyl groups attached to their surface, which plays a key role in their surface charge behaviour [20]. Zinc oxide nanoparticles are used considerably for its catalytic, electrical, optoelectronic and photochemical properties [21,22]. ZnO nanostructures have a great advantage to apply to a catalytic reaction process due to their large surface area and high catalytic activity [23]. One-dimensional nanostructures exhibit interesting electronic and optical properties due to their low dimensionality leading to quantum confinement effects [24].

2.2. Chalcone

Chalcone (Fig 2) is a generic term given to compounds bearing the 1, 3-diphenyl-2-propen-1-one framework and belong to the flavonoid family [25-27]. Chemically they are open-chain flavonoids in which the two aromatic rings are joined by a three carbon α, β-unsaturated carbonyl system. Chalcones are abundantly present in nature starting from ferns to higher plants [28] and a number of them are polyhydroxylated in the aryl rings. In plants, chalcones are converted to the corresponding (2S)-flavanones in a stereospecific reaction catalyzed by the enzyme chalconeisomerase. This close structural and biogenetic relationship between chalcones and flavanones explains why they often co-occur as natural products.

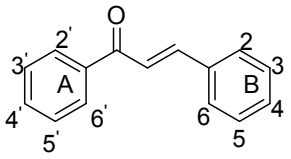
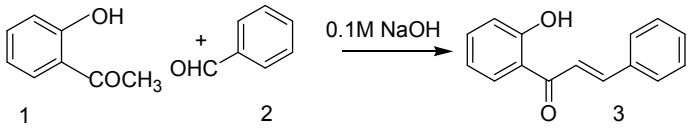


Figure 2**.** General structure of chalcone

All chalcones give pink coloration with concentrated sulphuric acid in Wilson's test and when a phenolic hydroxyl group is present, they give violet coloration with alcoholic ferric chloride solution [29]. Chalcones can be converted to their corresponding flavones up on heating with traces of iodine in dimethyl sulphoxide (DMSO) for two hours Chalcones can be converted into the corresponding flavonols by oxidation using hydrogen peroxide in methanolic sodium hydroxide solution and these flavonols showed a characteristic greenish yellow fluorescence in ethanolic solution as well as with concentrated sulphuric acid

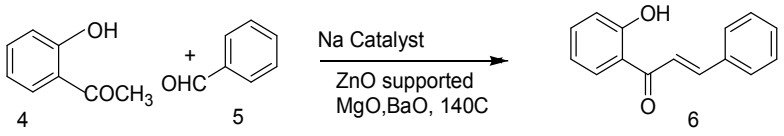
2.3. General methods of synthesis of chalcones

Chalcones can be obtained by acid or base catalyzed Aldol condensation of acetophenones with aromatic aldehydes [30-32]. 2'-hydroxyacetophenone (**1**) react with benzaldehyde (**2**) in the presence of 0.1M NaOH to give the chalcone (**3**) [33] (Scheme 2).



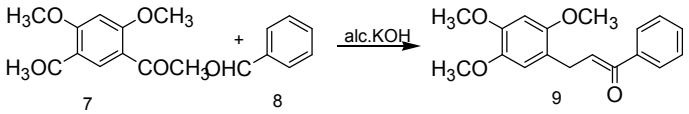
Scheme 2**:** Chalcone synthesized from 1.2'-hydroxyacetophenone react with benzaldehyde

Liquid phase Claisen–Schmidt condensation between 2'-hydroxyacetophenone (**4**) and benzaldehyde (**5**) was carried out over a zinc oxide supported metal oxide catalyst under solvent free conditions to form 2'-hydroxychalcone (**6**) [34] (Scheme 3).



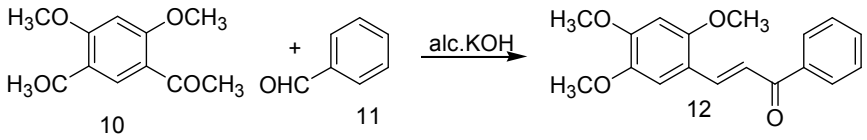
Scheme 3**:** Chalcone synthesized from 2'-hydroxyacetophenone and benzaldehyde

2',4',5'-trimethoxyacetophenone (**7**) when condensed with equimolar proportions of aromatic aldehydes (**8**) in the presence of 30 % alcoholic alkali at room temperature yield chalcones (**9**)[35](Scheme 4).



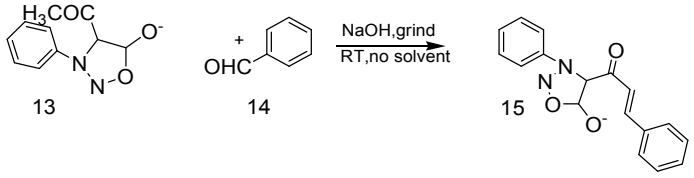
Scheme 4**:** Chalcone synthesized from 2'-hydroxyacetophenone and benzaldehyde

Claisen-Schmidt condensation between benzaldehyde (**11**) and acetophenone (**10**) by sonochemical and thermally activated reactions over zeolite as catalyst under solvent free conditions give chalcone (**12**) [36] (Scheme 5).



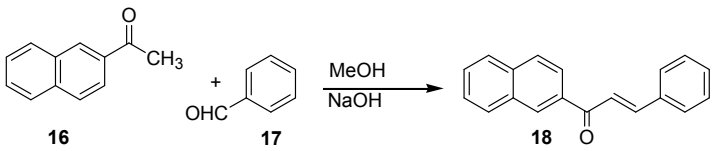
Scheme 5**:** Chalcone synthesized from benzaldehyde and acetophenone by sonochemical and thermally activated reactions

4-acetyl-3-aryl-syndones (**13**) when subjected to grinding with various arylaldehydes (**14**) in the presence of a base catalyst under solvent free conditions yield syndonechalcones (**15**) [37] (Scheme 6).



Scheme 6**:** Chalcone synthesized from 4-acetyl-3-aryl-syndones and arylaldehydes

Condensation of 2-naphthylmethyl ketones (**16**) with substituted arylaldehydes (**17**) in the presence of NaOH under methanol as solvent gave the corresponding chalcones (**18**) [38] (Scheme **7**).



16 17 18

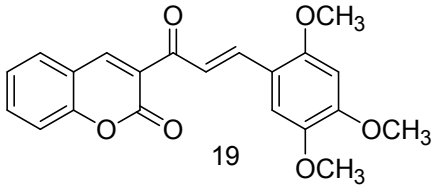
Scheme 7**:** Chalocones synthesized from 2-naphthylmethyl ketones with substituted arylaldehydes

2.3. Therapeutic potential of chalcones

Chalcone is a unique template that is associated with several biological activities and is well known intermediates for synthesizing various heterocyclic compounds [39]. The introduction of a halogen into the benzenoid part of these α, β- unsaturated ketones enhances their biological activity [40]. The compounds with chalcone as backbone have been reported to possess varied biological and pharmacological activities [41], including antimicrobiaanti-inflammatoryanalgesic, cytotoxic, antitumor, antimalarial, antitubercular, antiviral, anti-HIV, antiulcerativeantileishmanial, antioxidant, antiprotozoal, antihistaminic, antifedent, immunomodulatory, anticonvulsant, antihyperglycemic, antihyperlipidemic and antiplatelet activitiesThus chalcones continue to attract considerable scientific attention because of their association with a variety of biological activities.

2.3.1 Antimicrobial activity

The antimicrobial activity of chalcones is being increasingly documented. Many research groups either isolated or synthesized chalcones that possess antimicrobial activity. Prasad *et al.*[42] synthesized 3-[1-oxo-3-(2,4,5-trimethoxyphenyl)-2-propenyl]-2*H*-1-benzopyran-2-ones (**19**) that showed significant antimicrobial activity against *B.subtilis, B.pumilis* and *E.coli* when tested at a concentration of 1000 µg/mL.



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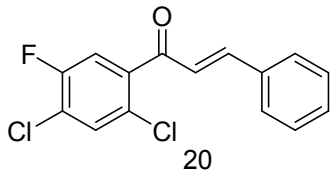
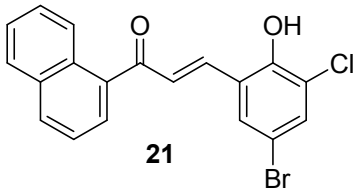
Karthikeyan *et al.*[43] synthesized 3-aryl-1-(2,4-dichloro-5- fluorophenyl)-2-propen-1-ones (**20**) showing antimicrobial activity,



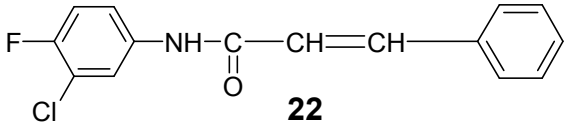
20

Prasad *et al*.[44] synthesized a chalcone (**21**) having a naphthalene moiety on one side and an aryl moiety having substituents on the other side, which showed significant antifungal activity against *A.niger* and *R.oryzae*.



21

Rao *et al*.[45] synthesized chalcones having chlorine and fluorine substitution (**22**), which showed antimicrobial activity.



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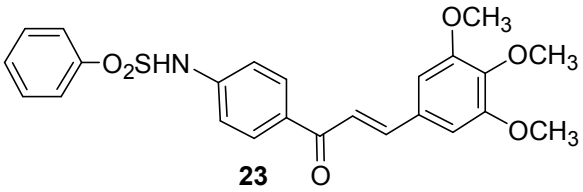
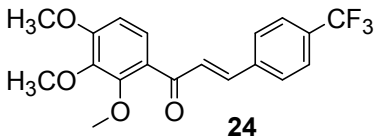
2.3.2 Antimalarial activity

Dominguez *et al.* [46] synthesized chalcone (**23**) with sulfonamide moiety possessing antimalarial activity.



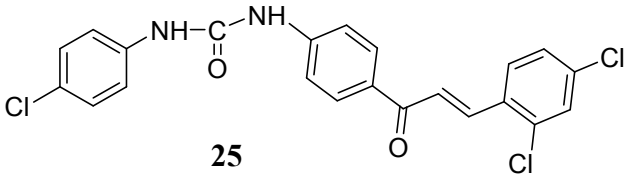
23

Liu *et al.* [47] synthesized a methoxychalcone (**24**) which showed significant antimalarial activity.



24

Dominguez *et al.*[48] synthesized phenylurenyl chalcones (**25**) that exhibited antimalarial activity.



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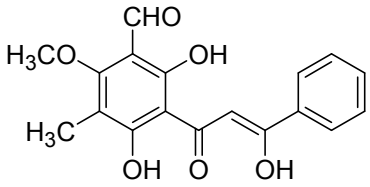
2.3.3 Anti-HIV activity

Wu *et al.*[49] isolated a chalcone (**26**) that exhibited the anti-HIV activity with a good therapeutic index.



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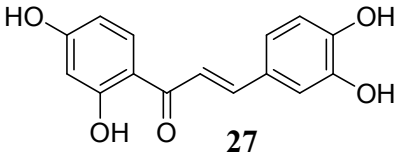
Xiu *et al.*[50] isolated butein (**27**) possessing anti-HIV activity.



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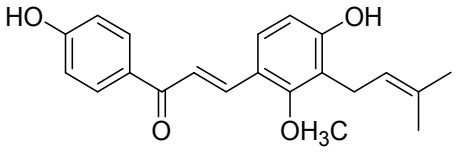
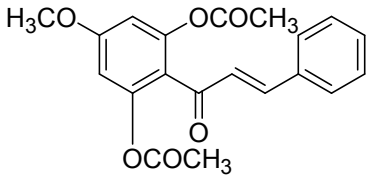
2.3.4 Antileishmanial activity

Nielsen *et al.*[51] synthesized licochalcone C (**28**) which showed antileishmanial activity.



28

Hermoso *et al.*[52] synthesized a dihydrochalcone (**29**) having antileishmanial activity.



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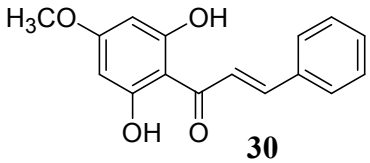
Santos *et al.*[53] synthesized 2', 6'-dihydroxy-4'-methoxychalcone (**30**) that showed significant antileishmanial activity.



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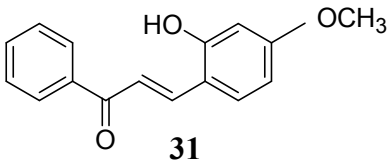
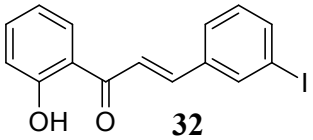
2.3.5 Antitubercular activity

Kumar *et al.*[54] synthesized a chalcone (**31**) that exhibited antitubercular activity.



31

Lin *et al.*[55] synthesized a 2'-hydroxychalcone (**32**) with iodine substitution, exhibiting antitubercular activity.



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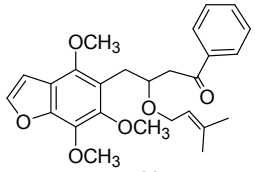
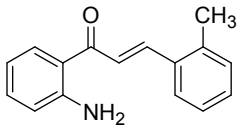
2.3.6 Antitumor activity

Francesco *et al.*[56] synthesized furanochalcones (**33**) possessing anticancer activity.



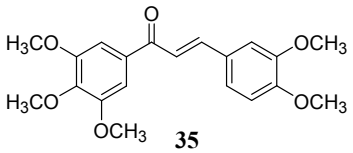
33

Yi *et al.*[57] synthesized substituted-2'-aminochalcones (**34**) which showed antitumor activity.



34

Lawrence *et al.*[58] synthesized a methoxylated chalcone (**35**) possessing good cytotoxic activity.



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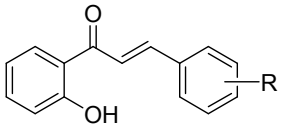
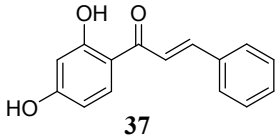
2.3.7 Antioxidant Activity

Kostova *et al.*[59] synthesized 2'-hydroxychalcones (**36**) which showed antioxidant activity.



36

Re *et al.*[60] synthesized 2',4'-dihydoxychalcone (**37**) which showed antioxidant activity.



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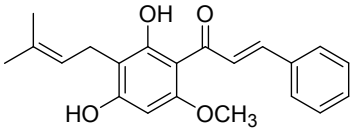
Miranda *et al.*[61] synthesized a prenylated chalcone (**38**) exhibiting antioxidant activity.



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3. MATERIALS AND METHODS

3.1 Reagent and Chemicals used

Distilled water, *n*-hexane, ethyl acetate ,zinc acetate dihydrated, Potassium hydroxide , ammonia, methanol, ethanol, silica gel and vanillin, acetophenone, *P*-hydroxyacetophenone, *P*-nitroacetophenone, *P*-phenylacetophenone *O*-hydroxylacetophenone and p-Nitrobenzaldehyde.

3.2 Apparatus and Instruments

Reactions were monitored by TLC using silica gel-G (Merck grade) as the adsorbent and the solvent systems are indicated at appropriate places. Silica gel (100-200 mesh, Merck grade) has been used for column chromatography. The column was subjected to gradient elution using n-hexane, mixtures of hexane and ethyl acetate (5%, 10%, 15 %, 25%, 50% and 75% hexane in ethyl acetate). Fractions each of 100 ml were collected. The separation of the compounds was checked on TLC under UV lamp. All the melting points were determined in open capillaries, using digital melting point apparatus, expressed in ^{o}C.Infrared spectra (FT-IR) were recorded on Perkin Elmer – 337 Infrared Spectrophotometer. Samples were screened in Potassium bromide (KBr) pellets and the values are expresses in cm^{-1}. The ^{1}H and ^{13}C NMR spectra of the compounds were recorded on Bruker avance 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm. The nanostructure of ZnO nanoparticle was studied at room temperature by using X-ray diffraction pattern.(Appendix 8)

3.3. Experimental Procedures

3.3.1. Preparation of Zno nanoparticles

ZnO nanoparticles were prepared according to a literature method developed by Pacholski et al. [62] Zinc acetate dihydrated [$Zn(CH_3COO)_2$].$2H_2O$, 2.4g) and 126 mL of water was added into a round bottom flask. The solution was heated to 60°C with magnetic stirring. The stock solution of potassium hydroxide (KOH) was prepared by dissolving 1.2g of KOH in to 70mL of double distilled water. The stock solution was dropped into the flask within 10-15min. At a constant temperature of 60°C for 2hr. The solution was condensed to about 10-15mL and reheated while stirring for another 5 hr before stopping the heating. The upper fraction of the solution was removed after 30min and water (50mL) was added to the solution and stirred for 5 min. The

upper fraction of the solution was discarded again after 30min and dried under vacuum to obtain ZnO nanoparticle.

3.3.2. General procedure for preparation of Chalcone using ZnO NPs as catalyst

A mixture of equimolar quantities of vanillin and acetophenone derivative (substituted) were mixed with ZnO (0.1mmol.) $SnC1_2.H_2O$ (10 %) and 5mL H_2O and heated at 100°C for 2hr. The progress of the reaction was monitored via TLC using *n*-hexane/ethyl acetate as eluent in (7:3) combinations. The reaction mass was cooled to 15-20°C and stirred with ethanol (50mL) for 30min and centrifuged for 10 min at 5000rpm. The supernatant was collected and concentrated under reduced pressure. The obtained product was checked by TLC and purified using silica gel column chromatography (230-400 mesh, Merck), with increasing gradient of ethyl acetate in *n*-hexane as the mobile phase. Finally the product yield was recorded [63].

3.3.3. General procedure for preparation of Flavonones using ZnO NPs as catalyst

Chalcone [2'-hydroxychalcone (**44**)] and Chalcone [(2'-hydroxy-4-nitrochalcone (**45**)] were mixed with ZnO (0.1 mmol.) $SnC1_2.H_2O$ (10 %) and 5 mL H_2O and heated at 100°C for 2 hr. The progress of the reactions were monitored via TLC using *n*-hexane/ethyl acetate eluent in (7:3) combinations. The reaction mass was cooled to 15-20°C and stirred with ethanol (50 mL) for 30min and centrifuged for 10 min at 5000 rpm. The supernatant was collected and concentrated under reduced pressure. The obtained product was checked by TLC and purified by silica gel column chromatography (230-400 mesh, Merck), with increasing gradient of ethyl acetate in *n*-hexane as the mobile phase. Finally the product yield was recorded.

3.3.4. Procedure for preparation of Flavonone via conventional methods

20% of aqueous KOH (2 mmol) was added to a solution of Chalcone [2'-hydroxy-4-nitrochalcone (**45**)] **(**4 mmol)) in C_2H_5OH (25 mL). The reaction mixture was stirred at room temperature for 8 hr**.** The progresses of the reactions were monitored via TLC using *n*-hexane/ethyl acetate eluent in (7:3) combinations. The reaction mass was then poured into ice water and neutralized with aqueous 10% HCl solution. Finally the product yield was recorded.

3.3.5. Spectroscopic Techniques

Characterizations of the synthesized compounds were governed by spectroscopic techniques through the overdue conditions. NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C at room temperature using DMSO-d_6. A region from 0 to 12 ppm for ^{1}H and 0 to 205 ppm for ^{13}C was employed for scanning. Signals were referred to an internal standard tetra methyl silane (TMS). Chemical shifts are reported in δ units and coupling constants (*J*) in Hz. Multiplicities of ^{1}H NMR signals are indicated as *s* (singlet), *d* (doublet), *dd* (doublet of doublets), *t* (triplet), and *m* (multiplate). IR spectra were recorded between 400-4000 cm^{-1} in KBr pellets.

3.3.6. Determination of Antibacterial Activity

Antibacterial activities of test samples were evaluated using disc diffusion method against the test strains .The plates were allowed to solidify for 5min and 0.1% inoculums suspension of tested organisms were swabbed uniformly and the inoculums were allowed to dry for 5min. 20µL of synthesized compound (**39** to **45**) were loaded on 6 mm sterile individual paper discs (HiMedia) and thoroughly dried in air draft to remove traces of the solvent. Negative control was prepared using respective solvent which is DMSO. Ceftraxone was used as reference standard for comparing the results. Nutrient broth was used for the preparation of inoculum of the bacteria and nutrient agar was used for the screening method. The test organisms were sub cultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with the respective bacterial strain.

After incubation at 37ºC ±1ºC for 24 hours, they were stored in a refrigerator. The nutrient agar medium 101 was sterilized by autoclaving at 121ºC (15 lb/sq.inch) for 15 min. The petriplates, tubes and flasks plugged with cotton were sterilized in hot-air oven at 160ºC, for an hour. Into each sterilized petriplate (20cm diameter), was poured about 125mL of molten nutrient agar medium which was already inoculated with the respective strain of bacteria (5 ml of inoculum to 250 mL of nutrient agar medium) aseptically. The plates were left at room temperature aseptically to allow the solidification. After solidification, the cups of each of 6 mm diameter were made by scooping out medium with a sterilized cork borer from a petridish and labeled accordingly. Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5mL Analar

grade) to give a concentration of 1000μg/mL. Ceftraxone solution was also prepared to give a concentration of 1000 μg/ml in sterilized distilled water. The pH of all the test solutions and control was maintained in between 2 to 3 by using conc.HCl. All the compounds were tested at dose levels of 50 μg (0.05mL) and 100μg (0.1 ml) and DMSO used as a control. The solutions of each test compound, control and reference standard (0.05 ml and 0.1 ml) were added separately in the cups and the plates were kept undisturbed for at least 2 hours in a refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37±1 0C for 24 hr. After incubation, the diameter of zone of inhibition was measured. All the experiments were carried out in triplicate.[64].

4. RESULTS AND DISCUSSION

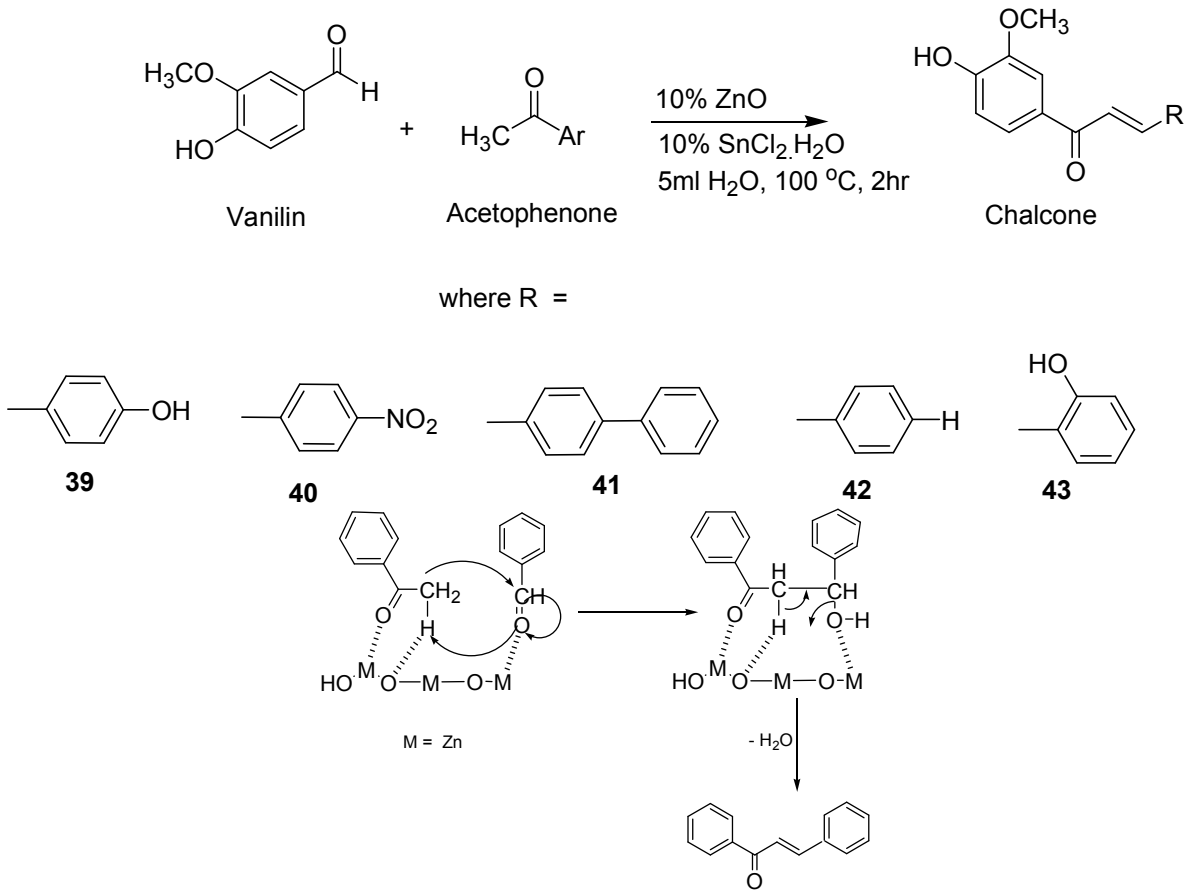
4.1. Synthesis of Chalcones and Flavanones

4.1.1 Synthesis of Chalcone (39) 3-(4-Hydroxy-3-methoxy-phenyl)-1-(4-hydroxy-phenyl)-propenone

A mixture 4'-hydroxyacetophenone (0.013145 mol, 1.7897 g) and vanillin (0.013145 mol, 2.001g) was stirred with ZnO (0.1 mmol.) NPs , $SnC1_2.H_2O$ (10 %) and 5 mL H_2O and heated with stirred at 100°C for 2 hr. The reaction mass was cooled to (15-20°C) and stirred with ethanol (50 mL) for 30 min and centrifuged for 10 min at 5000 rpm. The supernatant was collected and concentrated under reduced pressure. The mixture was purified by column chromatography on silica gel (100-200 mesh, Merck), with a mixture of ethyl acetate and hexane as the mobile phase.By adopting the above synthetic procedure, compounds **39 to 45** were also synthesized.

The list of chalcones synthesized include 3-(4-Hydroxy-3-methoxy-phenyl)-1-(4-hydroxy-phenyl)-propenone(**39**), 3-(4-Hydroxy-3-methoxy-phenyl)-1-(4-nitro-phenyl)-propenone(**40**), 1-Biphenyl-4-yl-3-(4-hydroxy-3-methoxy-phenyl)-propenone(**41**),3-(4-Hydroxy-3-methoxy-phenyl)-1-phenyl-propenone(**42**),3-(4-Hydroxy-3-methoxy-phenyl)-1-(2-hydroxy-phenyl)-propenone (**43**).

The overall reaction involving the formation of chalcones and the proposed mechanism is indicated below (schemes 8) .

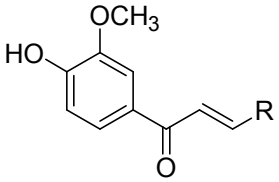


Scheme 8. Mechanism of formation of chalcones by Claisen-Schmidt condensation [67]

4.1.2 Synthesis of Favonones 2-(4-Hydroxy-3-methoxy-phenyl)-chroman-4-one (44)

Chalcone (2'-hydroxychalcone 3.0011g) was mixed with ZnO (0.1 mmol) $SnC1_2.H_2O$ (10 %) and 5 mL H_2O and heated at 100°C for 2 hr. The progresses of the reactions were monitored via TLC using *n*-hexane/ethyl acetate eluent in (7:3) combinations. The reaction mass was cooled to (15-20°C) and stirred with ethanol (50 mL) for 30 min and centrifuged for 10 min at 5000 rpm. The supernatant was collected and concentrated under reduced pressure. The obtained product was checked by TLC and purified by silica gel column chromatography (230-400 mesh, Merck), with increasing gradient of ethyl acetate in *n*-hexane as the mobile phase. Finally the product yield was recorded. By adopting the above synthetic procedure, compounds 2-(4-Nitro-phenyl)-chroman-4-one was also synthesized.

Table 1. Physical characterization data of chalcones



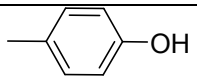
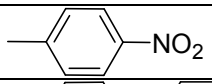
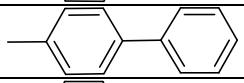
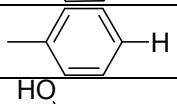
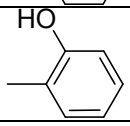
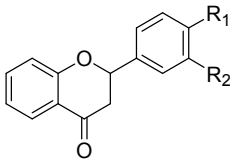
Compound	R	Molecular Formula	Color	Melting point In ^{0}C	Yield %
39		$C_{16}H_{16}O_3$	Solid Pale yellow	216 - 218	80.5
40		$C_{16}H_{13}NO_5$	Solid Yellow	78 - 81	88.9
41		$C_{22}H_{18}O_3$	Solid Yellow	80 -83	85.3
42		$C_{16}H_{15}O_3$	Solid Yellow	86 - 87	80.5
43		$C_{16}H_{16}O_3$	Solid Yellow	79 -82	85.4

Table 2. Physical characterization data of Flavonoes



Compound	R_1 and R_2	Molecular formula	Color	Melting point In ^{0}C	Yield %
44	R_1 = OH , R_2 = OCH_3	$C_{16}H_{14}O_4$	Soild yellow	89 - 90	89.8
45	R_1= NO_2 , R_2 = H	$C_{15}H_{11}NO_4$	Solid yellow	87-90	85.5

Table 3: Method comparison of % yield of the Conventionally and ZnO NPS for synthesis of 2-(4-Nitro-phenyl)-chroman-4-one (**45**)

Method	Time taken for reaction	yield %	Temperature
Conventional	8 hrs	55.7	100 ^{0}C
ZnO NPS	2 hrs	89.6	100 ^{0}C

Yield was estimated on the basis of initial weight of the raw materials taken and the final weight of the Chalcone and Flavonones obtained after the complete drying. It was observed that % yield of the reaction in case of ZnO NPS synthesized Flavonone (89.6%) was higher as compared to conventionally synthesized Flavonone (55.7 %) (Table 3)

Furthermore effect of $SnCl_2,H_2O$ and ZnO NPS variation on the % yield of synthesized Flavonone and Reaction time was also evident.

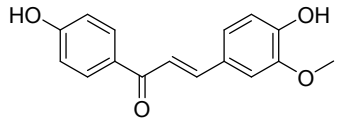
4.1.3 Characterization of compound 39

Compound **39** was obtained as Solid Pale yellow (melting point 216-218^oC) and its percent yield was 80.5%. The IR (KBr pellet) spectrum showed (v in cm^{-1}, Appendix 1A) broad absorption at 3338 cm^{-1} attributed to hydroxyl group. The intense absorption bands at 1651 cm^{-1}, medium bands at 1504 cm^{-1} and intense absorption band at 1170 cm^{-1} showd the presence of carbonyl carbon, aromatic C-C double bond, and the presence of -O-CH_3, respectively .

The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz, Appendix 1B) showed a singlet spectra at δ 3.81 (s, 3H) corresponding to a methoxyl group. The presence of aromatic ring with ABX multiplicity pattern (ring B) was confirmed on the basis of peaks observed at δ 7.33 (H-2, *d*, *J*= 2 Hz), δ 6.83 (H-5, *d, J* = 7.2 Hz), δ 7.31 (H-6, *dd*, *J* = 7.2, 2.0 Hz). The presence *trans* ethylenic protons (αH and Hβ) adjacent to the carbonyl were evident from ^{1}H NMR spectrum δ7.77(1H, *d*, *J*=12.0Hz), δ7.90 (1H, *d*, *J*=12.0Hz) respectively. The presence aromatic ring with AA'BB' spin system (ring A) was confirmed on the basis of peaks at δ 7.33 (H-2',6', 2H, *dd*, *J*=8.4, 2.1Hz)and δ 6.75 (H-3',5', 2H, *dd*, *J*=7.6, 2.1Hz) (table 4).

The ^{13}C NMR spectrum revealed peak resonating at δ 192.9 attributed to carbonyl carbon (Appendix 1C). The presnec of methoxy group, and carbon-carbon (Cα and Cβ) confimed on the basis of peaks at δ 56.5, 120.8 and 147.9, respectively. The peaks observed at δ 148.7 and 149.7 indicate the presence of two vicinal oxygenated sp2 quaternary carbons on ring B (C-3, C-4) whereas the peak observed at δ154.7 indicated the presence of sp2 oxygenated quaternary carbon at C-4' (ring A) (table 4). Thus, on the basis of ^{1}H NMR, ^{13}C NMR and IR spectra data, the

structure of compound (**39)** was established as chalcone 3-(4-Hydroxy-3-methoxy-phenyl)-1-(4-hydroxy-phenyl)-propenone



39

Table 4. ^{1}H NMR and ^{13}C NMR Compound (39)

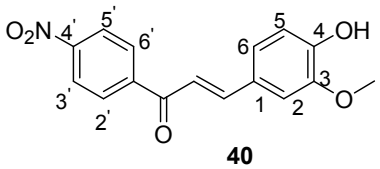
Position of atom	^{1}H NMR	^{13}C NMR
1	-	127.9
2	7.33(1H;d;J=2.0Hz)	116.4
3	-	149.7
4	-	148.7
5	5 6.83(1H;d;*J*=7.2Hz)	116.2
6	7.31(1H;dd;*J*=7.2 , 2.0Hz)	132.1
C=O	-	192.9
α	7.77(1H;d;*J*=12.0Hz)	120.8
β	7.90(1H;d;*J*=12.0Hz)	147.9
1’	-	131.1
2’	7.32(1H;d;*J*=8.4Hz)	130.7
3’	6.75(1H;d;J=7,6Hz)	115.9
4’	-	154.2
5’	6.75(1H;;J=7.6Hz)	115.8
6’	7.32(1H;d;J=8.4Hz)	130.7
CH_3O-	3.74(1H; s)	56.5

4.1.4 Characterization of compound 40

Compound **40** was obtained as yellow solid (melting point 7881 ^{o}C) and its percent yield was 88.9%. The IR (KBr pellet) spectrum showed (ν in cm^{-1}, Appendix 2A) broad absorption at 3338 cm^{-1} attributed to hydroxyl group whereas the presence of carbonyl carbon, aromatic C-C double bond and presence of –O-CH_3 were evident from intense absorption bands observed at 1651 cm^{1}, 1504 cm^{-1} and 1170 cm^{-1}, respectively .The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz, Appendix 2B) showed a singlet spectra which resonates at δ 3.81, corresponding to a methoxyl group. The presence of aromatic ring with ABX multiplicity pattern was confirmed on the basis of peaks at δ 7.33 (H-2, *d*, *J*=2 Hz), δ 6.83 (H-5, *d*; *J* = 7.2, 2.0 Hz), and δ 7.31 (H-6, *dd*, *J* = 7.2, 2.0 Hz). The presence *trans* ethylenic protons (αH and Hβ) adjacent to the carbonyl were evident from ^{1}H

NMR spectrum δ 7.77(1H, *d*, *J*=12.0Hz) and 7.90 (1H, *d, J*=12.0Hz), respectively. The presence of aromatic ring with AA'BB' spin system was observed at δ 7.33 (H-2',H-6', 2H, *dd, J*=8.4, 2.0Hz) and δ 6.75 (H-3',H-5', 2H, *dd, J*=8.4, 2.0Hz) (table 5).

The ^{13}C NMR spectrum revealed (Appendix 2C, table 6) peak at δ 191.5 attributed to carbonyl carbons. The presence of methoxyl group, and carbon-carbon (Cα and Cβ) double bonds were observed at δ 56, 120.8 and 147.9, respectively. The peaks observed at at δ 148.7 (C-4) and 147.7 (C-3) revealed the presence of vicinal sp^2 oxygenated quaternary carbons on ring B whereas the presence of peak at δ 154.7 confirmed the presence of sp2 carbon conneted to nitro group in ring A. Thus, on the basis of the ^{1}H NMR, ^{13}C NMR and IR spectra data of compound (**40)**,its structure was established as chalcone 3-(4-Hydroxy-3-methoxy-phenyl)-1-(4-hydroxy-phenyl)-propenone



40

Table 5. ^{1}H NMR and ^{13}C NMR Compound **40** (Appendix Figure 2)

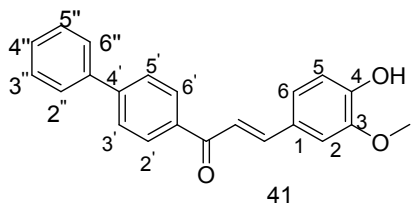
Position of atom	^{1}H NMR	^{13}C NMR
1	-	126.53
2	7.33(1H;d;J=2.0Hz)	115.8
3	-	147.7
4	-	148.9
5	6.83(1H;d;*J*=7.2Hz)	No peak on spectra
6	7.31(1H;dd;*J*=7.2 , 2.0Hz)	No peak on spectra
C=O	-	192.9
α	7.77(1H;d;*J*=12.0Hz)	120
β	No peak on spectra	148
1'	-	No peak on spectra
2'	7.32(1H;d;*J*=8.4Hz)	No peak on spectra
3'	6.75(1H;d;J=7,6Hz)	No peak on spectra
4'	No peak on spectra	153.4
5'	6.75(1H;;J=7.6Hz)	No peak on spectra
6'	7.32(1H;d;J=8.4Hz)	No peak on spectra
CH_3O-	3.84(1H; s)	56.5

4.1.5 Characterization of compound 41

Compound **41** was obtained as yellow solid (melting point 80–83 ^{o}C) and its percent yield was 85.3%. The IR (KBr pellet) spectrum showed (v in cm^{-1}, Appendix 3A) broad intensity band at 3338 cm^{-1} attributed to hydroxyl group. The intense absorption bands observed at 1651 cm^{-1}, 1504 cm^{-1} and 1170 cm^{-1} suggest the presence of carbonyl carbon, aromatic C-C double bond and presence of –O-CH_3 moiety .

The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz, Appendix 3B, table 7) showed a singlet spectra which resonates at δ 3.81, corresponding to a methoxyl group. The presence of aromatic ring with ABX mupltiplicity pattern was confirmed on the basis of peaks observed at δ 7.33 (H-2, *d*, *J*= 2 Hz), δ 6.83 (H-5, *d*, *J* = 7.2 Hz) and δ 7.31 (H-6, *dd*, *J* = 7.2, 2.0 Hz). The presence *trans* ethylenic protons (αH and Hβ) adjacent to the carbonyl were evident from ^{1}H NMR spectrum δ 7.77 (1H, d, *J*=12.0Hz), and δ 7.90 (1H, d, *J*=12.0Hz), respectively. The presence of 1,4 substituted aromatic ring was confirmed on the basis of peaks observed at δ 7.33 (H-2',6', 2H, *dd*, *J*=8.4, 2.1Hz), δ 8.2(H-3',5', 2H, *dd*, *J*=7,6, 2.1Hz). Additional monosubstituted aromatic ring conneted at C-4' position of ring A was confirmed on the basis of peaks observed at δ 7.5 (H-2'',6'', 2H, dd *J*=8.4, 2Hz), δ 7.5 (H- 3'',5'', 2H, dd *J*=8.4, 2Hz) and δ 7.4 (H-4'', 1H, m).

The ^{13}C NMR spectrum showed peak (Appendix 3C, table 6) at δ 191.5 attributed to carbonyl carbon. The peaks observed at δ 56.3, 120.8 and 147.9 suggest the presence of a methoxyl group and carbon-carbon (Cα and Cβ) double bonds adjacent to carbonyl carbon. The presence of vicinal sp2 oxygenated quaternary carbons on ring B was confirmed from peaks observed at 147.7 and 148.6 (C-3,4) whereas the peak at δ133.7 suggests that a phenyl ring is connected at C-4' position of ring A (table 6). Thus, on the basis of ^{1}H NMR, ^{13}C NMR and IR spectral data of compound (**41)**, its structure was established as chalcone 3-(4-Hydroxy-3-methoxy-phenyl)-1-(4-hydroxy-phenyl)-propenone



41

Table 6. ^{1}H NMR and ^{13}C NMR of Compound 41 **(**Appendix 3)

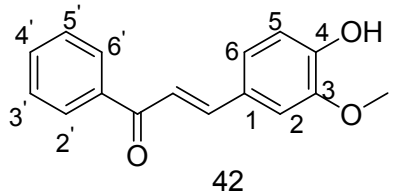
Position of atom	^{1}H NMR	^{13}C NMR
1	-	126.53
2	7.33(1H;d;J=2.0Hz)	111.1
3	-	148.7
4	-	149.7.6
5	6.83(1H;d;*J*=7.2Hz)	115.8
6	7.31(1H;dd;*J*=7.2 , 2.0Hz)	119.1
C=O	-	191.54
α	7.77(1H;d;*J*=12.0Hz)	124.5
β	No peak on spectra	145.7
1’	-	138.4
2’	7.32(1H;d;*J*=8.4Hz)	129.1
3’	6.75(1H;d;J=7,6Hz)	128.5
4’	No peak on spectra	153.4
5’	6.75(1H;J=7.6Hz)	128.5
6’	7.32(1H;d;J=8.4Hz)	129.1
1”		138..4
2”	7.5(1H;d; *J*=2Hz)	127.3
3”	7.5(1H:dd; *J*=8.4)	129.2
4”	7.4(1H;m; J=1.5Hz)	133.7
5”	7.4(1H;d; J=8.4)	129.2
6”	7.3(1H;d;J=2Hz)	127.4
CH_3O-	3.84(1H; s)	56.3

4.1.6 Characterization of compound 42

Compound **42** was obtained as yellow solid (melting point 86-87^{o}C) and its percent yield was 80.5%. The IR (KBr pellet) spectrum showed (ν in cm^{-1}, Appendix 4A) broad absorption peak at at 3338 cm^{-1} attributed to hydroxyl group. The intense absorption bands at 1651 cm^{-1}, 1504 cm^{-1} and 1170 cm^{-1} suggests the presence of carbonyl carbon, aromatic C-C double bond, and O-CH_3 .The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz, Appendix 4B) showed a singlet spectra which resonates at δ 3.87, corresponding to a methoxyl group. The presence of aromatic ring with ABX multiplicity pattern was confirmed on the basis of peaks observed at δ 7.33 (H-2, *d*, *J*= 2 Hz), δ 6.83 (H-5, *d*, *J* = 7.2,2 Hz) and δ 7.31 (H-6, *dd*, *J*= 7.2,.0 Hz). The presence *trans* ethylenic protons (αH and Hβ) adjacent to the carbonyl were evident from ^{1}H NMR spectrum δ 7.77(1H, d, *J*=12.0Hz), δ7.90 (1H, d, *J*=12.0Hz), respectively. The presence of monosubstituted phenyl

ring was observed at δ 8.1 (H-2',H- 6', 2H, *dd*, *J*=8.4,2Hz), δ 7.7(H-3',5', 2H, *dd*, *J*=8.4, 2Hz) and 7.3 (1H, m, H-4') (table 7).

The ^{13}C NMR spectrum (Appendix 4C) showed peak at δ191.4 attributed to carbonyl carbon. The peaks observed at δ56.2, 124.5 and 145.4 suggets a methoxyl carbon signal and carbon-carbon (C_α and C_β) double bonds adjacent to carbonyl carbon. The two vicinal sp^2 quaternary carbons were observed at δ 147.8 and 148.6 (C-3,4). The peaks observed at δ 138..4 (C-1'), 129.1 (C-2',6'), 115.9 (C-3', 5') and 133.2 (C-4') suggest the presence of monosubstituted aromatic ring (ring A). Thus, based on ^{1}H NMR, ^{13}C NMR and IR spectral data the structure of compound **42** was proposed as shown below (table 7**)**.



42

Table 7. ^{1}H NMR and ^{13}C NMR Compound **42** (Appendix 4)

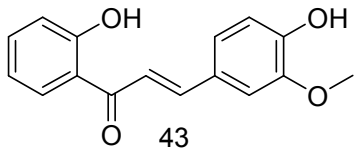
Position of atom	**^{1}H NMR**	**^{13}C NMR**
1	-	128.5
2	7.33(1H;d;J=2.0Hz)	112.1
3	-	149.7
4	-	148.7
5	6.83(1H;d;*J*=7.2Hz)	115.8
6	7.31(1H;dd;*J*=7.2 , 2.0Hz)	119.1
C=O	-	191.4
α	7.77(1H;d;*J*=12.0Hz)	124.5
β	7.90(1H;d;*J*=12.0Hz)	145.4
1'	-	138..4
2'	7.32(1H;d;*J*=8.4Hz)	129.1
3'	6.75(1H;d;J=7.6Hz)	115.9
4'	8.3(1H;m;J=1.5Hz)	133.2
5'	6.75(1H;;J=7.6Hz)	115.8
6'	7.32(1H;d;J=8.4Hz)	129.1
CH_3O-	3.74(1H; s)	56.2

4.1.7 Characterization of compound 43

Compound **43** was obtained as yellow solid (melting point 79-82 ^{0}C) and its percent yield was 85.4%. The IR (KBr pellet) spectrum showed (v in cm^{-1}, Appendix 5A) broad absorption band at 3338 cm^{-1} attributed to hydroxyl group.The intense absorption bands at 1651 cm^{-1}, 1504 cm^{-1}, and 1170 cm^{-1} suggest the presence of carbonyl carbon, aromatic C-C double bond, and O-CH_3 moeity .

The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz, Appendix 5B) showed a singlet spectra which resonates at δ 3.87, corresponding to a methoxyl group. The presence of aromatic ring with ABX multiplicity pattern was confirmed at δ 7.33 (H-2, *d*, *J*= 2 Hz), δ 6.87 (H-5, *d*; *J* = 7.2 Hz) and δ 6.98 (H-6, *dd*, *J* = 7.2, 2.0 Hz). The presence *trans* ethylenic protons (αH and Hβ) adjacent to the carbonyl were evident from ^{1}H NMR spectrum δ 7.77(1H, d, *J*=12.0Hz) and 8.2 (1H, d, *J*=12.0Hz), respectively . The presence of another disubstituted aromatic ring (ring A) was confirmed on the basis of peaks observed at δ7.8(H-6',1H, *dd*, *J*=8.4, 2Hz), δ7.5 (H-4',1H, *dd*, *J*=;8.4, 2Hz), δ7.3 (H-3',1H, *dd*, *J*=8.4, 2Hz) and δ6.9 (H-5',1H, *dd*, *J*=;8.4, 2Hz) (table 8).

The ^{13}C NMR spectrum of showed (Appendix 5C) peak at δ194.1 attributed to carbonyl carbon. The presence of peaks at δ56.3, 126.5 and 146.6 ppm suggests the presence of methoxyl carbon signal,and carbon-carbon (C_α and C_β) double bonds adjacent to carbonyl. The presence of sp2 oxygenated quaternary carbon at at δ 162.6 (C-2') confirms the presence of hydroxyl group at C-2' position. Thus, based on the IR, ^{1}H NMR and ^{13}C NMR spectral data the followed structure was suggested for compound **43**.



43

Table 8. ^{1}H NMR and ^{13}C NMR Compound **43** (Appendix 5)

Position of atom	^{1}H NMR	^{13}C NMR
1	-	126.5
2	7.33(1H;d;J=2.0Hz)	116.4
3	-	140.8
4	-	148.7
5	6.83(1H;d;*J*=7.2Hz)	116.2
6	7.31(1H;dd;*J*=7.2 , 2.0Hz)	119.5
C=O	-	192.9
α	7.77(1H;d;*J*=12.0Hz)	120.9
β	7.90(1H;d;*J*=12.0Hz)	146.6
1’	-	125.3
2’	7.32(1H;d;*J*=8.4Hz)	131.1
3’	7.3(1H;dd;J=8.4Hz,2Hz)	120.9
4’	7.5(1H;dd;J=8.4Hz,2Hz)	136.6
5’	6.9(1H;dd;J=8.4Hz,2Hz)	116.1
6’	7.8(1H;dd;J=8.4Hz,2Hz)	162.6
CH_3O-	3.74(1H; s)	56.5

4.1.8 Characterization of compound 44

Compound **44** was obtained as yellow solid (melting point 80-89 ^{0}C) and its percent yield was 89.8%. The IR (KBr pellet) spectrum showed (v in cm^{-1}, Appendix 5A): broad absorption at 3043 cm^{-1} attributed to hydroxyl group. The intense absorption bands observed at 1706 cm^{-1}, 1589 cm^{-1}, 1170 cm^{-1} and 1103 cm^{-1} suggest the presence of carbonyl carbon, aromatic C-C double bond, O-CH_3, and –C-O-C- group moiety .

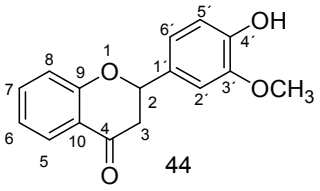
The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz, Appendix 6B, table10) showed a singlet spectra which resonates at δ 3.87, corresponding to a methoxyl group. The presence of oxygenated sp3 methine at δ 5.35 ((H-2,; dd; J = 2.79 and J =12.31) and methylene peak at δ 2.70 (H-3, *dd*; J =2.79, 16.72). The presence of aromatic ring with ABX multiplicity pattern was confirmed on the basis of peaks observed at δ 6.76 (H-5’,; d, J = 8.9), δ 6.83(H-6’, d, J = 8.82) and δ 6.96(H-2’, d, J=2Hz). The presence of disubstituted aromatic ring (ring A) was confirmed δ 6.90 (H-

7,*dd*, *J*=2.79, 8Hz), δ 7.27 (H-5, *dd*, J = 8.9, 2Hz), δ6.60(H-6, *dd*,J=8, 2Hz), and δ6.8(H-6, *dd*, J=8, 2Hz).

The ^{13}C NMR spectrum (Appendix 6C) showed peaks at 181.8and 56.5attributed to carbonyl carbon and methoxyl carbon. The presence of sp3 oxygenated methine and methylene alpha to carbonyl carbon was confirmed at δ78.3 (C-2) and δ60 (C-3). The presence of peaks at δ147.7 and 148.1 confirm the presence of vicinal sp2 oxygenated quaternary carbons (C-3', 4'). Thus, based on the above spectral data the structure of compound 44 was confirmed as shown below.

Table 9. ^{1}H NMR ^{13}C NMR Compound **44** (Appendix 6)

Position of atom	^{1}H NMR	^{13}C NMR
1	-	
2	5.33(1H;dd;J=2.79Hz,16.72Hz)	78.3
3	2.70(IH;dd;J=2.79Hz,16.72Hz	60.3
4	-	181.8
5	7.27(1H,d,*J*=8.94Hz	120.7
6	6.60(1H,d,J=8.82Hz)	112.3
7	6.90(1H; dd, J=2.79, 8Hz)	136.6
8	6.8(1H,d,J=8.94Hz,)	114.9
1'	-	158.7
2'	6.96(1;d;J=1.86Hz)	116.3
3'		147.7
4'		148..1
5'	6.76(1H;d;J=8.13,)	116.3
6'	6.83(1H;dd;J=1.86,J=8.13)	120.7
CH_3O-	3.74(1H; s)	56.5



44

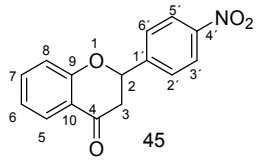
4.1.9 Characterization of compound 45

Compound **45** was obtained as yellow solid (melting point 87-90 ^{0}C) and its percent yield was 85.0%. The IR (KBr pellet) spectrum showed (*v* in cm^{-1}, Appendix 5A): broad absorption at 3043 cm^{-1} attributed to hydroxyl group. The intense absorption bands observed at 1706 cm^{-1},

1650 cm^{-1} , 1589 cm^{-1}, 1170 cm^{-1} and 1103 cm^{-1} suggest the presence of carbonyl carbon, NO_2 ,aromatic C-C double bond, O-CH_3, and –C-O-C- group moiety .

The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz, Appendix 6B, table10) showed presence of oxygenated sp3 methine at δ 5.35 ((H-2,; dd; *J* = 2.79 and *J* =12.31) and methylene peak at δ 2.50 (H-3, *dd*; *J* =2.79, 16.72). The presence of aromatic ring with ABX multiplicity pattern was confirmed on the basis of peaks observed at δ 8.12 (H-5',; d, *J* = 8.9), δ 7.45(H-6', d, *J* = 8.82) and δ 7.12(H-2', d, J=2Hz). The presence of disubstituted aromatic ring (ring A) was confirmed δ 6.90 (H-7,*dd*, *J*=2.79, 8Hz), δ 7.27 (H-5, *dd*, J = 8.9, 2Hz), δ6.60(H-6, *dd*,J=8, 2Hz), and δ6.8(H-6, *dd*, J=8, 2Hz).

The ^{13}C NMR spectrum (Appendix 6C) showed peaks at 191.37 attributed to carbonyl carbon The presence of sp3 oxygenated methine and methylene alpha to carbonyl carbon was confirmed at δ78.3 (C-2) and δ60 (C-3). The presence of peaks at δ147.7 and 148.1 confirm the presence of quaternary carbons (C-1', 4'). Thus, based on the above spectral data the structure of compound **45** was confirmed as shown below



45

Table 10. ^{1}H NMR and ^{13}C of Compound 45 (Appendix 7)

Position of atom	^{1}H NMR	^{13}C NMR
1	-	
2	5.35(1H;dd;J=2.79Hz,16.72Hz)	78
3	2.50(IH;dd;J=2.79Hz.16.72Hz	60.3
4	-	191.3
5	7.27(1H,dd,*J*=8.9Hz,2Hz	120.7
6	76.6(1H,dd,J=8.8HZ, 2Hz)	121.1
7	6.90(1H, dd, J=2.79Hz,12.31Hz)	136.6
8	7.27(1H,d,J=8.94Hz,)	118.5
9		161.1
1'	-	128.1
2'	7.12(1H;d;J=2Hz)	124.2
3'	6.76(1H;d;J=8.13,)	122.1
4'		148..1
5'	8.12(1H;d;J=8.9)	124.2
6'	7.45(1H;dd;J=8Hz,2Hz)	129.1

4.2 Antibacteria Activity

All the chalcones and flavonones **(39** to **45)** have been evaluated for their antibacterial activity against *S. aureus* **(**Gram-positive) and *E. coli, K. Pneumonia* and *P.aeroginosa* (Gram-negative) using disk diffusion method. The results of this evaluation were compared with cefraxone as reference standard. It is interesting to note from the results that almost all the compounds exhibited some degree of inhibition zones . The chalcones **42** and **43** are active on against S. aureus were as Flavonones **44** active on against *E. coli* and **45** active on against S. aureus *and*

*P.aeroginosa***.**

Table 11. Antibacterial activity of chalcones and Flavonones

Compound	**Zone of inhibition in mm**			
	S.aureus	***E.coli***	***K.pneumonia***	***P.aeroginosa***
39	6	9	8	9
40	7	8	8	8
41	6	10	8	6
42	13	8	8	9
43	11	6	6	6
44	6	10	6	6
45	10	6	6	10
(Ceftraxone) Standard				
DMSO	6	6	6	6

5. CONCLUSION AND RECOMMENDATION1

5.1. Conclusion

The study explored the scope of application of ZnO nanoparticle as a catalyst for synthesis of chalcone derivatives of vanillin and different substituted acetophenone derivatives with electron donating and withdrawing groups at para position of acetophenone. The Claisen-Schmidt condensation reaction proceeded smoothly to furnish chalcones and flvanones derivatives in good yield. The study reported inexpensive and easily available solid base catalyst, shorter reaction time, high yield, easy work up procedure, and no side product comparedto conventional method using potassium hydroxide. Thus, green synthesis protocol was developed to synthesize chalcones and flvanones derivatives of vanillin and acetophenone. Moreover, the method can also be applied to synthesis of chalcone derivatives comprising base sensitive functional groups with high yield, short reaction time and easy work up procedure**.**

5.2. Recommendation

Based on the present study the following recommendations are forwarded:

- Related research work is recommended to synthesize additional series of chalcones and flavonones derivatives of vanilline and acetophenone and conduct structure-activity relationship (SAR) analysis

- Related research work is recommended to synthesize a series of pyrazole and oxazole derivatives of vanilline and acetophenone chalcone derivatives andconduct structure-activity relationship (SAR) analysis

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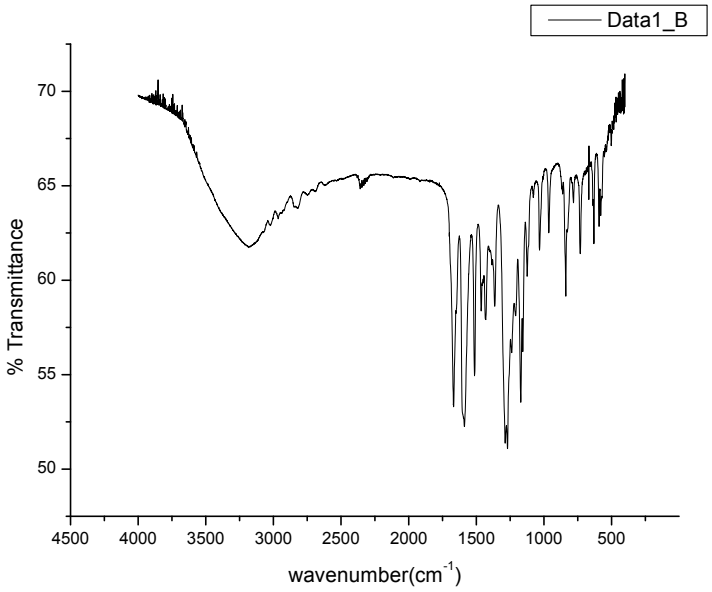
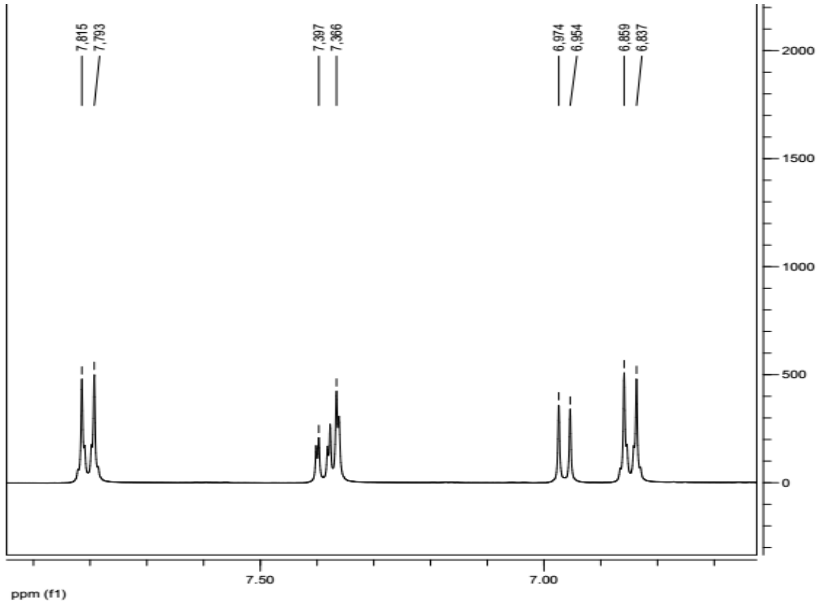
Appendix 1 A: FT-IR spectrum of Compound **39**



Appendix IB: ^{1}H NMR spectrum of Compound **39**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7,793
7,397
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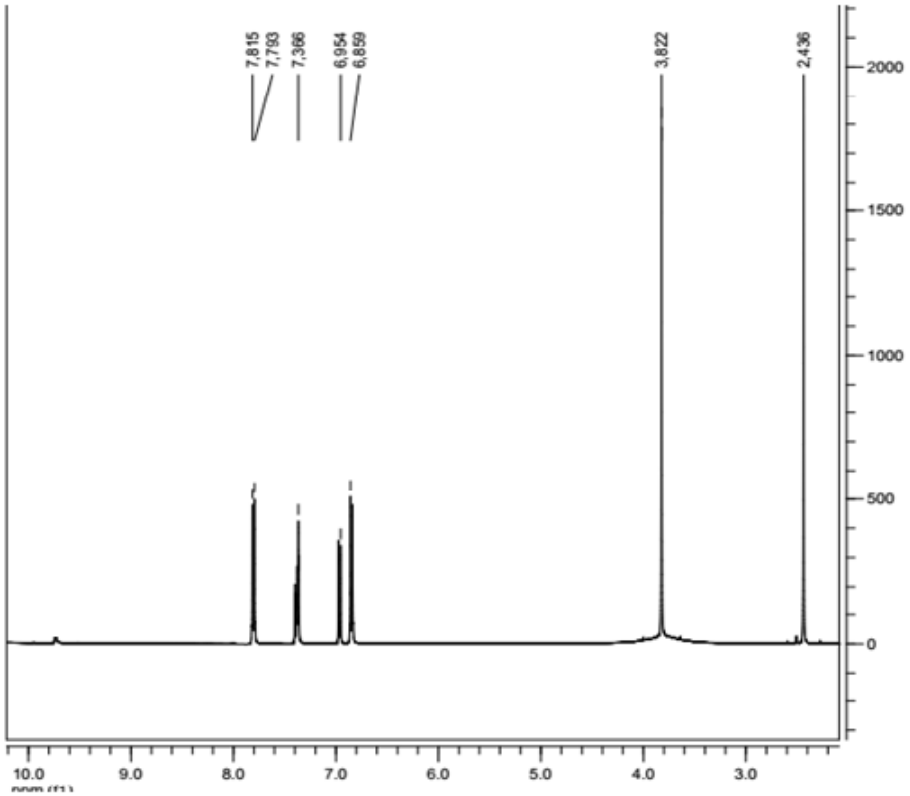
6,954
6,859
6,837
2000
1500
1000
500
0
7.50
7.00
ppm (f1)


7,815
7,793
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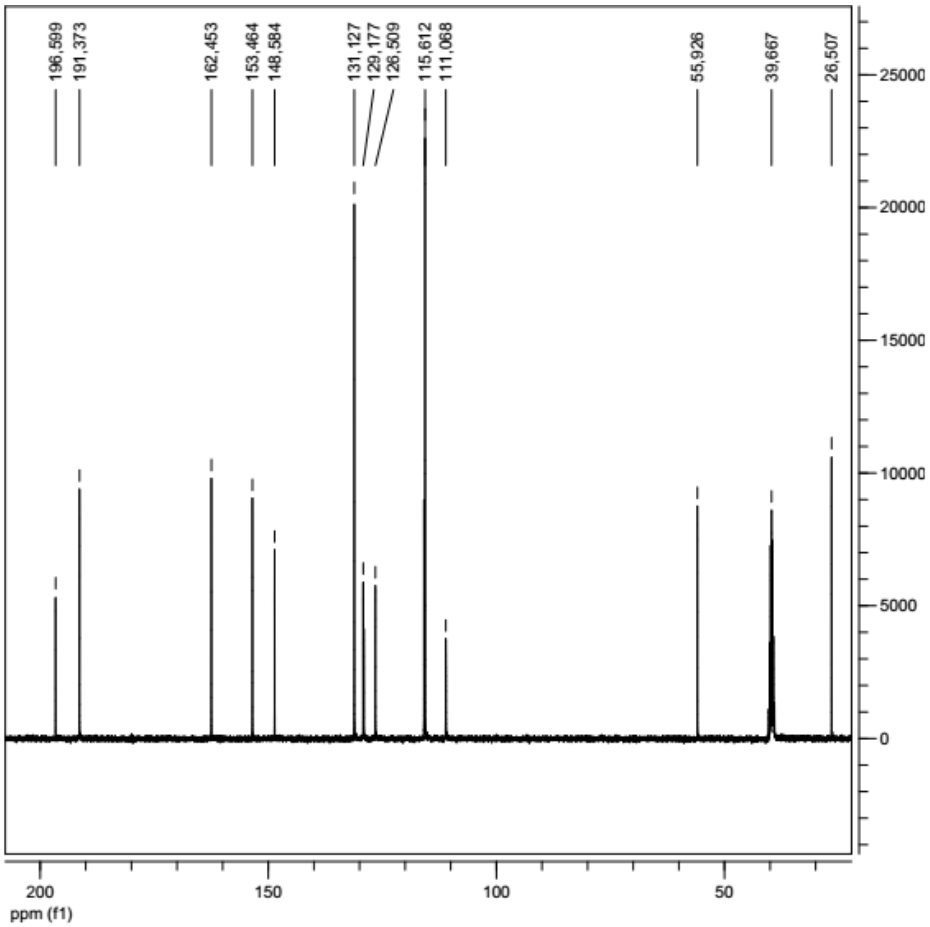
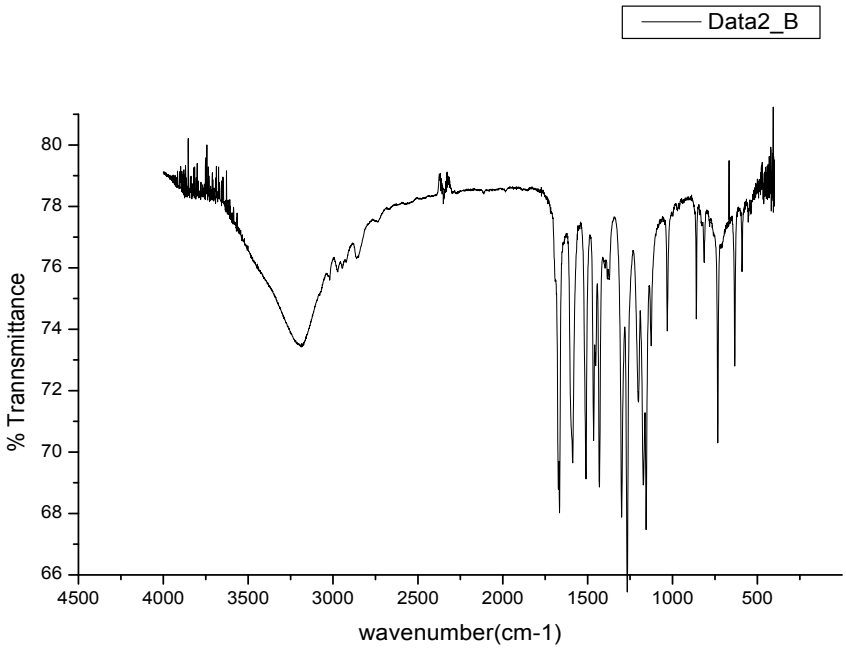
6,954
6,859
3,822
2,436
2000
1500
1000
500
0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0

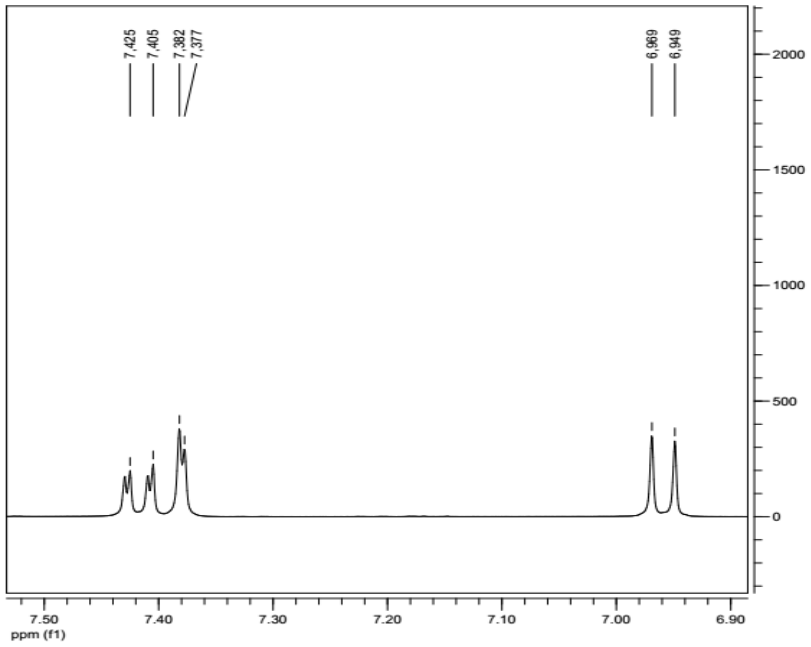
Appendix 1 C: ^{13}C NMR spectrum Compound **39**

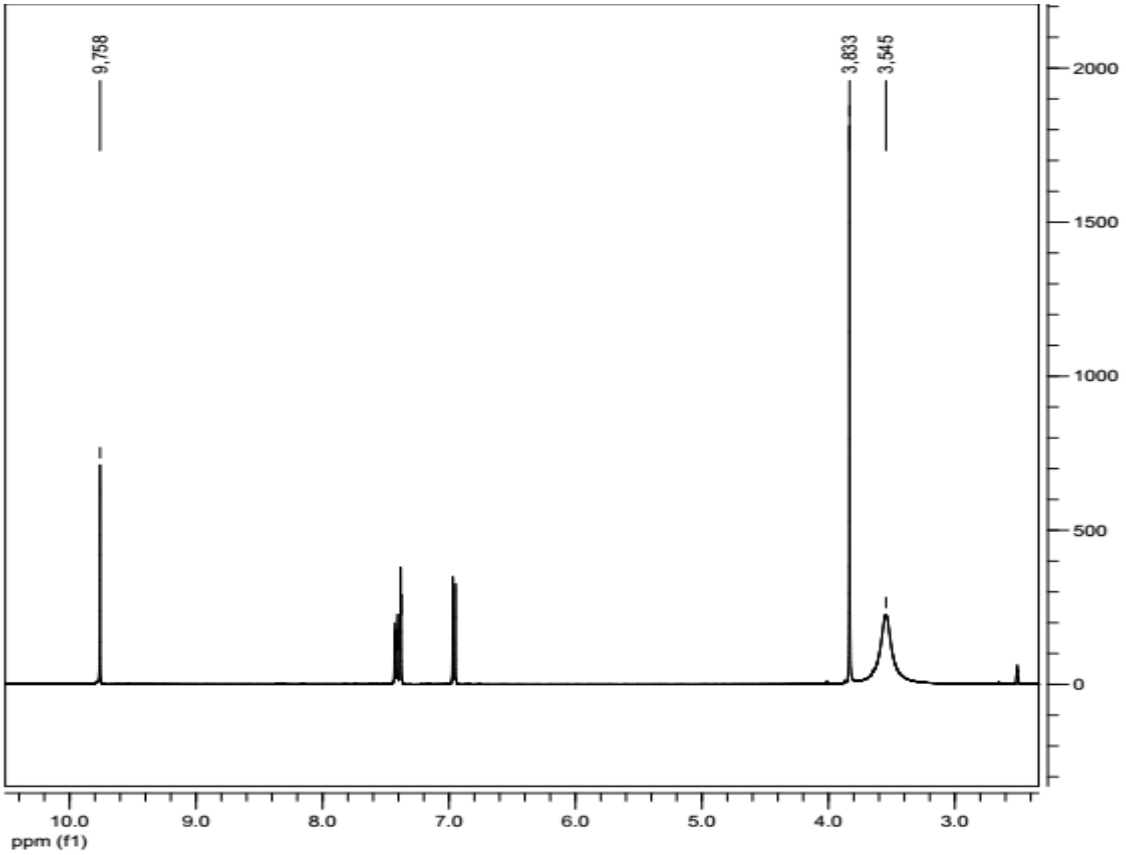


Appendix 2A: FT-IR spectrum of Compound **40**

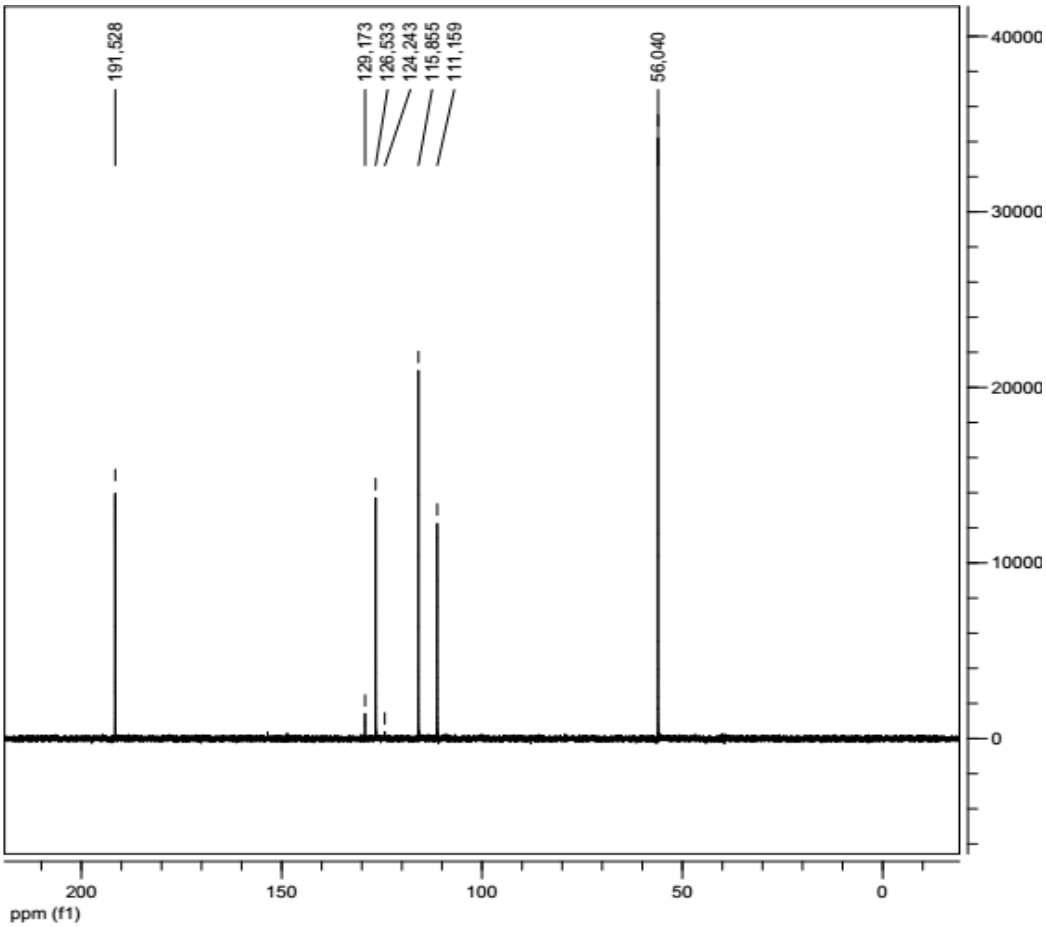


Appendix 2 B: ^{1}H NMR spectrum of Compound 40

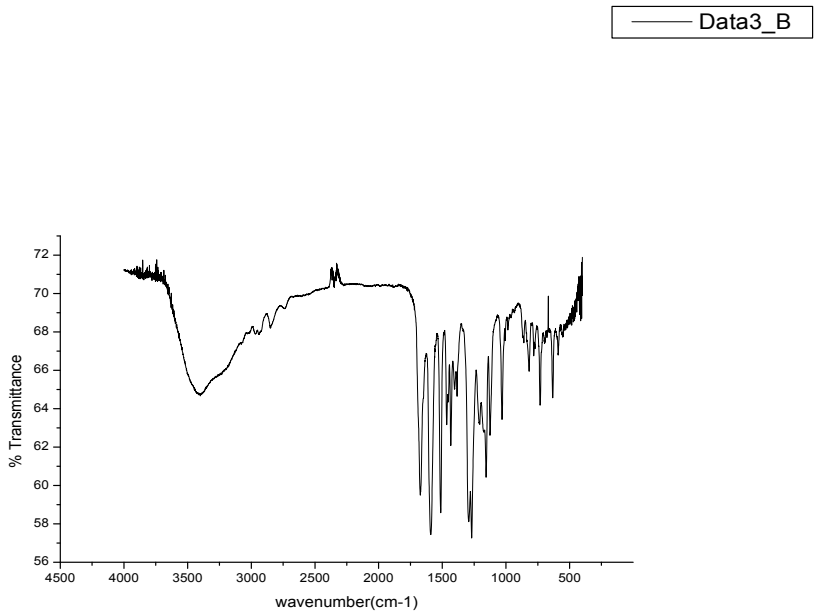




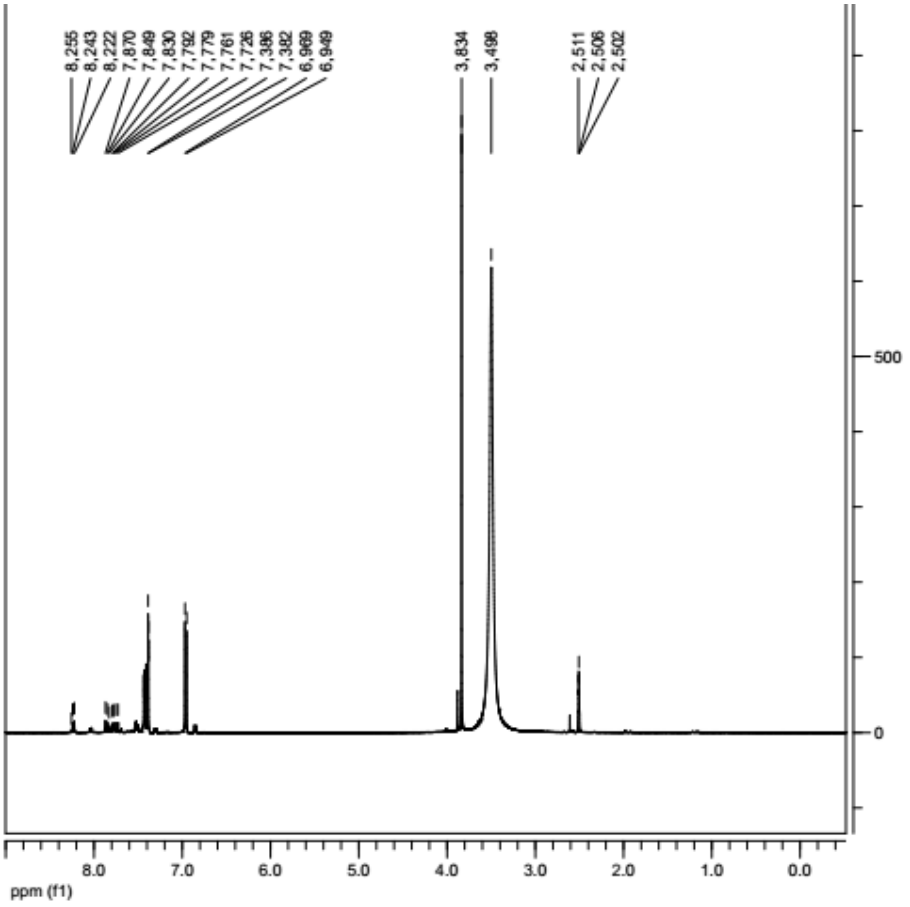
Appendix 2 C: ^{13}C NMR spectrum Compound **40**



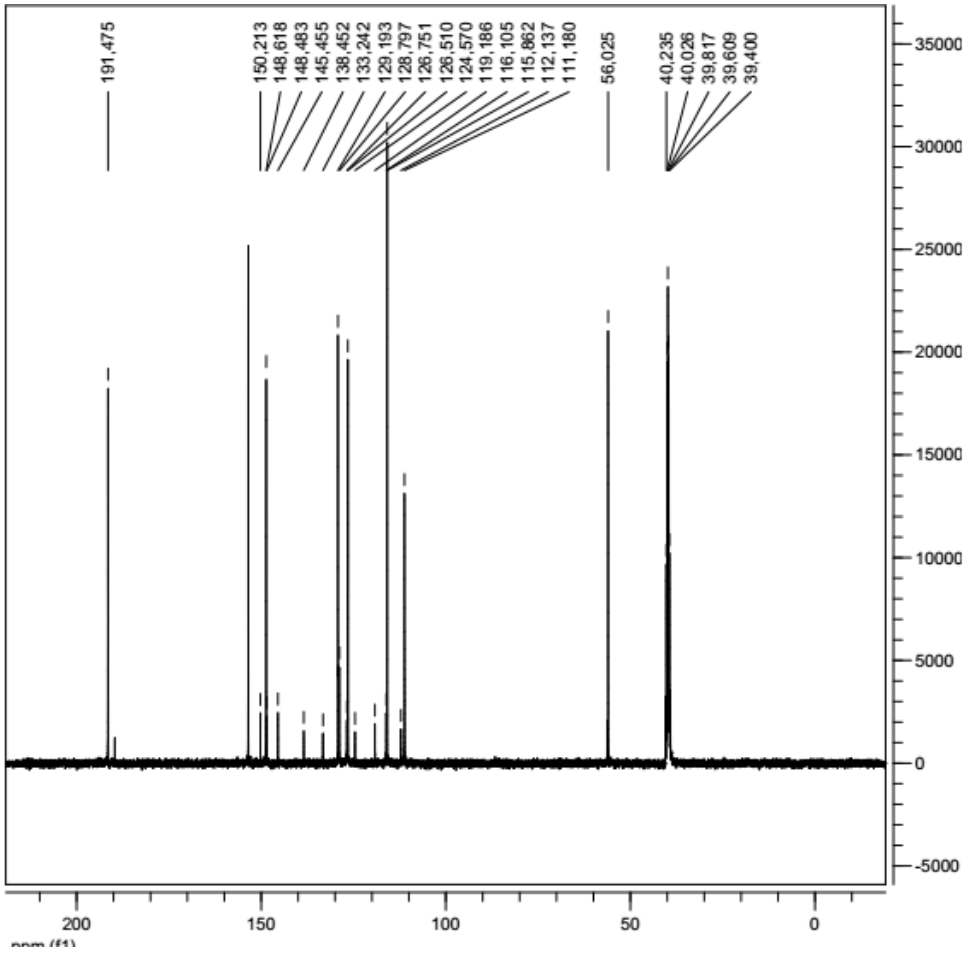
Appendix 3 A: FT-IR spectrum of Compound **41**



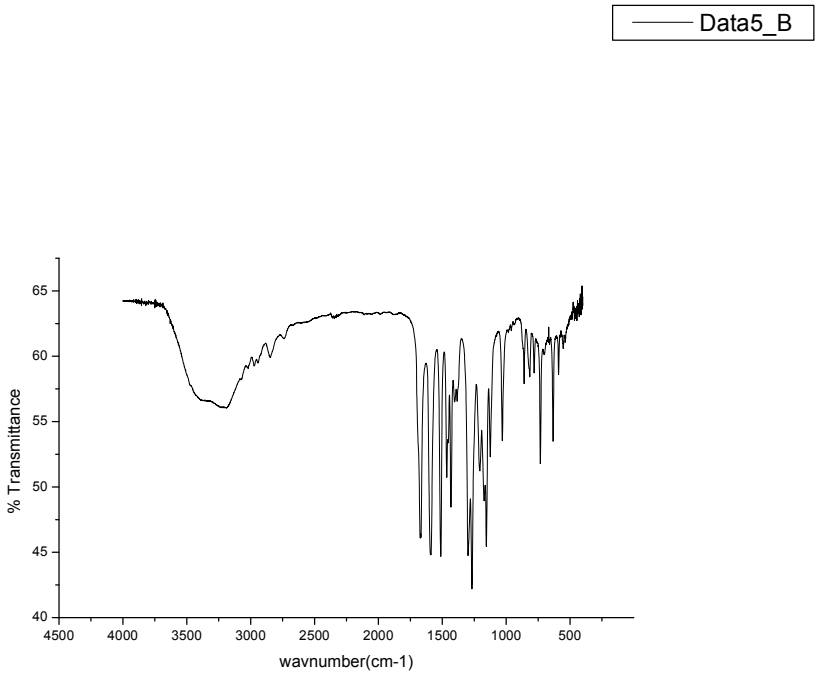
Appendix 3 B: ^{1}H NMR spectrum of Compound **41**



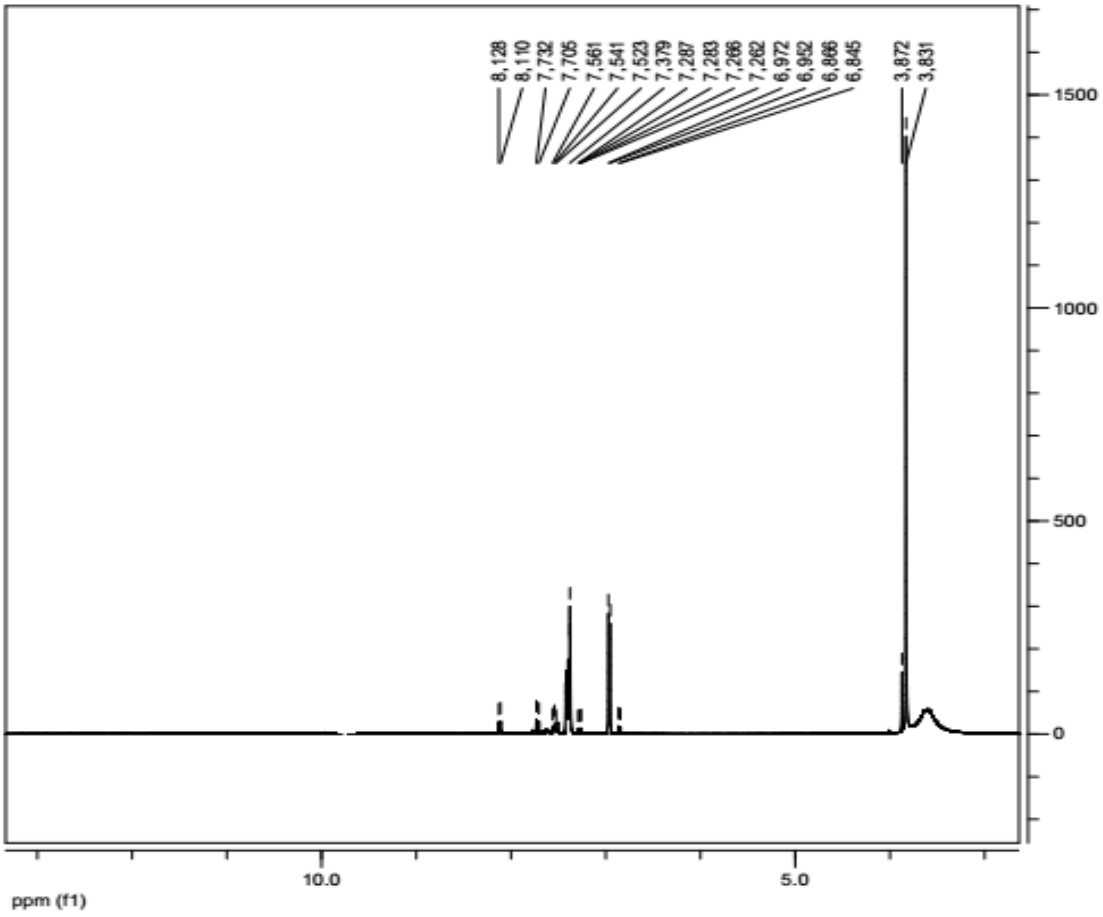
Appendix 3 C: ^{13}C NMR spectrum Compound **41**



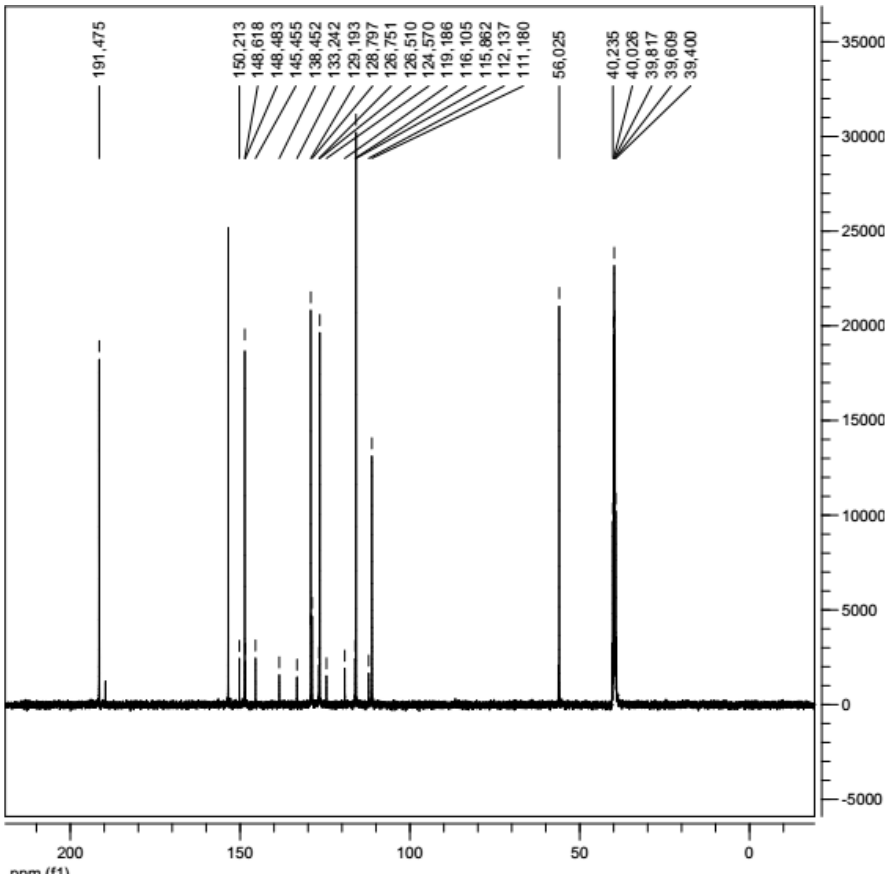
Appendix 4 A: FT-IR spectrum of Compound **42**



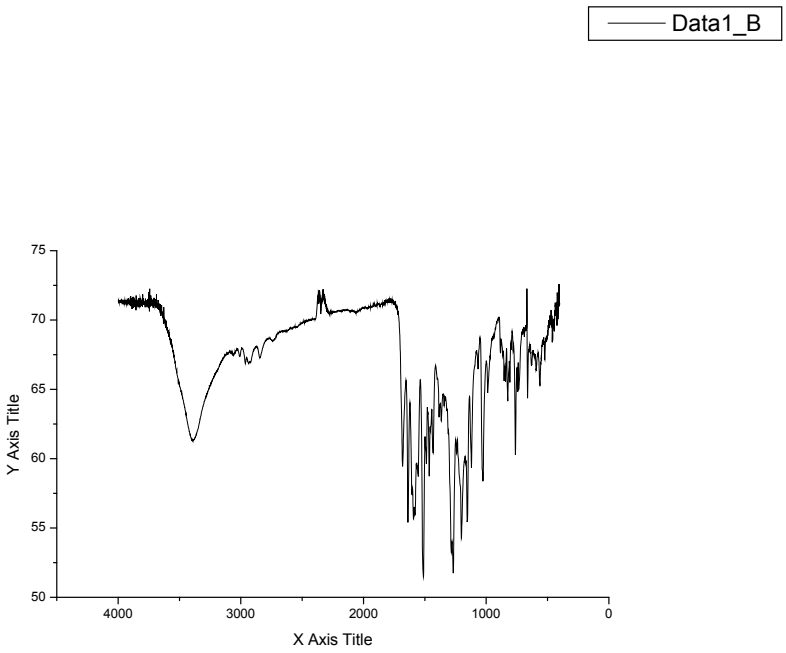
Appendix 4 B: ^{1}H NMR spectrum of Compound **42**



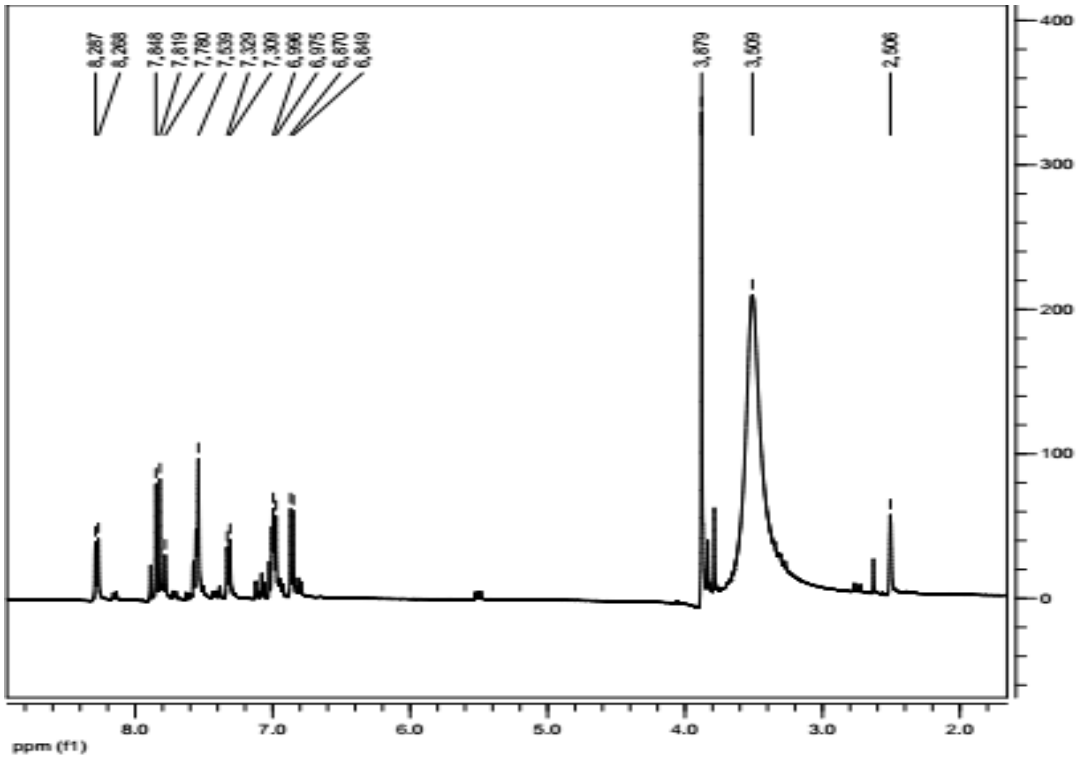
Appendix 4 C: ^{13}C NMR spectrum Compound**42**



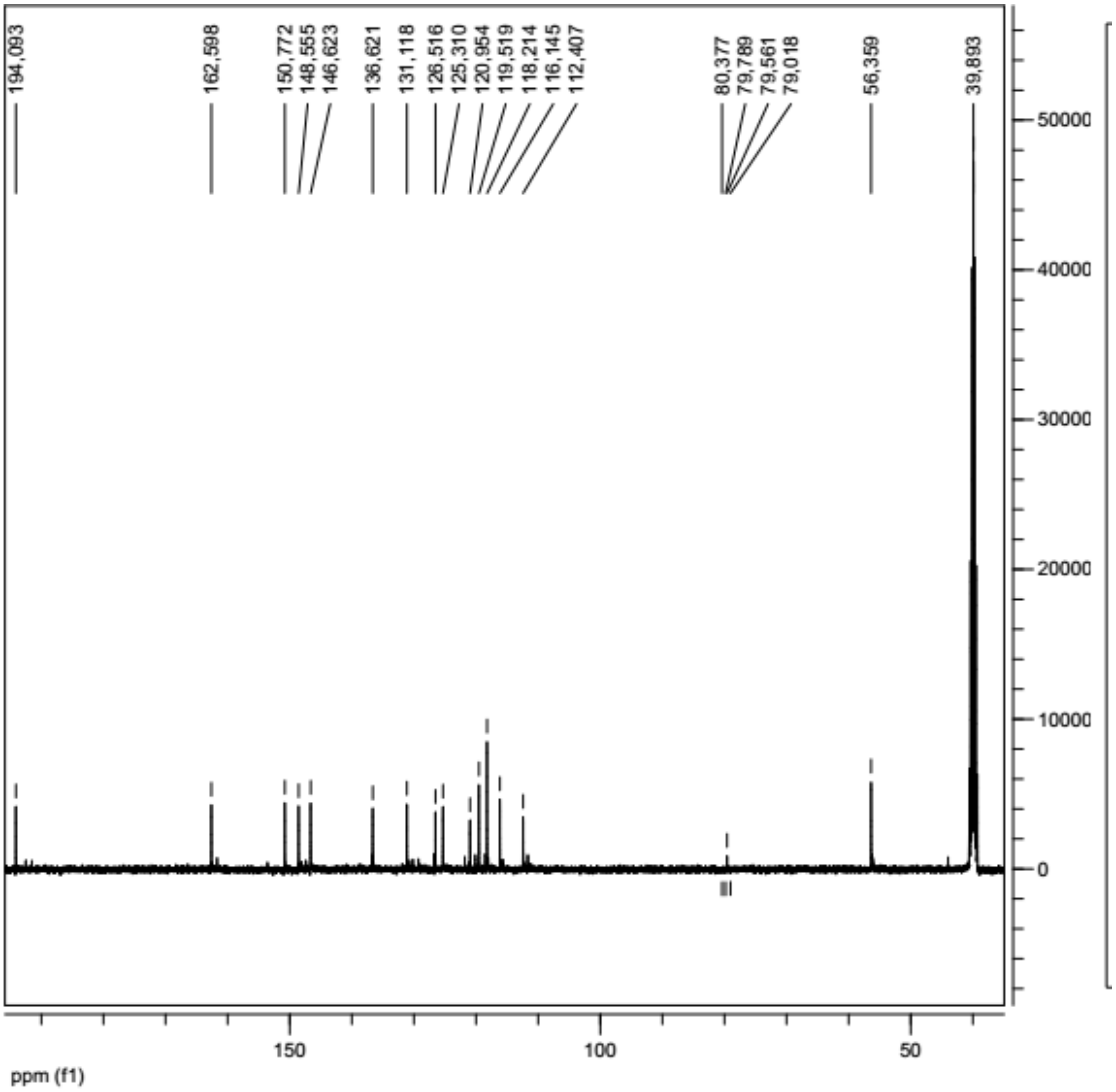
Appendix 5 A: FT-IR spectrum of Compound **43**



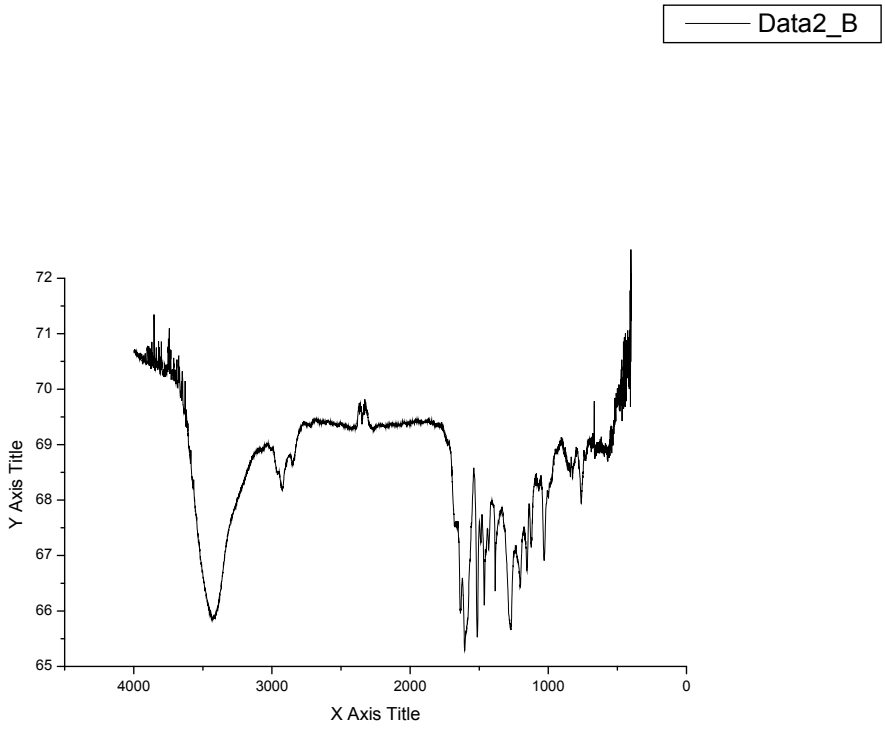
Appendix 5 B: ^{1}H NMR spectrum of Compound **43**



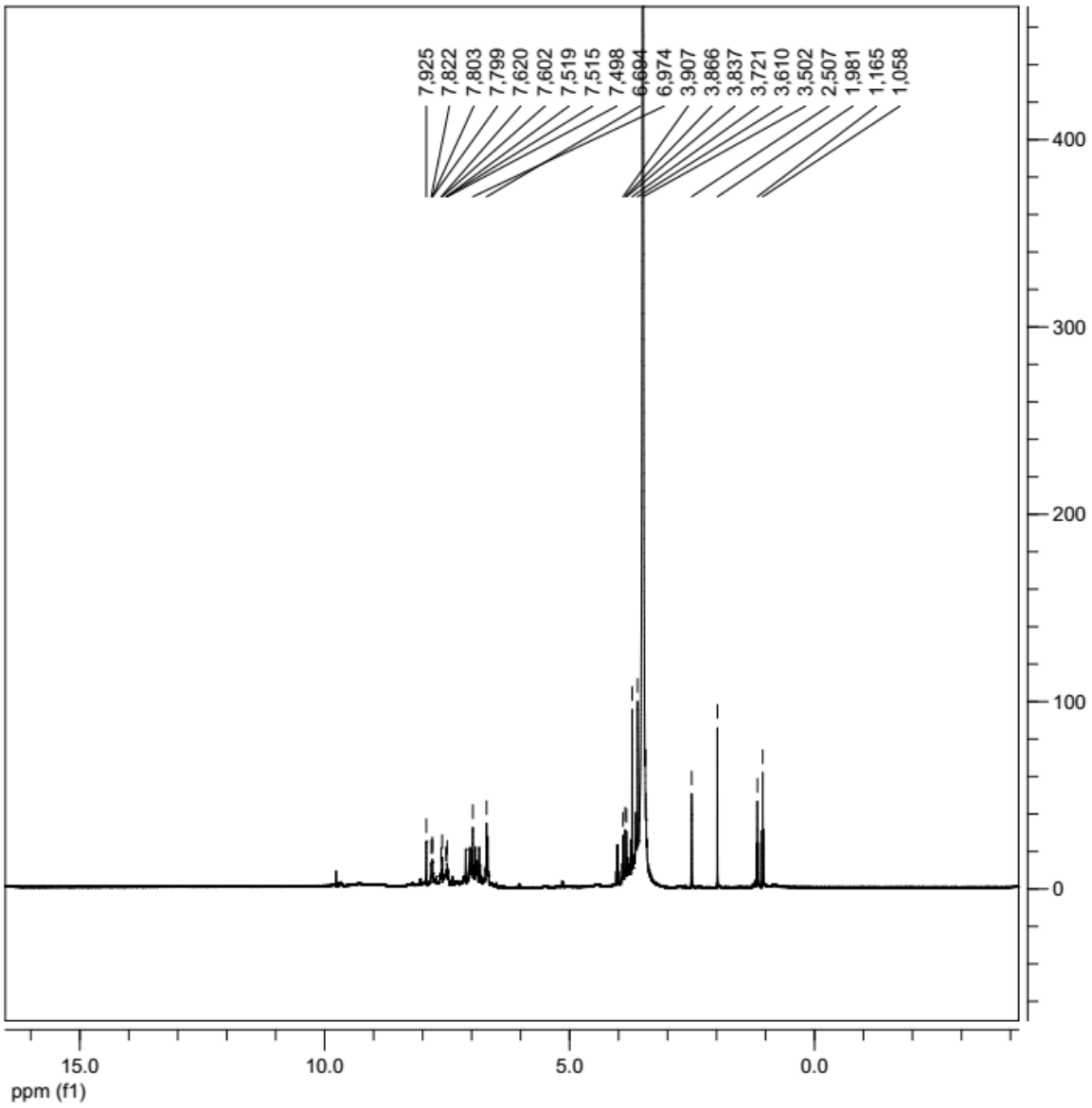
Appendix 5 C: ^{13}C NMR spectrum Compound **43**



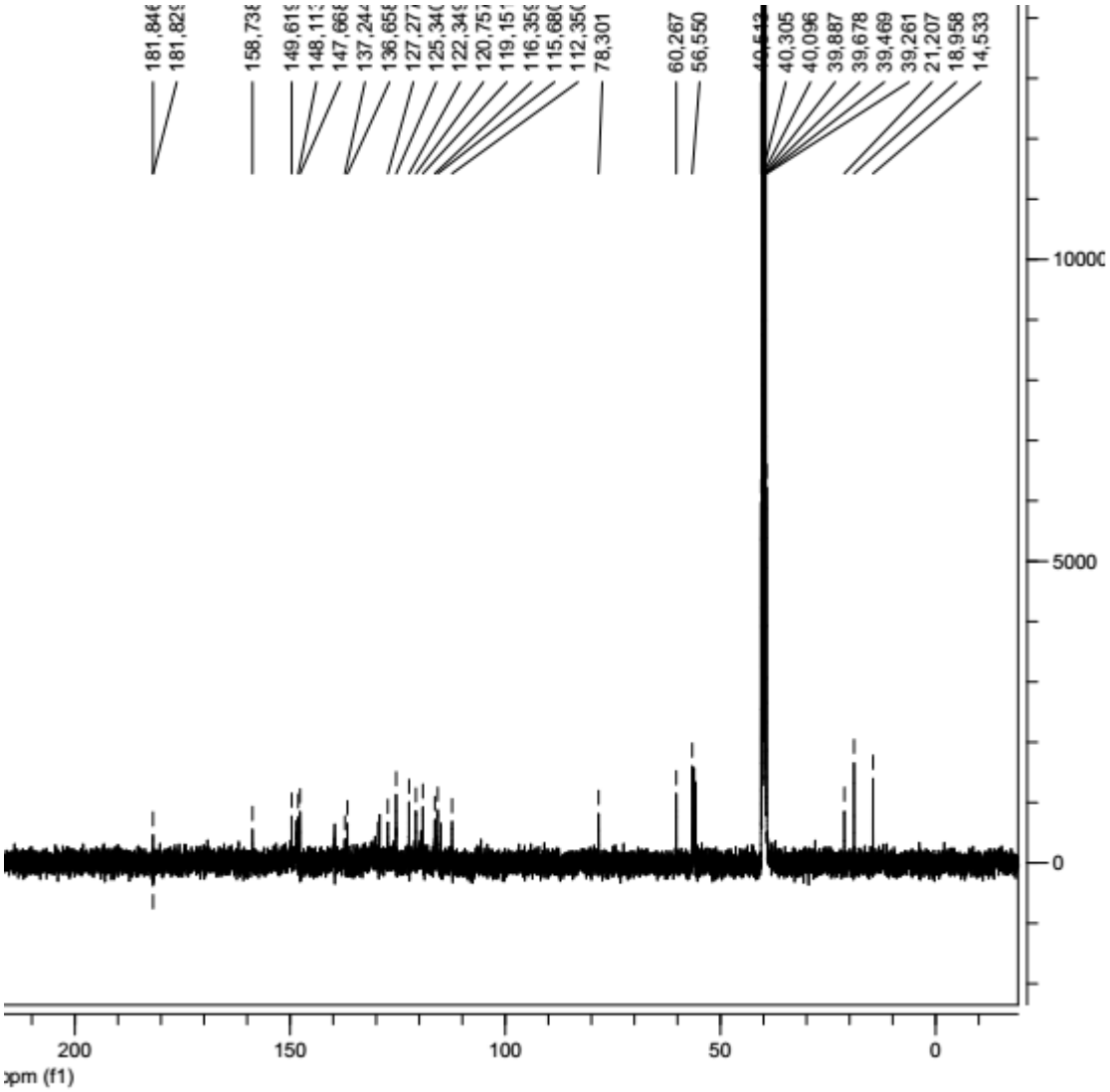
Appendix 6 A: FT-IR spectrum of Compound **44**



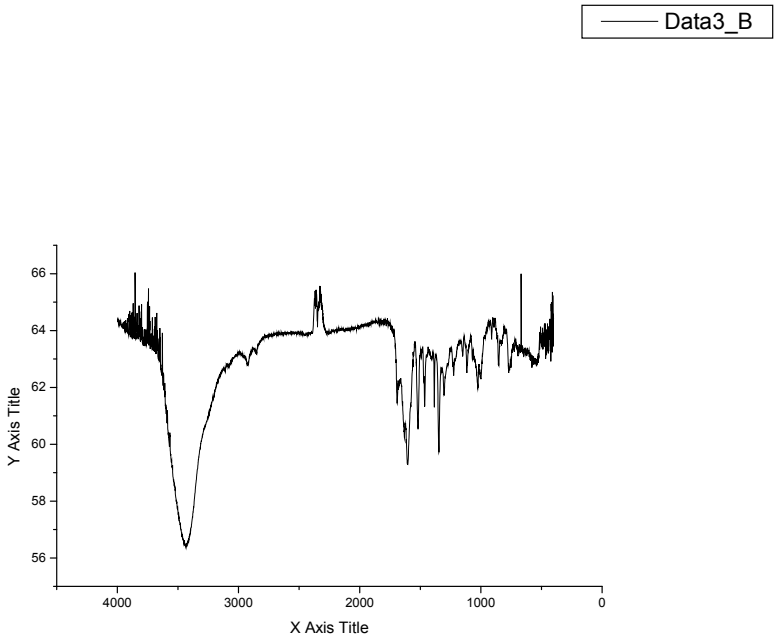
Appendix 6 B: ^{1}H NMR spectrum of Compound **44**



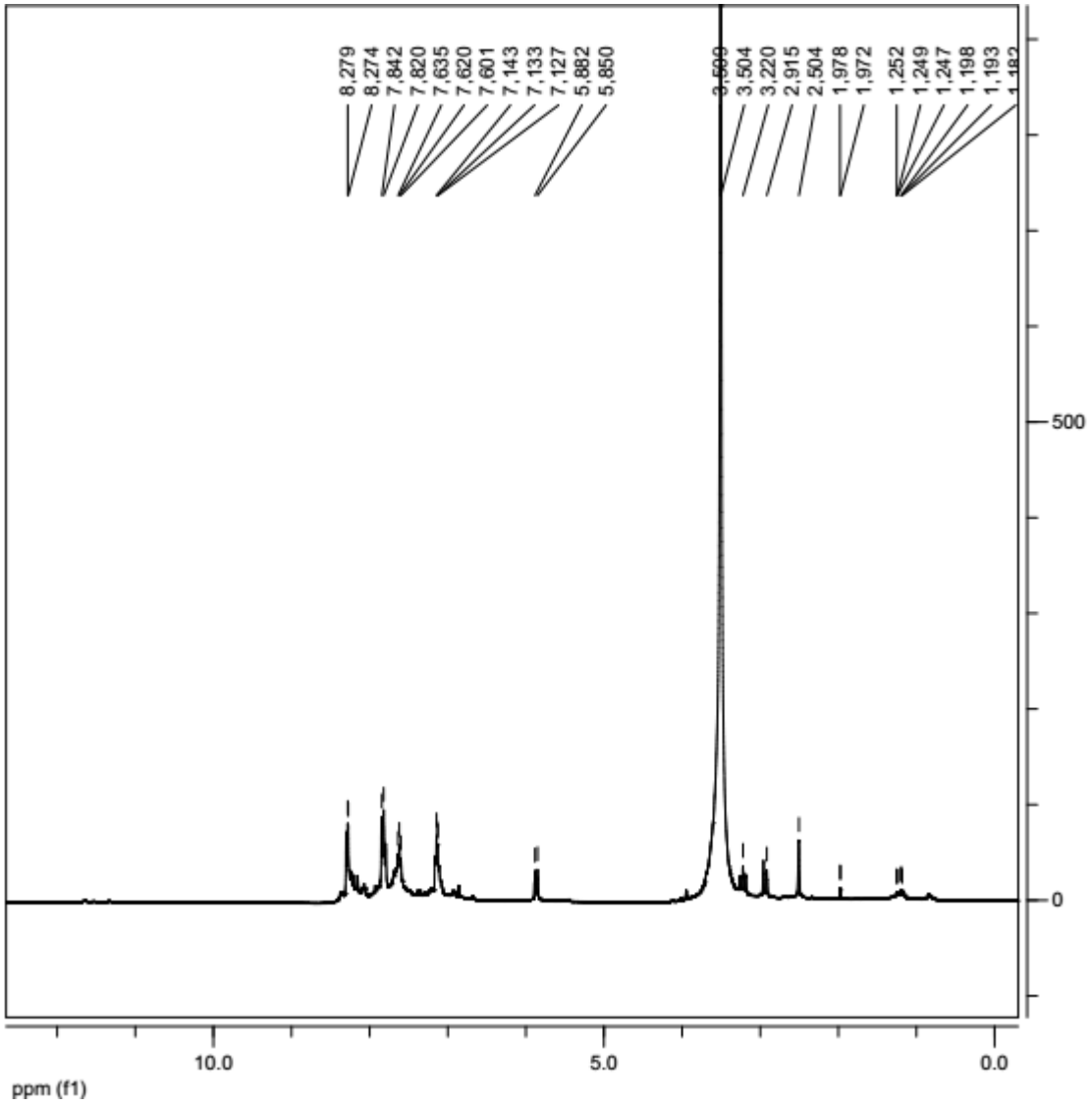
Appendix 6 C: ^{13}C NMR spectrum Compound **44**



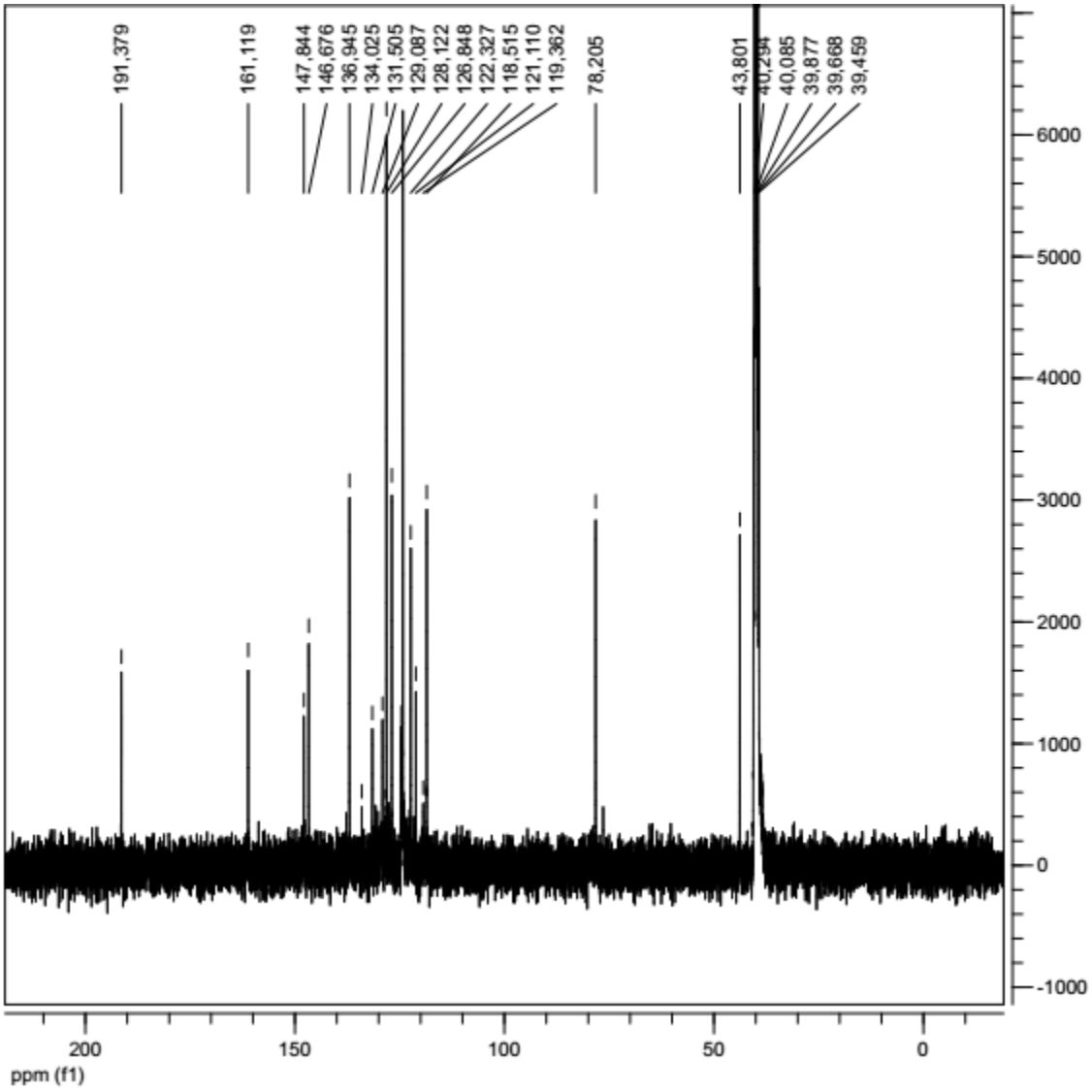
Appendix 7 A: FT-IR spectrum of Compound **45**



Appendix 7 B: : ^{1}H NMR spectrum of Compound **45**



Appendix 7 C : ^{13}C NMR spectrum Compound **45**



Appendix 8 : XRD spectrum of ZnO