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# Effect of Methoxy and Lipophilic Prenyl Substituents on Structure Activity Relationship of Some Synthesized Hydroxychalcones; Determined by Antimicrobial, Antioxidant and Cytotoxic Activity

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University of Rajshahi

A Thesis for the M. Phil. Degree

# Effect of Methoxy and Lipophilic Prenyl Substituents on Structure Activity Relationship of Some Synthesized Hydroxychalcones; Determined by Antimicrobial, Antioxidant and Cytotoxic Activity

*Submitted to the Department of Chemistry,*
*University of Rajshahi, in fulfillment of the requirement for the degree of*
*M. Phil. in chemistry*


**SUBMITTED BY**

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**Exam Roll No.: 12232**
**Registration No. : 2830**
**M. Phil. Session: 2012-2013**



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## DECLARATION

I, Mst. Marzina Khatun, hereby declare that this thesis has been written independently. It has not been published anywhere before. Due references and acknowledgement have been stated propery where materials of other Authors were used.

Marzina Khatun

Mst. Marzina Khatun

# CERTIFICATE

This is to certify that the thesis entitled "Effect of Methoxy and Lipophilic Prenyl Substituents on Structure Activity Relationship of Some Synthesized Hydroxychalcones; Determined by Antimicrobial, Antioxidant and Cytotoxic Activity" submitted to the Department of Chemistry, University of Rajshahi for the degree of Master in Philosophy (M.Phil.) embodied the result of a piece of bona fide research work carried out by Mst. MarzinaKhatun bearing Roll No. 12232, Registration No. 2830 under our supervision and guidance. No Part of the thesis has been submitted for any other degree.

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# Abstract

Natural chalcones and their derivatives, plant pigments are abundantly present in fern to higher plants especially in different parts of edible plants. They are belonging to flavonoid family and also considered to be main precursors in the biosynthesis of flavones, isoflavones and other biologically essential heterocycles. Flavonoids are the most common naturally occurring antioxidants and found ubiquitously in plants as pigments for flower coloration, in fruits and vegetables and play an ecological role in nature. A series of 2′- hydroxychalcones and their derivatives are found to have potential therapeutic agents against bacterial diseases. Chalcones are 1, 3-diphenyl-2-propene-1-one, in which two aromatic rings are linked by a three carbon α, β-unsaturated carbonyl system (-CO-CH=CH-). These possess conjugated double bonds and a completely delocalized π-electron system on both benzene rings. Molecules possessing such system have relatively low redox potentials and have a greater probability of undergoing electron transfer reactions. During the last sixty years synthetic as well as isolation works on the chalcones are being done throughout the world. They have been demonstrated to possess unique templates associated with very diverse application mainly biological and pharmacological activities such as antibacterial, antiviral, antioxidant, anti-inflammatory, antimutagenic, antiallergic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activities and inhibitory activities in several enzymes. Structure activity relationship (SAR) is also an important factor which has aroused considerable interest to the chemist. Different functional groups which are attached to the benzene ring of chalcones can be varied to enhance activity. Chalcones and its derivatives are still an object of sustained interest thus the present study has been extended by synthesizing eight substituted chalcones; 2′ - hydroxychalcone **(3a)**, 2′ - hydroxy - 4 - methoxychalcone **(3b)**, 2′ -

hydroxy - 2, 4, 5 - trimethoxychalcone **(3c)**, 2′, 5′ - dihydroxy - 2, 4, 6 - trimethoxychalcone **(3d)**, 4 - hydroxy - 3′, 4′, 5′ - trimethoxychalcone **(3e)**, 2′- hydroxy - 3′ - C - prenylchalcone **(4a)**, 2′- hydroxy - 5′ - C - prenylchalcone **(4b)**, 2′, 5′ - dihydroxy - 2, 4, 6 - trimethoxy - 3′ - C - prenylchalcone **(4c)** based on Claisen-Schimdt condensation method from substituted benzaldehyde and acetophenone in good yield. They have been characterized by spectral data. The potency of the development of new antibiotic or chemotherapeutic agents is highly based on *in vitro* antimicrobial screening. The synthesized chalcones **(3a-3e, 4a-4c)** were screened *in vitro* for their antibacterial activity against four pathogenic bacteria viz. *Bacillus caerius* ($G^+$, $B_1$), *Staphylococcus aureus* ($G^+$, $B_2$), *Eschericia coli* ($G^-$, $B_3$), *Agrobacterium Species* ($G^-$, $B_4$). The primary assay was performed by disc diffusion technique to classify the microorganism susceptible as well as resistant towards particular compounds. The bioactivity is expressed by the diameter of zone of inhibition in mm. Synthesized chalcones 3d, 3b, 3b, 4c in 250 μg/disc showed very high activity against $B_1$ (38), $B_2$ (40), $B_3$ (30), $B_4$ (40) respectively comparing to those of the standard drug (ciprofloxacin, C-50). The rest of the chalcones showed moderately good activity in different condition. In addition the synthesized chalcones **(3a-3e, 4a-4c)** were evaluated for *in vitro* antioxidant activity using diphenylpicrylhydrazyl (DPPH) model. Observation for antioxidant activity is expressed in terms of percent scavenging of DPPH radical and the inhibitory concentration 50% ($IC_{50}$) is lowest for structure **3d**, 0.923 (μg/mL) at DPPH conc. 0.02% indicating highest activity. The compound **3d** contains two phenolic -OH group and suppose to produce phenoxide free radical easily and stabilized by electronic group methoxy occupied ortho and para position of aromatic ring attached to benzaldehyde part. Other structures showed good antioxidant activity as compared to the standard, ascorbic acid,

$IC_{50}$ 0.08 (μg/mL). The antioxidant activity was also measured in another method using reducing power capacity. All the chalcones especially prenylated chalcone showed appreciable reducing activity and **3c** showed the highest value. Cytotoxic activities of the same compounds were undertaken *in vivo* by brine shrimp lethality test (BST) and expressed by lethal concentration 50%, ($LC_{50}$). The compound **3d** showed the least toxicity having $LC_{50}$ 71.75 (μg/mL) whereas $LC_{50}$ values less than 30 ppm for pure compounds were considered toxic. Increment the no. of methoxy and hydroxy group causes higher $LC_{50}$ values among the compounds **(3a-3d)**. As steric hindrance effect causes better tolerance by reducing their killing ability. On the contrary prenylation causes cell membrane permeability and enhance toxicity. In **3e** three methoxy groups are occupied ring A and in three adjacent carbon. It shows poor activities in any sort of biological screening undertaken.

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# Abbreviations

| | |
|---|---|
| IR | Infra red |
| IUPAC | International Union of Pure and Applied Chemistry |
| $IC_{50}$ | The Inhibitory Concentration 50% |
| $LC_{50}$ | Lethal concentration 50% |
| NMR | Nuclear Magnetic Rsonance |
| TLC | Thin Layer Chromatography |
| TMS | Tetramethylsilane |
| UV | Ultra Violet |

# List of Symbols and Units

| Symbol | Full Expression |
|---|---|
| asym | Asymmetric Stretching |
| bs | Broad Singlet |
| b.p. | Boiling point |
| β | Bronsted Coefficient |
| °C | Degree Celsius |
| cm | Centimeter |
| d | Doublet |
| dd | Doublet of doublet |
| δ | Delta, Chemical Shift in NMR Spectrum |

| | |
|---|---|
| g | Gram |
| hrs. | Hours |
| Hz | Hertz |
| $J$ | Coupling Constant |
| $\lambda_{max}$ | UV absorbance maxima |
| m | Multiplate |
| mL | Mili ($10^{-3}$) litre |
| mins. | Minutes |
| m.p | Melting point |
| MHz | Mega Hertz |
| μ | Micro ($10^{-6}$) |
| μL | Microlitre |
| μg | Microgram |
| nm | Nano Meter |
| ppm | Parts per million |
| s | Singlet |
| Str | Stretching |
| Sym | Symmetric Stretching |
| t | Triplet |

# CHAPTER ONE

# INTRODUCTION

# CHAPTER ONE

## *Introduction*

### 1. General Discussion on Flavonoid and related compounds

A natural product is a chemical compound or substance produced by a living organism, found in nature. In the broadest sense, natural products include any substance produced by life. Natural products can also be prepared by chemical synthesis and have played a central role in the development of the field of organic chemistry by providing challenging synthetic targets. The term natural product has also been extended for commercial purposes to refer to cosmetics, dietary supplements, and foods produced from natural sources without added artificial ingredients.

Within the field of organic chemistry, the definition of natural product is usually restricted to mean purified organic compounds isolated from natural sources that are produced by the pathways of primary or secondary metabolism. Within the field of medicinal chemistry, the definition is often further restricted to secondary metabolites. Secondary metabolites are not essential for survival, but nevertheless provide organisms that produce them an evolutionary advantage. Natural products sometimes have pharmacological or biological activity that can be of therapeutic benefit in treating diseases. As such, natural products are the active components not only of most traditional medicines but also many modern medicines. Furthermore, the structural diversity of natural products can be readily achievable by chemical synthesis and thus synthetic analogues can be prepared with improved potency. Hence natural products are often used as starting points for drug discovery.

The world of nature abounds in organic compounds of every conceivable structural class. The cells of living organisms, plants, fungi, bacteria, insects, other animals are the sites of intricate and complex biosyntheses that result in the fragmentation of many varieties of organic compounds of great practical importance to mankind. The structures of these naturally occurring compounds are often extremely complex and elucidation of their structures being a major challenge to organic chemists and biochemists alike. Structural and synthetic study of natural products constitute one of the most fascinating and fruitful field of study open to organic chemists.

Flavonoids, the most common naturally occurring antioxidants are found ubiquitously in plants as pigments for flower coloration, in fruits, vegetables and beverages.[1] They are prominent plant secondary metabolites that have been found in dietary components including fruits, vegetables, olive oil, tea, and red wine.[2] Chalcone is one of distinctive subclass of flavonoid being generated high interest in scientific study all over the world as it is associated with many different biological and pharmacological activities.[2, 3]

## 1.1 Chalcone

Benzylideneacetophenones (IUPAC name 1, 3- diphenyl-2-propen-1-one) constitute a class of naturally occurring pigments, which are often referred to as 'Chalcones' [Fig.1, also see Table-1]. The term was first coined by Kostanecki.[4] An interesting feature of Chalcones (polyhydroxylated) is that they serve as the precursors for the synthesis of another class of naturally occurring and widely distributed flavonoid pigments called 'Flavones'.

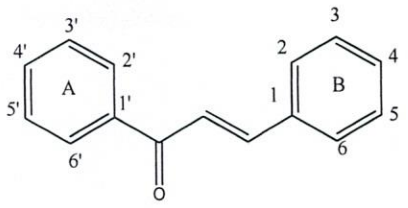


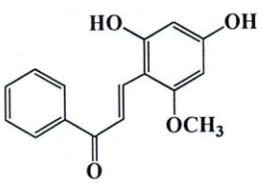

Figure 1: General structure of chalcone

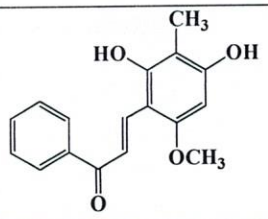
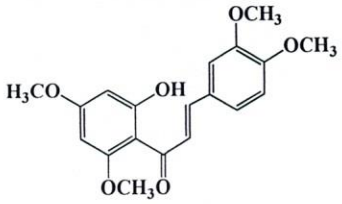
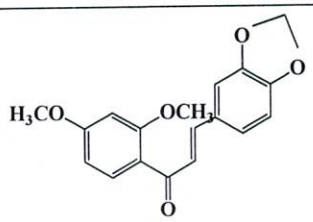
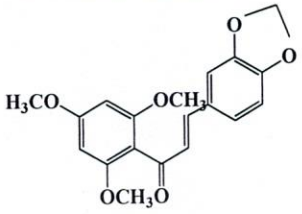
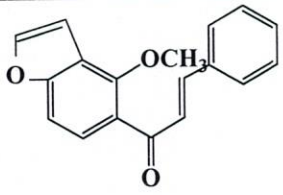
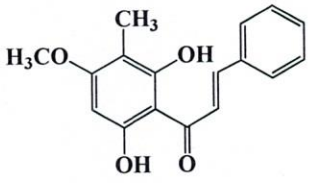
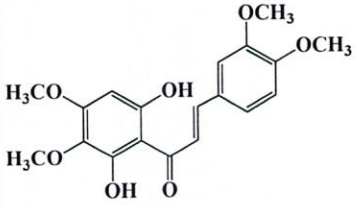
The chemistry of chalcones has generated intensive scientific studies throughout the world. Especially interest has been focused on the synthesis and biodynamic activities of chalcones. These compounds are also known as benzalacetophenone or benzylidene acetophenone. In chalcones, two aromatic rings are linked by an aliphatic three carbon chain. Chalcone bears a very good synthon so that variety of novel heterocyclic derivatives with good pharmaceutical profile can be designed. Chalcones are α, β-unsaturated ketone containing the reactive ketoethylenic group. These are colored compounds because of the presence of the chromophore -CO-CH=CH-, which deepens in the presence of other auxochromes, also used as non azo dye industrially.[5] Chalcones, bright yellow colored compounds are found most conspicuously in flowers and are known to play an ecological role in nature in relation to colors of the leaves and flowers of plants. Naturally occurring chalcones are all hydroxylated to a greater or lesser extent. Chemically chalcones are polyphenolic heteronuclear compounds include two aromatic ring bounds by an α, β unsaturated carbonyl group, a unique templates associated with very diverse application as antioxidant and antimicrobial activity.[2] During last sixty years synthetic as well as isolation works from plant material of chalcone being carried out throughout the world. Structural modification leads structure activity relationship (SAR) is also an important factor which has aroused considerable interest to the chemist.

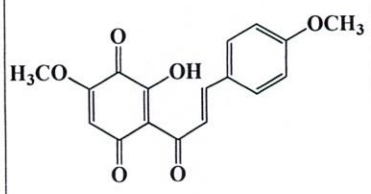
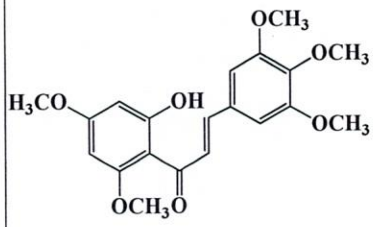
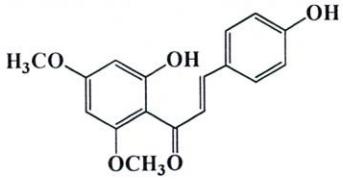
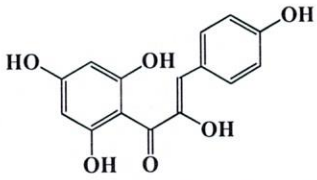
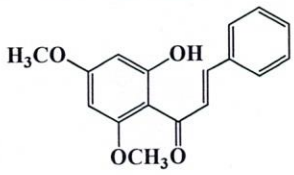
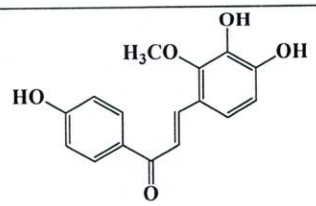
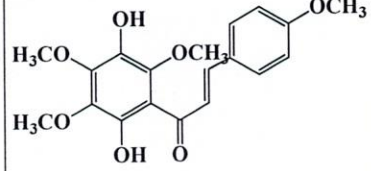
### 1.1.1 Natural chalcones and biological activity

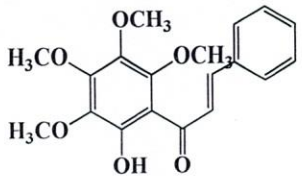
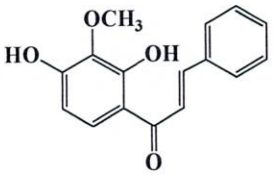
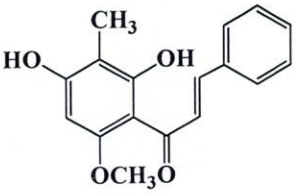
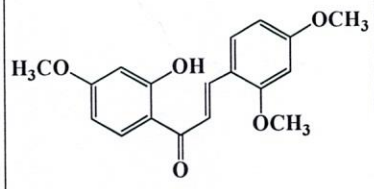
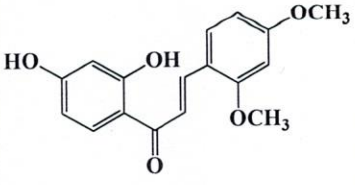
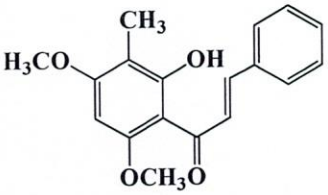
Natural Chalcones and their derivatives, plant pigments are abundantly present in fern to higher plants especially in different parts of edible plants. They are belonging to flavonoid family and also considered to be main precursors in the biosynthesis of flavones, isoflavones and other biologically essential heterocycles. [6] The chalcones have been found useful in elucidating structure of natural products like hemlock tannin, cyanomaclurin, ploretin, eriodictyol and homo eriodictyol, naringenin etc.[5] Chalcone and its derivatives have also attracted vast attention due to numerous pharmacological properties. The compounds with chalcone's skeleton have been reported to possess a broad spectrum of biological activities such as anti-inflammatory, chemopreventive, anticancer, antiproliferative, antimalarial, antiparasitary and anti-HIV activity. Some of their derivatives exhibit analgesic, antipyretic, antitumor and cytotoxic activity.[6] Chalcones are isolated and separated from natural resources or dye yielding plants and are known to be used as a natural dye, a good alternative to nitrogenous toxic azo dye. The list of some naturally occurring chalcones given by Mabry *et al.*[7] can be supplemented with new chalcones which have been isolated recently as tabulated below.

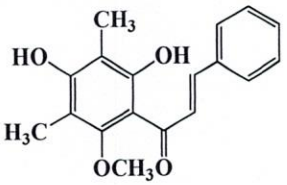
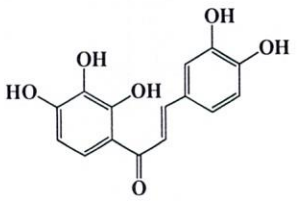
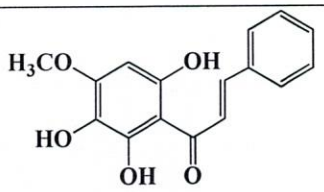
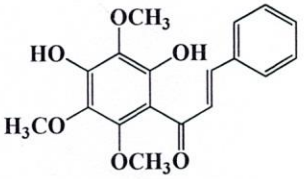
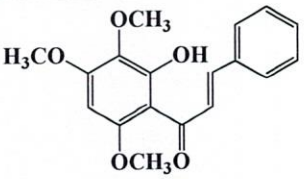
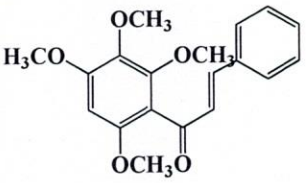
Table 1: The list of naturally occurring chalcones and their sources

| Name | Structure | Source | Reference |
|---|---|---|---|
| 2, 4- dihydroxy - 6 - methoxychalcone |   | *Dragon's blood Resin* | Cardillo, *et al.*, (1971), *J. Chem. Soc., C*, 3967-3970 |

| | | | |
|---|---|---|---|
| 2, 4- dihydroxy-3-methyl - 6 - methoxychalcone |  | -do- | -do- |
| 2′-hydoxy - 4′, 6′, 3, 4 tetramethoxy chalcone |  | *Pongamia Pinata* | Tanaka *et al.*, (1992), *Phytochem*, 31 (3), 998 |
| 2′, 4′- dimethoxy - 3, 4 -methylene dioxy chalcone |  | -do- | -do- |
| 2′, 4′, 6′- trimethoxy - 3, 4 - methylenedioxy- chalcone |  | -do- | -do- |
| 2′-methoxyfurono [2″, 3″: 4′, 3′]- chalcone |  | -do- | -do- |
| Triangularin |  | *Pityrogramma triangularis* | Mabry *et al.*, (1978) *Phytochemistry*, 17, 586 |
| 2′- hydroxy - 4′, 5′, 6′, 3, 4 - pentamethoxy chalcone |  | *Chromo-laena odorata* | Barua *et al.*, (1978) *Phytochemistry*, 17, 1807 |

| | | | |
|---|---|---|---|
| Quinochalcone-C |  | -do- | -do- |
| 2′- hydroxy - 3, 4, 5, 4′, 6′- pentamethoxy chalcone |  | *Merrillea caloxylon* | Fraser *et al.*, (1972), *Phytochemistry,* 11, 868 |
| Flavokawain-C |  | *Piper Methylsticum* | Dutta *et al.*, (1973), *Indian J. Chem.* 11, 509 |
| 2′, 4′, 6′, 4, α - pentahydroxychalcone |  | *Berchemia zeyheria* | Volsteedt *et al.*, (1973), *Tet. Lett.*, 1001 |
| Pashanone |  | *Didymoca rups pedicellantus* | Agarwal *et al.*, (1973), *Indian J. Chem.* 11, 9. |
| Licochalcone-B |  | *Glycyrrhiza glabra* | Saitoh *et al.*, (1975), *Tet. Lett.*, 50, 4461 |
| 3′, 6′- dihydroxyl- 2′, 4′, 5′, 4 - tetramethoxy - chalcone |  | *Flemingia strobilifera* | Bhatt (1975), *Indian J. Chem.* 13, 1105. |

| | | | |
|---|---|---|---|
| Kamakugiol |  | *Lindera erythrocarpa* | Liu *et al.*, (1975), *J. Pharm. Soc.* (Jpn), 19, 1114 |
| Lassein |  | *Lassea nitida* | Pederiva *et al.*, (1975), *An. Assoc. Quim. Argent*; 63, 85. |
| Aurentiacin |  | *Didymocarpus pedicellantus* | Chaudhury, *et al.*, (1976), *Phytochemistry*, 15, 224. |
| Cerasidin |  | *Prunus cerasus* | Parmer and Nagaranjan, (1977), *Phytochemistry*, 16, 1317 |
| Cerasin |  | -do- | -do- |
| 2′-hydroxy - 4′, 6′-dimethoxy-3′-methylchalcone |  | *Myrica gale* | Malterud, K. E., *et al.*, *Phytochemistry*, 16, 1805 |

| | | | |
|---|---|---|---|
| 2′, 4′ - dihydroxy - 6′-methoxy-3′, 5′-dimethyl chalcone |  | -do- | -do- |
| 2′, 3′, 4′, 3, 4 – penta-hydroxychalcone |  | *Albilizia adianthfolia* | Candy *et al.*, (1978) *Phytochem.*, 17, 1807. |
| Helilandin-B |  | *Helichrysum sutherlandii* | Bohlmann *et al.*, (1978), *Phytochem.*, 17, 1935. |
| Isodidymocarpin |  | *Didymo carpus pedicellata* | Bose and Chaudhury, (1978), *J. Indian Chem. Soc.*, 55, 1198 |
| 2′-hydroxy - 3′, 4′, 6′-trimethoxy-chalcone |  | *Popowia cauliflora* | Panichpol and Watermann, (1978), *Phytochem.*, 17, 1363. |
| 2′, 3′, 4′, 6′- tetra-methoxychalcone |  | -do- | -do- |

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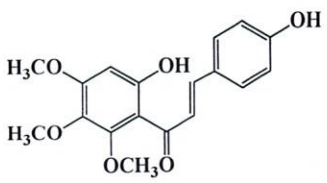
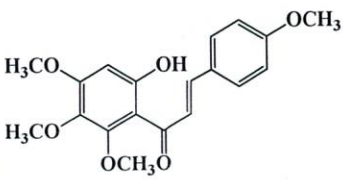
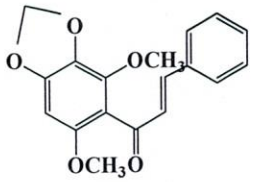
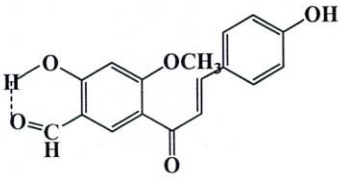
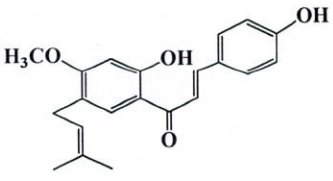
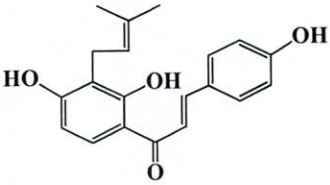
| 2′, 4- dihydroxyl- 4′, 5′, 6′- trimethoxychalcone |  | *Chromo- laena* | Barua *et al.*, (1978), *Phytochemistry*, 17, 1807. |
| 2′-hydroxy- 4, 4′, 5′, 6′- tetramethoxychalcone |  | -do- | -do- |
| Helilandin-A |  | *Helichrysum sutherlandii* | Bohlmann *et al.*, (1978), *Phytochem.*, 17, 1935. |
| Isonebara chalcone |  | *Psoralea corylifolia* | Gupta, *et al.*,(1980), *Phytochem.*, 19 (9) |
| 2′, 4-dihydroxy-3′-methoxy-5′-C-prenyl chalcone. |  | -do- | Bhalla, V. K, *et al.*, (1968) *Tett. Lett.*, 2401. |
| 2′, 4′, 4-trihydroxy-3′-C-prenylchalcone. |  | -do- | -do- |

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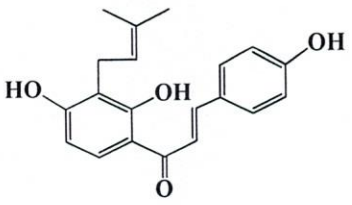
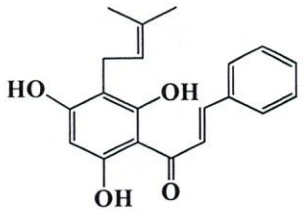
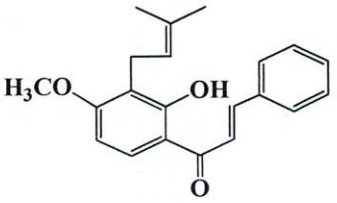
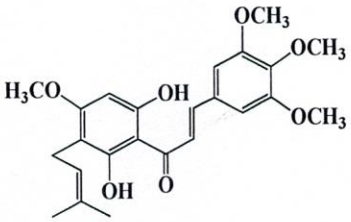
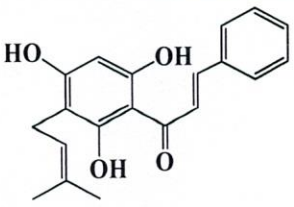
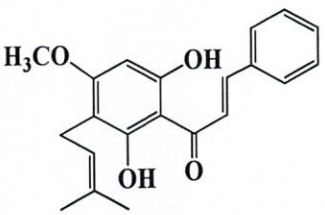
| 2′, 4′-dihydroxy-6′-methoxy-3′-C-prenylchalcone |  | Sophora angustifolia (Leguminosae) | Komatsu, M. *et al.*, (1970) Yakugaku. Zasshi, **90**, 463 |
| 2′, 4′, 6′-trihydroxy-3′-C-prenylchalcone |  | GlycyrrhiZaglabra (Leguminosae) | Kattav, N. S. *et al.*, (1972) Khim. Prir. Soedin, **8**, 805. |
| 2′-hydroxy-4′-methoxy-3′-prenyl chalcone. |  | Derris Sericea (Leguminosae) | Nascimento do, *et al.*, (1972) *Phytochem*, **11**, 3023. |
| 2′, 6′-dihydroxy-4′, 3, 4, 5-tetramethoxy-5′-C-prenylchalcone |  | Artocarpus Heterophyllus (Moraceae) | Dayal, R., *et al.*, (1974). *Indian J. Chem.*, **12** (8) 895. |
| 2′, 4′, 6′-trihydroxy-5′-C-prenylchalcone |  | Deriris rariflora. (Leguminosae) | Filho, R. B., *et al*, (1975). *Phytroche-mistry*, **14**, 261. |
| 2′, 6′-dihydroxy-4′-methoxy-5′-C-prenylchalcone |  | Deriris rariflora. (Leguminosae) | Filho, R. B., *et al*, (1975). *Phytroche-mistry*, **14**, 261. |

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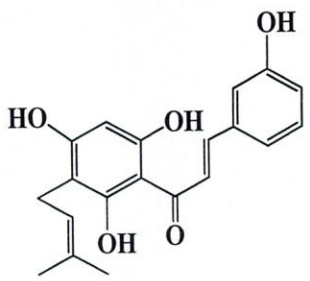
|---|---|---|---|
| 2′, 4′, 6′, 3-tetrahydroxy-5′-C-prenylchalcone |  | Sophora Tomentosa | Komatsu, M. *et al.*, (1978). *Chem. Pharm. Bull*, **26** (12) 3863. |

### 1.1.2 Synthetic chalcones and their importance

To improve the biological activities and to better understand the relation between chalcones intake and health outcomes, researches have been done continuously. Many analogues of naturally occuring chalcones introducing new substituent groups and their derivatives have been synthesized and reported. Chalcones possess conjugated double bonds and a completely delocalized π-electron system on both benzene rings. Molecules possessing such system have relatively low redox potentials and have a greater probability of undergoing electron transfer reactions.[8] Furthermore chalcones are important intermediate in many addition reactions of nucleophiles owing to inductive polarization of carbonyl group at the β position.[6] Besides these compounds have broad industrial application as artificial sweeteners, scintillator polymerization catalyst, fluorescent whitening agent, organic brightening agent, stabilizer against heat, visible light, ultraviolet light. So the chemistry of chalcones has generated intensive scientific studies throughout the world specially interest has been focused on the synthesis and biodynamic activities of chalcones. There are several methods for the syntheses of chalcones have been investigated. The major synthetic schemes are including Claisen-Schmidt condensation, Suzuki coupling reaction, Wittig reaction, Friedel-Craft acylation, photo-Fries rearrangement, carbonylative Heck reaction also unconventional method via

microwave irradiation. [6] On the Suzuki coupling reaction, benzoyl chloride was reacted with phenyl vinyl boronic acid using anhydrous toluene and catalyzed by tetrakis (triphenylphosphine) and cesium carbonate as base, gave 3′, 4′, 4-trimethoxychalcone. In photo-Fries rearrangement, phenyl cinnamate undergoes rearrangement and provided two chalcones molecules, ortho and para-hydroxychalcones. In carbonylative Heck reaction, chalcone was produced by reaction of arylhalide and styrene that added by carbon monoxide and catalyzed by palladium and a phosphine-amine ligand, N-heterocyclic carbene. [9]

### 1.1.3 Prenylated chalcones and their biological importance

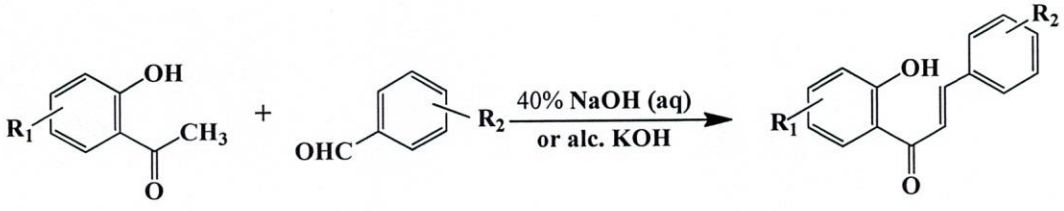
3-methyl-but-2-en-1-yl substituent is named as prenyl functional group. The prenylchalcones are a group of natural products found in fruits, vegetables, nuts, seeds and flowers as well as in teas and wines are important constituent of human diet. Prenyl group is lipophilic by nature and assume to facilitate attachment to cell membrane. Thirty seven naturally occuring prenyl compounds were investigated for their inhibitory activities on the mouse brain monoamino oxidase (MAO) *in vitro*. Three prenylchalcones Lupinifolinol ($IC_{50}$, 1μmol), Prenylnaringenin ($IC_{50}$, 4 μmol) and Topazolin ($IC_{50}$, 5 μmol) were judged to be very active.[5] Thus in the present study we are interested to extend our studies by the introduction of hydrophobic functionality like prenyl (3-methyl-but-2-en-1yl) group at benzene nucleus moiety of chalcone which may give an important information about positional effect of prenyl group and its role as auxochromes to show enhance and stabilized color effect also to study its bioactivity.

## 1.2 Synthesis of chalcones through various methods

### 1.2.1 The Claisen-Schmidt reaction [10]

The Claisen-Schmidt condensation is a classical method to synthesize chalcones which employs cross aldol condensation of appropriate aldehyde and ketone by base catalyzed (such as NaOH, KOH, $Ba(OH)_2$, $NaCO_3$, hydrotalcities, etc.) or acid catalyzed (such as HCl, $BF_3$, $B_2O_3$, *p*-toluenesulfonic acid, $SOCl_2$, etc.) and then followed by dehydration.[8]

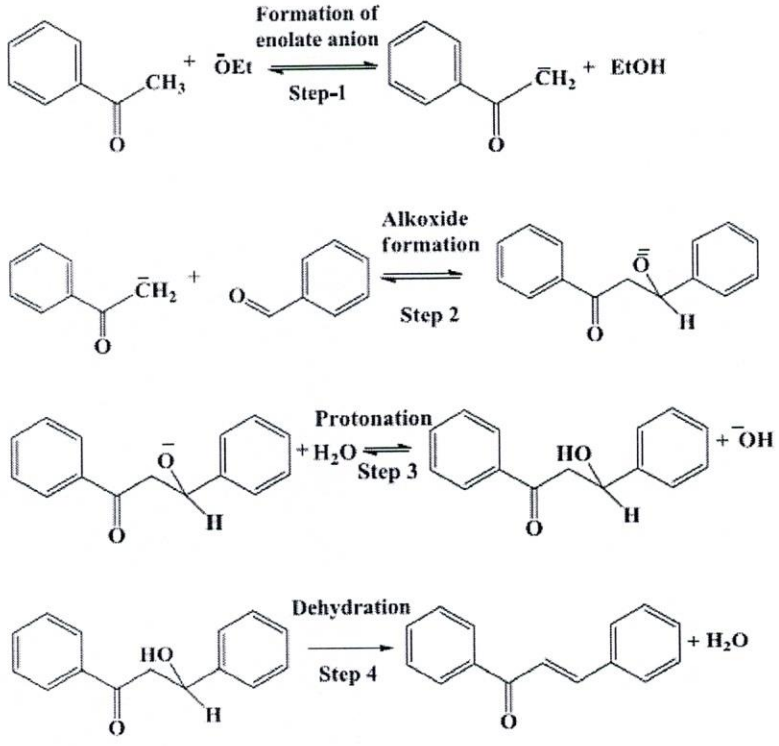
The Claisen-Schmidt condensation (scheme 1) between acetophenone and benzaldehyde derivatives is an important C-C bond forming reaction which allows α, β-unsaturated ketone such as chalcone to be obtained. It is the most frequently used means of establishing the $C_6$-$C_3$-$C_6$ flavonoids nucleus owing to the availability of starting materials such as 2-hydroxyacetophenone and benzaldehyde derivatives to obtain a 2′-hydroxychalcone. Chalcone (Figure 1) bears A-ring substituent provided by the acetophenone (indicated as $R_1$) and B-ring substituent provided by benzaldehyde (indicated as $R_2$). The classical Claisen-Schmidt reaction is routinely carried out using aqueous sodium or potassium hydroxide, otherwise ethanolic potassium hydroxide at 50 °C over a period of several hrs. The benzaldehyde derivative is often used in slightly more than equivalent amounts. The extensive conjugation of the products causing absorbance of light in the visible region, leading this yellow color.




Scheme 1: The Claisen-Schmidt condensation reaction scheme.

### 1.2.1.1 Mechanism of chalcone formation [6, 11, 12]

Sodium hydroxide in an alcoholic solvent which used in this experiment leads to fast and reversible formation of intermediate compound. For that reason, it was used to increase the reaction rate of Claisen-Schmidt condensation. Base removed acidic alpha hydrogen from acetophenone, producing a resonance–stabilized enolate ion. This enolate ion then attacked aldehyde molecule, yielding a neutral condensation product and followed by dehydration to generate chalcones in good yields.

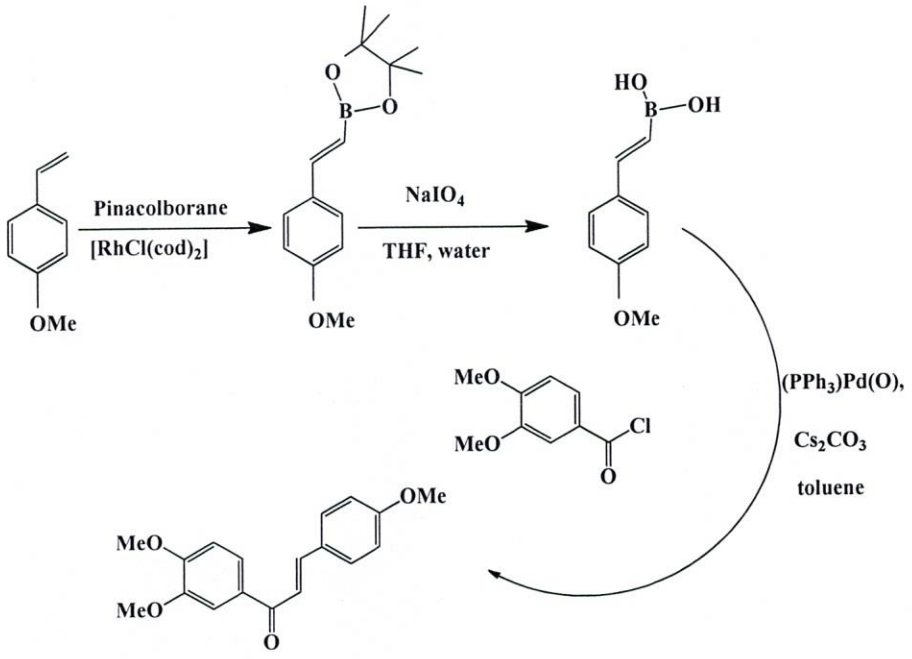



Scheme 2: Mechanism for the base-catalyzed Claisen-Schmidt condensation between acetophenone and benzaldehyde

### 1.2.2 Synthesis of chalcone via Suzuki coupling reaction [8]

An efficient synthesis of chalcones was carried out based on the Suzuki coupling reaction between benzoyl chlorides and phenyl vinyl boronic acid.

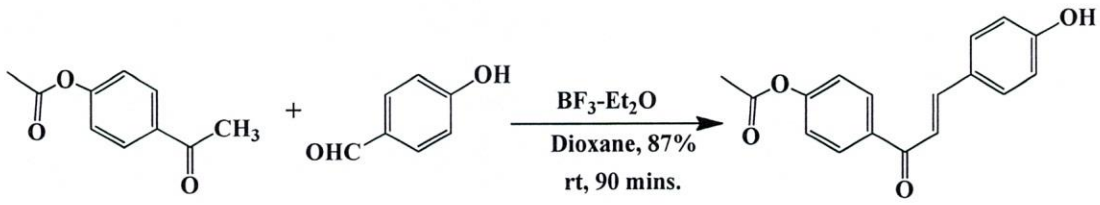
Phenyl vinyl boronic acid was prepared by dehydrogenative borylation of para-methoxystryne by pinacoleborane oxidative addition-dehydrogenation, catalyzed by the rodium complex, $RhCl(cod)_2$ to give paramethoxy phenyl ethenylboronic acid pinacol ester (Scheme 3)




Scheme 3: Synthesis of chalcone via Suzuki coupling reaction

### 1.2.3 Synthesis of chalcone Using Borontrifluoride-etherate

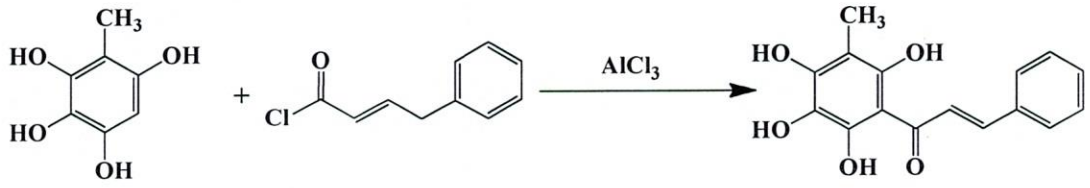
Narender and Reddy [13] developed a new methodology by using $BF_3$-$Et_2O$ to synthesize several substituted chalcones. The advantages of this method over the existing methods are high yield, simple work-up, short reaction times, no side reactions and separation is needed to get the products. This method is applicable for reactions involving liquid reactants which have base sensitive functional groups such as esters and amides. A condensation between 2-acylated acetophenone and the respective aromatic aldehyde produce 2-acylated chalcone in high yields by using $BF_3$-$Et_2O$ as shown in Scheme 4




Scheme 4: Synthesis of chalcone using Borontrifluoride-etherate

### 1.2.4 Synthesis of chalcone via Friedel-Crafts acylation [8]

Besides the Claisen-Schmidt reaction, chalcone can also be synthesized by direct Friedel-Crafts acylation of a phenol. In this approach the phenol becomes the A-ring while the acylation agent provides both the B-ring and the three carbon bridge to form $C_6$-$C_3$-$C_6$ unit. Friedel-Crafts acylation of 2, 4-dimethyl-1, 3, 5-triolbenzene with 3-phenylpropionyl chloride gave 2′, 4′, 6′-trihydroxy-3′, 5′-dimethylchalcone as shown in Scheme 5.

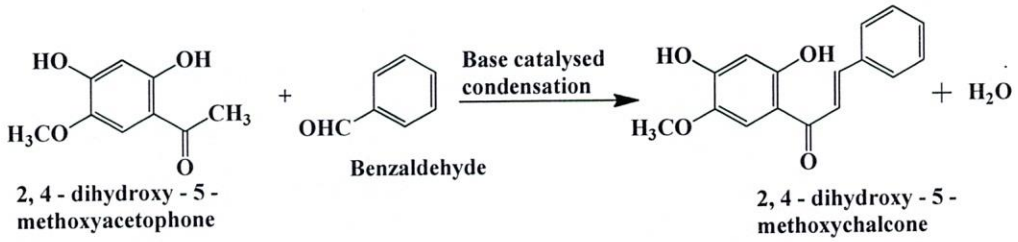



Scheme 5: Synthesis of chalcone via Friedel-Crafts acylation

### 1.2.5 Synthesis of chalcones by using some modified methods

The substituted benzylidene acetophenones have likewise been obtained by condensing the appropriately substituted acetophenones with substituted benzaldehydes in the presence of alkali. The 2-hydroxyacetophenones on acid or base catalyzed condensation with aldehydes yield either a chalcone or a flavones or a mixture of both. In Kostanecki's method [4], the starting materials are suitably substituted 2- hydroxyactophenone (furnishing A-ring) and benzaldehyde (furnishing B-ring) which are condensed in the

presence of alcoholic costic potash to give chalcones. If only 2′-hydroxyl group of the chalcone is free, the condensation proceeds smoothly in 10% alcoholic costic potash in as high yield as 85% but in the case of compounds with a large number of free hydroxyl groups, higher concentration of alcoholic costic potash (50%-60%) is required. The yields are highest when alcoholic costic potash (50%-60%) is used at 0-20 °C for 15-72 hours and when starting materials are methoxylated or benzylated. But for polyhydroxy acetophenones good yields are obtained only when the condensation is performed at 0 °C. Chaudhury *et al.*[14] synthesized flemichapparin by the base condensation of 2, 4-dihydroxy-5 methoxyacetophenone with benzaldehyde at 0-5 °C for 8 days.




Scheme 6: Base catalyzed condensation for synthesis of chalcone.

A modification of the Kostanecki's method was worked out by Russel and Todd. [15] It involves the condensation of benzoylated derivatives of 2-hydroxyacetophenone with benzoylated derivatives of 2-hydroxy-benzaldehyde in the presence of dry hydrogenchloride at 0 °C followed by hydrolysis with alkali to the free chalcone. Thus 2, 4-dibenzoyloxy acetophenone condenses with dibenzoyloxy protocatechuic aldehyde to give the tetrabenzoyloxy chalcone which on hydrolysis yields 2′, 4′, 3, 4-tetrahydroxy chalcone. The synthesis of some chalcones has been accomplished by using sodium hydride as the base for the condensation. This method gives good yields even if the hydroxyl groups are not protected. Thus 2′, 3, 4-trihydroxy-3′, 4′, 5′-trimethoxychalcone has been synthesized by

Stout and Stouf[16] by using sodium hydroxide as the base. Chalcone has also been synthesized by irradiating a solution of phenyl- cinnamate in methanol, with UV light (253 nm) for 1.5 hrs. under nitrogen atmosphere. Synthesized two isomeric chalcones were reported by this method. [17] Synthesis of chalcones using organo phosphorus activated reagent [18] has been reported. Lupi and co-workers [19] while synthesizing various prenylated and chromeno chalcones under alkaline conditions have noticed the yield variation by changing the temperature, solvent and using different bases for condensation. Sodium hydroxide, sodium ethoxide and piperidine were used as bases in solvents like absolute alcohol, dry DMSO, anhydrous dioxane at a temperature varying between 25-100 °C for a period ranging from 1-30 hrs. The best yield was obtained when piperidine was used as a base in absolute alcohol for a period of 6 hrs. at a temperature between 60-70 °C using a little excess of aldehyde than the stoichiometric amount. Obera *et al.* [20] have reported the synthesis of 2′, 3′, 4′ ,6′, 4-pentahydroxy chalcone is very good yield by the base catalyzed condensation of 2, 3, 4, 6-tetramethoxy acetophenone and methoxybenzaldehyde followed by demethoxy methylation using methanolics hydrochloric acid of the many methods available for protection of hydroxyl groups alkylation with methoxy methyl chloride appeared to be attractive since excellent yields were obtained by heating in acid medium.

Nevertheless, many of these methods require prolonged reaction times, gave poor yields, low selectivity and also suffered from harsh reaction condition for instance toxic reagent, strong acidic and strong basic condition. From many literatures, Claisen-Schmidt condensation still occupies leading positions for synthesizing chalcone, since that method is very simple, inexpensive and easy to conduct.

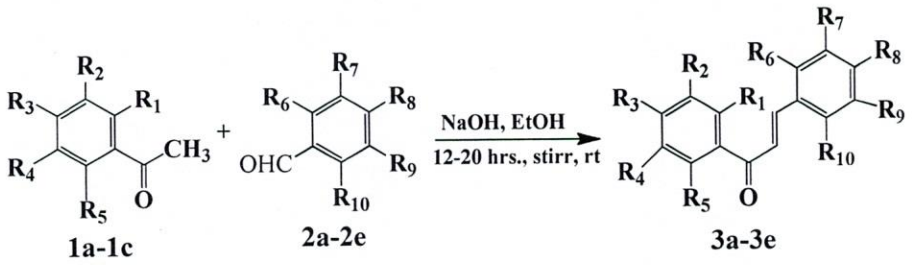
## 1.3 Aim of the present study [5, 21, 22]

Natural chalcones and their derivatives, plant pigments are abundantly present in fern to higher plants especially in different parts of edible plants. They are belonging to flavonoid family and also considered to be main precursors in the biosynthesis of flavones, isoflavones and other biologically essential heterocycles. Flavonoids are the most common naturally occurring antioxidants and found ubiquitously in plants as pigments for flower coloration, in fruits and vegetables and play an ecological role in nature. A series of 2′- hydroxychalcones and their derivatives are found to have potential therapeutic agents against bacterial diseases. Chalcones are 1, 3-diphenyl-2-propene-1-one, in which two aromatic rings are linked by a three carbon α, β-unsaturated carbonyl system (-CO-CH=CH-). These possess conjugated double bonds and a completely delocalized π-electron system on both benzene rings. Molecules possessing such system have relatively low redox potentials and have a greater probability of undergoing electron transfer reactions. During the last sixty years synthetic as well as isolation works on the chalcones are being done throughout the world. They have been demonstrated to possess unique templates associated with very diverse application mainly biological and pharmacological activities such as antibacterial, antiviral, antioxidant, anti-inflammatory, antimutagenic, antiallergic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activities and inhibitory activities in several enzymes. Structure activity relationship (SAR) is also an important factor which has aroused considerable interest to the chemist. Different functional groups which are attached to the benzene ring of chalcones can be varied to enhance activity. Chalcones and its derivatives are still an object of sustained interest. Besides chalcones are isolated and separated from natural resources or dye yielding plants and is known to be used as a natural dye, a good alternative to

nitrogenous toxic azo dye. Synthesis of new naturally occuring organic compounds with basic skeleton of chalcones, flavones and oxygenated flavones and their biological activity were reported by this research groups for long.[5, 21, 22] In the present study we are interested to extend our studies by the introduction of hydrophilic functionality like hydroxy, methoxy and lipophilic functionality like prenyl (3-methyl-but-2-en-1yl) group at benzene nucleus moiety of chalcone which may give an important information about positional effect of hydroxy, methoxy and prenyl group and its role as auxochromes to show enhance and stabilized color effect also to study its bioactivity.

## 1.4 Statement of the present study

In the present study eight substituted chalcones were synthesized: 2′-hydroxychalcone **(3a)**; 2′-hydroxy- 4-methoxychalcone **(3b)**; 2′ - hydroxy - 2, 4, 5 - trimethoxychalcone **(3c)**; 2′, 5′- dihydroxy - 2, 4, 6 - trimethoxychalcone **(3d)**; 4 - hydroxy - 3′, 4′, 5′ - trimethoxychalcone **(3e)**; 2′- hydroxy - 3′ - C - prenylchalcone **(4a)**; 2′- hydroxy - 5′ - C - prenylchalcone **(4b)**; 2′, 5′ - dihydroxy -2, 4, 6 - trimethoxy - 3′ - C - prenylchalcone **(4c)** using Claisen-Schmidt condensation method, a ciassical, very simple, inexpensive and easy to conduct synthetic strategy. The chalcones **(3a-3e and 4a-4c)** were obtained from their corresponding acetophenones **(1a-1c)** as well as aldehydes **(2a-2e)** in presence of NaOH/EtOH as shown in scheme 7. The structures of the above compounds were assigned on the basis of spectral data; UV, IR, $^{1}H$ and $^{13}C$ NMR together with their elemental analysis.




1a-1c 2a-2e 3a-3e

1a) $R_1$=-OH, $R_2$=$R_3$=$R_4$ = $R_5$=H
1b) $R_1$=$R_4$=-OH, $R_2$= $R_3$=$R_5$=H
1c)$R_1$=$R_5$=H,$R_2$=$R_3$= $R_4$=-$OCH_3$

2a) $R_6$=$R_7$= $R_8$= $R_9$=$R_{10}$=H
2b) $R_6$=$R_7$= $R_9$=$R_{10}$= H $R_8$= -$OCH_3$
2c) $R_6$= $R_8$=$R_9$=-$OCH_3$, $R_7$= $R_{10}$=H
2d) $R_6$=$R_8$=$R_{10}$=-$OCH_3$, $R_7$=$R_9$=H
2e)$R_6$=$R_7$=$R_9$=$R_{10}$=H $R_8$=-OH

3a) $R_1$= -OH, $R_2$=$R_3$=$R_4$=$R_5$=$R_6$= $R_7$=$R_8$=$R_9$=$R_{10}$=H
3b) $R_1$= -OH, $R_2$=$R_3$=$R_4$= $R_5$ =$R_6$=$R_7$=$R_9$=$R_{10}$=H,$R_8$=-$OCH_3$
3c) $R_1$= -OH, $R_2$=$R_3$=$R_4$=$R_5$= $R_7$ =$R_{10}$=H, $R_6$=$R_8$=$R_9$=-$OCH_3$
3d) $R_1$=$R_4$=-OH, $R_2$=$R_3$=$R_5$ =$R_7$=$R_9$ = H,$R_6$=$R_8$=$R_{10}$=-$OCH_3$
3e) $R_1$=$R_5$=$R_6$=$R_7$=$R_9$=$R_{10}$=H, $R_2$=$R_3$=$R_4$=-$OCH_3$, $R_8$=-OH

Scheme 7: Synthetic route for five synthesized chalcones **(3a-3e)** from their corresponding acetophenones **(1a-1c)** and aldehydes **(2a-2e)**

2-methyl-but-3-en-2-ol in dry dioxane was treated with chalcone **(3a, 3d)** followed by gradual addition of boron trifloride-etherate to get prenylated chalcone **(4a, 4b, 4c).**

The potency of the development of new antibiotic or chemotherapeutic agents is highly based on *in vitro* antimicrobial screening. The synthesized chalcones **(3a-3e, 4a-4c)** were screened *in vitro* for their antibacterial activity against four bacteria viz. *Bacillus caerius* ($G^+$, $B_1$), *Staphylococcus aureus* ($G^+$, $B_2$), *Eschericia coli* ($G^-$, $B_3$), *Agrobacterium Species* ($G^-$, $B_4$). The primary assay was performed by disc diffusion technique to classify the microorganism susceptible as well as resistant towards particular compounds. The bioactivity is expressed by the diameter of zone of inhibition in mm. Synthesized chalcones **3d, 3b, 3b, 4c** in 250µg/disc showed very high activity against $B_1$ (38), $B_2$ (40), $B_3$ (30), $B_4$ (40) respectively comparing to those of the standard drug (ciprofloxacin, C-50). The rest of the chalcones showed moderately good activity in different

condition. In addition the synthesized chalcones **(3a-3e, 4a-4c)** were evaluated for *in vitro* antioxidant activity using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) model. Observation for antioxidant activity is expressed in terms of percent scavenging of DPPH radical and the inhibitory concentration 50% ($IC_{50}$) is lowest for structure **3d**, 0.923 (µg/mL) at DPPH conc. 0.02% indicating highest activity. The compound **3d** contains two phenolic -OH group and suppose to produce phenoxide free radical easily and stabilized by electronic group methoxy occupied ortho and para position of aromatic ring attached to benzaldehyde part. Other structures showed good antioxidant activity as compared to the standard, ascorbic acid, $IC_{50}$ 0.08 (µg/mL). The antioxidant activity was also measured in another method using reducing power capacity. All the chalcones especially prenylated chalcone showed appreciable reducing activity and **3c** showed the highest value. Cytotoxic activities of the same compounds were undertaken *in vivo* by brine shrimp lethality test (BST) and expressed by lethal concentration 50%, ($LC_{50}$). The compound **3d** showed the least toxicity having $LC_{50}$ 71.75 (µg/mL) whereas $LC_{50}$ values less than 30 ppm for pure compounds were considered toxic. Increment the no. of methoxy and hydroxy group causes higher $LC_{50}$ values among the compounds **(3a-3d)**. As steric hindrance effect causes better tolerance by reducing their killing ability. On the contrary prenylation causes cell membrane permeability and enhance toxicity. In **3e** three methoxy groups are occupied ring A and in three adjacent carbon. It shows poor activities in any sort of biological screening undertaken.

# CHAPTER TWO

# EXPERIMENTAL

# CHAPTER TWO

## *Experimental*

### 2.1 Section A; General Methods [5, 21, 22]

#### 2.1.1 Melting Point Apparatus

Melting points are uncorrected and all solid samples were recorded on an electro-thermal melting point apparatus (EDU-LAB SRL MLTP 06018020).

#### 2.1.2 Spectral techniques (UV, IR, NMR)

**(i) UV:** Chemical reactions were monitored by thin-layer chromatography and spots were visualized with UV light (in nm) using UV-180, SHIMADZU, the spectrophotometer SHIMADZU UV-160, was used to measure absorbance maxima and antioxidant activity both by DPPH method and reducing capacity.

**(ii) IR:** Infrared spectra were recorded on a SHIMADZU "FTIR-8400 spectrophotometer using KBr pellets for solid samples in the Central Science Laboratory, University of Rajshahi, Rajshahi, Bangladesh and the characteristics peaks are expressed in $cm^{-1}$.

**(iii) NMR:** $^{1}$HNMR and $^{13}$C-NMR spectrum was recorded on a BRUKER 400 MHz NMR Spectrometer in $CDCl_3$ and $d_6$-DMSO solution at the Laboratories of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka and BRUKER 500 MHz SPOECTROMETER University Malaysia Pahang. Chemical shifts were quoted on the δ scale relative to tetra methyl silane (TMS, δ = 0) as an internal standard. The signals are

abbreviated s, d, t, q, m, dd, refer to singlet, doublet, triplet, quartet, multiplet, doublet of doublet respectively. The coupling constant is indicated by *J* and is given in Hz.

### 2.1.3 Thin Layer Chromatography (TLC)

The technique of thin layer chromatography (TLC) was extensively used to monitor the progress of the reaction. TLC plates (0.25 mm thickness) were prepared by spreading a layer of silica gel (60 $GF_{254}$, E. MERCK) on clean and thoroughly dried glass plates (2g gel was mixed with 4 mL of distilled water for each plate of size (5cm×20 cm). These were activated by drying at 110 °C before use.

#### 2.1.3.1 Development of TLC plates

**i) Using iodine tank:** The chromatograms were developed in iodine vapor by placing the plate in the iodine tank.

**ii) Using UV light:** The presence of the pure compound as a single spot or a mixture can be identified by several spots in UV light.

**iii) Using spraying agent:** The spots were appeared if the plate was spread with the following spraying reagents.

(i) 2% ferric chloride solution in water is used as a spraying reagent.

(ii) $H_2SO_4$ (50%) is also used as a spraying reagent and heating the plate.

$R_f$ value is measured from the TLC plate. $R_f$ means retardation factor which measures the velocity of movement of the solute zone relative to that of the solvent front.

$$R_f = \frac{\text{Distance (cm) travelled by solute}}{\text{Distance (cm) travelled by solvent}}$$

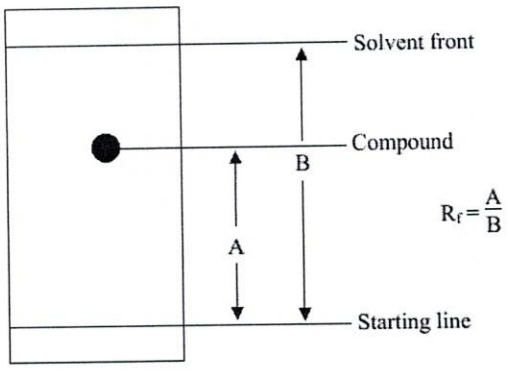



Figure 2: A plate for a calculation of $R_f$ value

Usually, the $R_f$ value is constant for any given compound and it corresponds to a physical property of that compound.

### 2.1.4 Preparative Thin Layer Chromatography (PTLC)

The preparative glass plates (20 cm × 20 cm) were cleaned and dried. The plates were coated with silica gel slurry (silica gel 60 mesh, E. MERCK, 16 g silica gel was mixed with 32 mL of distilled water) with the help of spreader to yield a coating of 0.5 mm thickness. The plates were left at room temperature until their surface become completely dry, the plates were then heated at 120 °C in an electrical oven before use to increase activation. The solutions of compounds to be purified were applied with special type of thin glass capillaries at about 3 cm from bottom of the plates. After drying, the plates were then placed vertically with the spotted end placed downward in chromatographic tanks so that the spotted end of the compounds remained above the solvent. The plates were removed from the tanks when the solvent front reached almost to the upper edge of the plates (1cm far from the upper edge). The plates were usually developed in appropriate solvent and were subsequently dried in the air. The plates were then viewed under UV light or the sides of the plates were exposed to iodine vapor to locate the position of

the compounds. The zones bearing the compounds were scratched off from the plates and subsequently extracted with suitable solvent.

### 2.1.5 Column Chromatography

Column Chromatography is a very useful technique for the separation of pure compounds from its mixture. For column chromatography silica gel (Kisel gel-66, 70-230 mesh, ASTM MERCK) used as adsorbent and solvent like n- hexane, petroleum ether, benzene, acetone, ethyl acetate etc. at different proportion was used as eluent. The column was prepared by slurry method silica gel being the stationary phase. The column was thoroughly cleaned and rinsed with acetone and dried. It was then clamped properly and rinsed with solvent used in the preparation of silica gel slurry and cotton plug was fitted at the bottom. The column was half filled with appropriate solvent and the slurry was then poured into it, so that the packing was compact and uniform. Air bubble was avoided by packing the column as quickly as possible. The column was allowed to settle for an hr. The mixture of the compound was completely dried and then was dissolved in eluting solvent and was carefully placed on the surface of the column, and eluted with desired solvent system. In case of polar compounds, the mixture was dissolved in suitable polar solvents and absorbed in small amount of silica gel in a flask. The solvent was removed completely under vacuum by rotary evaporator. From silica gel and this silica gel with absorbed material was put on the surface of the silica gel column. The compounds were then collected as distinct bands. The purity of each band was further checked by TLC examination.

### 2.1.6 Reagent and Solvent and their Purification

Starting materials, substituted acetophenone (1a, 1c), substituted benzaldehyde (2a-2e), resorsinol, $BF_3$-etharate were purchased from Sigma Aldrich and used without further purification.

The reagents and solvents were purified and dried before use where necessary and the rest were used as such from the bottle. All solutions in water, immiscible solvents which has been contact with water were dried over anhydrous sodium sulphate and magnesium sulphate, prior to evaporation. Solvent was usually removed with the help of a vacuum rotary evaporator. Dry hydrogen chloride gas (HCl) was produced by pouring conc. $H_2SO_4$ over a mixture of sodium chloride and ammonium chloride (5:1) moistened with conc. HCl gas thus produced were dried by passing them through the troughs containing good quality of conc. $H_2SO_4$. Pet. ether and benzene were dried by sodium wire and or phosphorus pentaoxide ($P_2O_5$). Acetone and dioxane were dried by keeping over anhydrous $K_2CO_3$ and distilling the solvents. The liquid reagents were sometimes distilled by following standard method before use.

**Acetone:** To 500 mL of acetone anhydrous $K_2CO_3$ (150 g) was added and it was kept overnight. Then acetone was decanted off form $K_2CO_3$ and distilled to get pure acetone and preserved in an air tight container.

**Acetic Acid:** About 100 mL of acetic acid was taken in a round bottomed flask and 50 g of $CaCl_2$ (anhydrous) was added to it. After 6 hrs. the acetic acid was decanted and it was distilled and collected at reduced pressure by maintaining its b.p.

**Diethyl ether ($Et_2O$):** Metallic sodium wire was added in diethyl ether and the mixture was kept overnight and was distilled over $P_2O_5$ at 35 °C.

**Chloroform ($CHCl_3$):** At first chloroform was shaked with concentrated sulfuric acid after this it was again shaked with distilled water to remove acid. The solvent was then kept in calcium chloride for two hours then it was distilled (b.p. 61 °C).

**Methanol:** To 500 mL of methanol CaO (50 g) was added and it was kept overnight. Then methanol was decanted off from CaO and refluxed it for 5-6 hrs. using $CaCl_2$ guard tube. After reflux it was distilled and collected to a round bottom flask. A white cake of Mg-turning was formed in another round bottom flask and the whole mass of methanol was added to it. Again it was refluxed for 4 hrs., distilled out and collected it into an air tight container.

**Benzene:** To 500 mL of benzene anhydrous $CaCl_2$ (20 g) was added and it was kept overnight. Then benzene was decanted off form $CaCl_2$, distilled and collected at 80 °C into an air tight container.

**Petroleum ether**: To 500 mL of petroleum ether anhydrous $CaCl_2$ (20 g) was added and it was kept overnight. Then petroleum ether was decanted off form $CaCl_2$, distilled and collected at 40-60 °C into an air tight container.

**Dioxane:** To 500 mL of dioxane anhydrous $K_2CO_3$ (100 g) was added and it was kept overnight. Then dioxane was decanted off form $K_2CO_3$ and distilled to get pure dioxane and preserved in an air tight container.

### 2.1.7 Cleaning and drying of glassware

All glassware was cleaned and for most purposes, dried before employed in preparative work in the laboratory. The glassware was washed with a commercial house hold washing powder, which does not scratch glass e.g. "Vim, Trix etc." The washing was either introduced directly into the apparatus or moistened with a little water or it was applied to the dirty surface with a wet test-tube brush, which has been dipped into the powder.

The operation was repeated if necessary. Finally, the apparatus was thoroughly rinsed with distilled water. The most widely used cleaning agent is the chromic acid, cleaning mixture. It is a mixture of chromic acid and concentrated sulphuric acid. It was prepared by the way 5 g of potassium dichromate were dissolved in 5 mL of water in a 250 mL beaker of 100 mL of concentrated sulphuric acid were then added slowly with constant stirring. The temperature will rise of 70-80 °C. The mixture was allowed to cool to about 40 °C and then transferred to a dry glass stopper bottle. Before using chromic acid mixture for cleaning the vessel was rinsed with water to remove organic matter. Sometimes chloroform, acetone, rectified spirit was used as a cleansing agent. Small and bulky both types of glass apparatus were dried by leaving it in an electrically heated oven maintained at 100-120 °C for one to two hrs. Sometimes the glassware was also dried with a commercial hair drier.

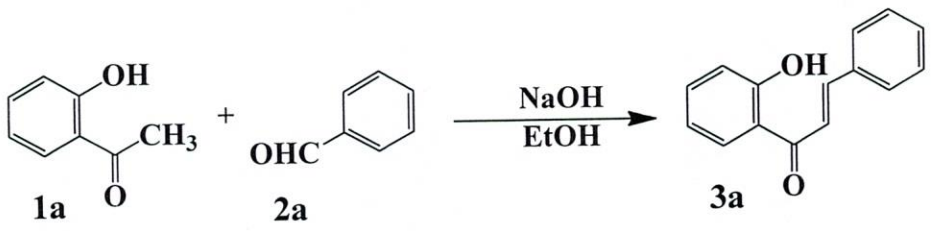
## 2.2 Section B; Synthesis and Characterization of eight 2′-hydroxychalcones (3a-3e, 4a-4c).

### 2.2.1 Preparation of β-resacetophenone (1b)

Anhydrous zinc chloride (16.5 g) was dissolved in glacial acetic acid (15.8 mL) by warming. To the hot solution at 140 °C, resorcinol (11.0 g) was added with constant stirring. The solution was heated in an oil-bath and the reaction mixture was allowed to complete itself at a temperature not more than 160 °C. After standing for 20 minutes the solution was poured over crushed ice, the precipitate, thus separated was filtered, washed with dil. HCl to remove zinc salts and then with water. It was crystallized from hot dil. HCl (10%, 150 mL), yield 10 g, m. p.142-143 °C.

### 2.2.2 Synthesis of 2′ - hydroxychalcone (3a) and its structural determination

A mixture of 2 - hydroxyacetophenone (**1a**, 0.01 mole) and benzaldehyde (**2a**, 0.01 mole) in ethanolic solution of NaOH (20%, 10 mL) with 30 mL ethanol was stirred at room temperature for about 12 hours. The reaction mixture was kept overnight at room temperature. Then it was diluted with ice cold water and acidified with ice cold dil. HCl. lemon yellow solid **(3a)**; yield 61%; melting point 84-85 °C; $R_f$ 0.97 (n-hexane : acetone, 10 : 1) was obtained.



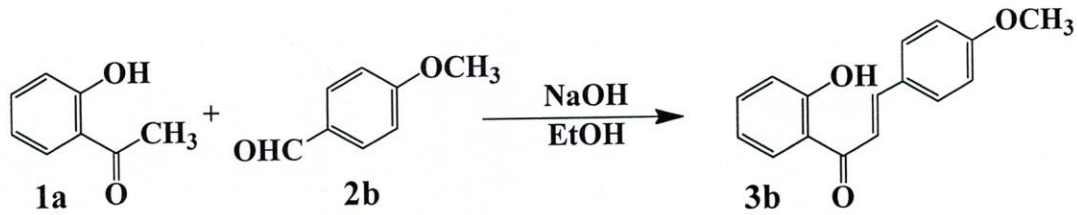

Scheme 8: Synthesis of 2′ - hydroxychalcone (**3a**) by condensation of 2 - hydroxyacetophenone (**1a**) with benzaldehyde (**2a**) under alkaline condition

$C_{15}H_{12}O_2$; Solid and lemon yellow, yield 61%; m. p. 84-85 °C, **UV:** $\lambda_{max}$ ($CH_3OH$): 196 and 233 nm, IR (KBr, $cm^{-1}$); 3414.06 (-OH), 3090 (C=C-H, aromatic, str.), 3046. 91 (C=C-H, olifinic, str.), 1959.40 (C=C, olifinic str.), 1640.01 (C=O, conjugated keto group), 1618.00 (C=C, aromatic), 1572.86, 1486.21, 1447.81, 1438.22, 1383.84, 1370.31, 1341.10 ($CH_3$, bending), 1267.72, 1236.07, 1205.71, 1181.52, 1153.06 (-C-O-C- str.), 1072.50, 1030.80 (C-O, str.), 994.70, 976.52, 864.79, 837.54 (C-H, bending aromatic) [See Appendix, Fig.1], $^1$H NMR, $\delta_H$ (400 MHz, $CDCl_3$, 12H); 12.803 (s, 1H, $C_2$-OH), 7.923 (d, $J$ = 8.4 Hz, 1H, $C_{6'}$ - H), 7.921 (d, $J$ = 14.8 Hz, 1H, $C_\beta$-H), 7.668 -7.661 (m, 2H, $C_2$ and $C_6$ -H), 7.657 (d, $J$ = 14.8 Hz, 1H, $C_\alpha$-H), 7.498 ( t, 1H, $C_{4'}$-H), 7.441-7.436 (bs, 3H, $C_3$, $C_4$ and $C_5$-H) 7.029 (d, 1H, $J$ = 8.4 Hz,

$C_{3'}$–H), 6.944 (t, 1H, $C_{5'}$- H), [See Appendix, Fig.2], $^{13}$C-NMR (100 MHz, $CDCl_3$, 15C); $\delta_c$ 193.77 (1C, >C=O), 163.62 (1C, C-2′), 145.49 (1C, $C_\beta$), 136.42 (1C, C-4′), 134.63 (1C, C-1′), 130.94 (1C, C-1), 129.67 (1C, C-5′), 129.06 (2C, C-2, C-6), 128.68 (3C, C-3, C-4, C-5), 120.17 (1C, Cα), 120.4 (1C, C-6′), 118.87 (1C, C-3′) [See Appendix, Fig.3]

### 2.2.3 Synthesis of 2′ - hydroxy - 4 - methoxychalcone (3b) and its structural determination

A mixture of 2 - hydroxyacetophenone (**1a**, 0.01 mole) and 4 - methoxybenzaldehyde (**2b**, 0.01 mole) was treated similar as procedure in 2.2.2 which yielded pineapple yellow solid (**3b**, 61%); m. p. 87-88 °C; $R_f$ 0.88 (n-hexane : acetone, 10:1) was obtained :



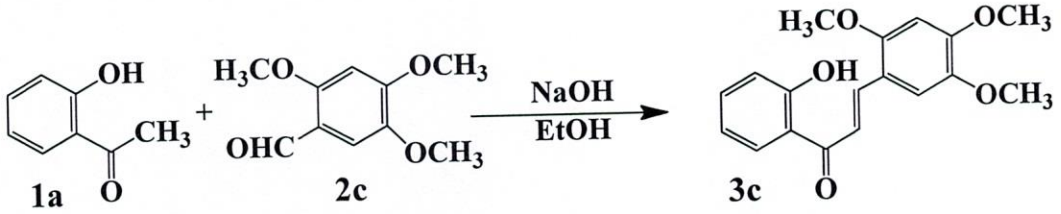

Scheme 9: Synthesis of 2′ - hydroxy - 4 - methoxychalcone (**3b**) by condensation of 2 -hydroxyacetophenone (**1a**) with 4 - methoxybenzaldehyde (**2b**) under alkaline condition

$C_{16}H_{14}O_3$; Solid and pineapple yellow, yield 61%; m. p. 87-88 °C **UV:** $\lambda_{max}$ ($CH_3OH$): 197 nm, IR (KBr, $cm^{-1}$); 3414.07 (-OH), 3080 (C=C-H, aromatic, str.), 3024.51 (C=C-H olifinic str.), 2969.85 (aliphatic C-H, asym.-str.), 2839.64 (aliphatic C-H, sym-str.), 1922.67 (C=C olifinic str.), 1640.50 (C=O, conjugated keto group), 1609.00 (C=C, aromatic, str.), 1584.52, 1513.59, 1493.97, 1442.00, 1423.64, 1376.12, 1344.03, 1303.72, ($CH_3$, bending), 13275.45, 1261.57, 1211.77, 1177.35, 1164.13, 1130.55 (-C-O-C-

str.), 1032.41(C-O, str.), 985.91, 866.88, 828.34, 804.08 (C-H, bending aromatic) [See Appendix, Fig.4], $^1$H NMR, $\delta_H$ (500 MHz, $CDCl_3$, 14H); 12.803 (s, 1H, $C_{2'}$-OH), 7.925 (d, $J$ = 8Hz, 1H, $C_{6'}$-H), 7.910 (d, $J$ = 16 Hz, 1H, $C_\beta$-H ), 7.640 (dd, $J$ = 8 Hz, 2H, $C_2$ and $C_6$-H), 7.545 (d, $J$ =16 Hz, 1H, $C_\alpha$-H), 7.490 ( t, 1H, $C_{4'}$-H), 7.030 ( d, 1H, $J$ = 8 Hz, $C_{3'}$-H) , 6.960-6.940 (m, 1H, $C_{5'}$-H), 6.950 (d, 2H, $C_3$ and $C_5$- H), 3.870 (s, 3H, $C_4$-O$\underline{CH_3}$) [See Appendix, Fig.5], $^{13}$C-NMR (125 MHz, $CDCl_3$, 16C); $\delta_c$ 192.80 (1C, >C=O), 163.60 (1C, C-2′), 159.80 (1C, C-4), 145.10 (1C, $C_\beta$), 135.90 (1C, C-4′), 130.20 (2C, C-2 and C-6), 129.60 (1C, C-6′), 127.50 (1C, C-1), 121.80 (1C, C-5′), 121.60 (1C, C-1′), 118.70 (1C, $C_\alpha$), 118.20 (1C, C-3′), 114.20 (2C, C-3 and C-5), 55.80 (1C, $C_4$-O$\underline{CH_3}$) [See Appendix, Fig.6]

## 2. 2. 4 Synthesis of 2′ - hydroxy - 2, 4, 5 - trimethoxychalcone (3c) and its structural determination

A mixture of 2 - hydroxyacetophenone (**1a**, 0.01mole) and 2, 4, 5 - trimethoxybenzaldehyde (**2c**, 0.01mole) was treated similar as procedure in 2.2.2 which yielded a dark yellow solid (**3c**, 61%); $R_f$ 0.84 (benzene: acetone, 5:1) was obtained.

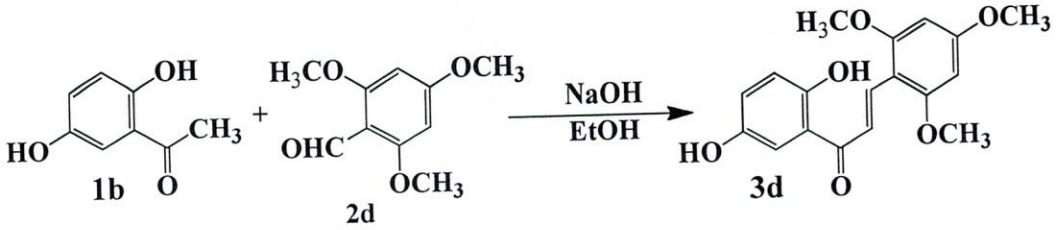



Scheme 10: Synthesis of 2′ - hydroxy - 2, 4, 5 - trimethoxychalcone (**3c**) by condensation of 2 -hydroxyacetophenone (**1a**) with 2, 4, 5 - trimethoxybenzaldehyde (**2c**) under alkaline condition

$C_{18}H_{18}O_5$, Solid and dark yellow, yield 61%; m. p. 120-122 °C, **UV:** $\lambda_{max}$ ($CH_3OH$): 196, 259 and 307 nm, IR (KBr, $cm^{-1}$); 3416.30 (-OH), 3040 (C=C-H, aromatic, str.), 3009.49 (C=C-H olifinic str.), 2964.99 (aliphatic C-H, asym.-str.), 2864.28 (aliphatic C-H, sym-str.), 2034.08 (C=C, olifinic str.), 1660.01 (C=O, conjugated keto group), 1609.36 (C=C, aromatic), 1520.00, 1479.67, 1412.91, 1358.89 ($CH_3$, bending), 1292.76, 1218.55, 1187.92 (-C-O-C-, str.), 1128.89, 1026.80, (C-O str.), 995.68, 864.04, 823.76, 757.15 (C-H, bending aromatic) [See Appendix, Fig.7], $^1$H NMR, $\delta_H$ (400 MHz, $CDCl_3$, 14H); 13.080 (s, 1H, $C_{2'}$-OH), 8.170 (d, $J$ = 16 Hz, 1H, $C_\beta$-H ), 7.640 (d, $J$ = 8.6 Hz, 1H, $C_{6'}$-H), 7.390 (d, $J$ = 16 Hz, 1H, $C_\alpha$-H), 7.370 ( m, 1H, $C_{4'}$-H), 7.010 (m, 1H, $C_{5'}$-H), 6.920 ( d, 1H, $J$ = 2.6 Hz, $C_{3'}$-H), 6.590 (s, 1H, $C_6$-H), 6.100 (s, 1H, $C_3$- H), 3.730 (s, 9H, $C_2$, $C_4$, $C_5$-OC<u>H</u>$_3$) [See Appendix, Fig.8], $^{13}$C-NMR (100 MHz, $CDCl_3$, 16C); $\delta_c$ 187.01 (1C, >C=O), 158.50 (1C, C-2′), 153.00 (1C, C-4), 147.80 (1C, C-3), 142.80 (1C, $C_\beta$), 139.81 (1C, C-4′), 135.71 (1C, C-2) , 131.11 (1C, C-6′), 123.90 (1C, C-5′), 123.30 (1C, C-1′), 121.61 (1C, Cα), 116.20 (1C, C-3′), 113.81 (1C, C-6), 108.20 (1C, C-1), 100.61 (1C, C-3), 56.40 ($C_2$- O<u>C</u>H$_3$), 56.30 (2C, $C_4$ and $C_5$-O<u>C</u>H$_3$) [See Appendix, Fig.9]

## 2. 2. 5 Synthesis of 2′, 5′ – dihydroxy - 2, 4, 6 - trimethoxychalcone (3d) and its structural determination

A mixture of 2, 5 - dihydroxyacetophenone (**1b**, 0.01 mole) and 2, 4, 6 - trimethoxybenzaldehyde (**2d**, 0.01 mole) was treated similar as procedure in 2.2.2 which yielded orange solid (**3d**, 61%). m. p. 175-176 °C; $R_f$ 0.37 (n-hexane : acetone, 3:1) was obtained.

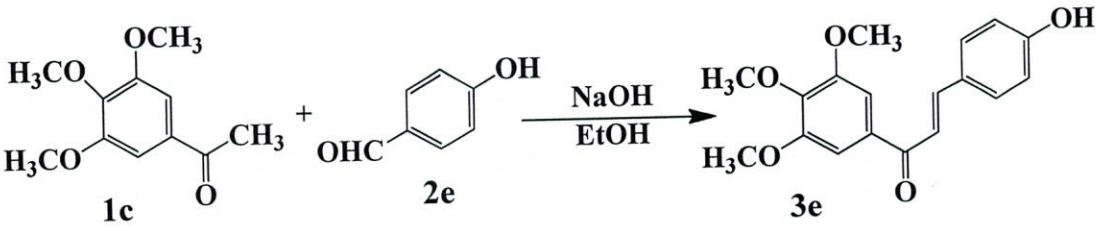



Scheme 11: Synthesis of 2′, 5′ - dihydroxy - 2, 4, 6 - trimethoxychalcone (**3d**) by condensation of 2, 5 - dihydroxyacetophenone (**1b**) with 2, 4, 6 - trimethoxy benzaldehyde (**2d**) under alkaline condition

$C_{18}H_{18}O_6$; Orange solid, yield 61%; m. p. 175-176 °C, **UV:** $\lambda_{max}$ ($CH_3OH$): 196, 242 and 384 nm, IR (KBr, $cm^{-1}$): 3415.26 (-OH), 3090.00 (C=C-H, aromatic, str.), 3000.50 (-C=C-H, olifinic, str.), 2940.52 (aliphatic C-H, asym.-str.), 2840.49 (aliphatic C-H, sym-str.), 1605.56 (C=O, conjugated keto group), 1548.81 (C=C, aromatic), 1488.73, 1456.61, 1384.30, 1309.40 ($CH_3$, bending), 1269.75, 1213.67, 1159.75, 1120.82 (-C-O-C-,str.), 1062.41, 1022.37 (C-O str.), 992.74, 921.57, 858.89, 828.30 (C-H, bending aromatic) [See Appendix, Fig.10], $^1$H NMR, $\delta_H$ (400 MHz, $CDCl_3$, 18H); 12.769 (s, 2H, $C_{2'}$ and $C_{5'}$-OH), 8.363 (d, $J$=15.6 Hz, 1H, $C_\beta$-H ), 7.906 (d, $J$ = 16 Hz, 1H, $C_\alpha$- H), 7.340 (dd, $J$ = 4.0 & 2.4 Hz, 1H, $C_{6'}$-H), 7.012-6.982 (m, 1H, $C_{4'}$-H), 6.894 ( t, 1H, $C_{3'}$-H), 6.877 (bs, 2H, $C_3$ and $C_5$-H), 3.916 (s, 6H, $C_2$ and $C_6$ - $OCH_3$), 3.861 (s, 3H, $C_4$-$OCH_3$) [See Appendix, Fig.11] $^{13}$C-NMR (100 MHz, $CDCl_3$, 16C); $\delta_c$ 194.89 (1C, >C=O), 163.75 (1C, C-4), 162.09 (2C, C-2 and C-6), 159.00 (1C, $C_{2'}$-OH), 157.69 (1C, $C_{5'}$-OH), 147.18 (1C, $C_\beta$) ,137.05 (1C, C-1′), 123.93 (1C, $C_\alpha$), 120.44 (1C, C-6′), 119.48 (1C, C-4′), 119.05 (1C, C-3′), 114.81 (1C, C-1′), 106.55 (1C, C-1), 90.61 (2C, C-3 and C-5), 55.85 (2C, $C_2$ and $C_6$-O<u>C</u>$H_3$), 55.40 (1C, $C_4$-O<u>C</u>$H_3$) [See Appendix, Fig.12]

## 2.2.6 Synthesis of 4 - hydroxy - 3′, 4′, 5′ - trimethoxychalcone (3e) and its structural determination

A mixture of 3, 4, 5 - trimethoxyacetophenone (**1c**, 0.01mole) and 4 - hydroxy benzaldehyde (**2e**, 0.01 mole) was treated similar as procedure in 2.2.2 which yielded brownish yellow solid (**3e**, 62%). m. p. 177-178 °C; $R_f$ 0.55 (petroleum ether: acetone, 2:1) was obtained and characterized.



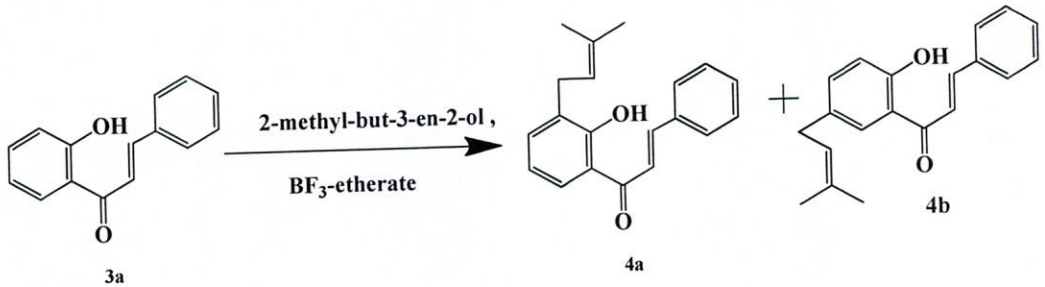

Scheme 12: Synthesis of 4 - hydroxy - 3′, 4′, 5′ - trimethoxychalcone (**3e**) by condensation of 3, 4, 5 - trimethoxyacetophenone (**1c**) with 4 - hydroxybenzaldehyde (**2e**) under alkaline condition

$C_{18}H_{18}O_5$; brownish yellow, yield 62%; m. p. 177-178 °C, UV: $\lambda_{max}$ ($CH_3OH$): 197 nm, IR (KBr, $cm^{-1}$): 3413.88 (-OH), 3232.28 (C=C-H, aromatic, str.), 3100.00 (-C=C-H, olifinic, str.), 2944.52 (aliphatic C-H, asym.-str.), 2834.07 (aliphatic C-H, sym-str.), 1964 (C-C, C=C), 1640.58 (C=O, conjugated keto group), 1610.44 (C=C, aromatic), 1594.25, 1555.80 (-$CH_2$ bending), 1464.29, 1439.66, 1413.71, 1384.32, 1349.04 ($CH_3$, bending), 1277.00, 1233.62, 1203.18 (-C-O-C, str), 1171.84, 1126.91, 1007.65 (C-O, str.), 986.16, 831.80, 720.69, 670.07 (C-H, bending aromatic). [See Appendix, Fig.13], $^1$H NMR, $\delta_H$ (400 MHz, $CDCl_3$, 18H) ; 9.850 (s, 1H, $C_4$–OH), 7.770 (d, $J$ = 16 Hz, 1H, $C_\beta$-H ), 7.550 (d, $J$ = 8.4 Hz, 2H, $C_2$ and $C_6$-H), 7.340 (d, $J$ =15.6 Hz, 1H, $C_\alpha$- H), 7.220 (d, 2H, $C_{2'}$ and $C_{6'}$-H), 6.890 ( d, $J$ = 8.4 Hz, 2H, $C_3$ and $C_5$-H), 3.900 (s, 9H, $C_{3'}$, $C_{4'}$ and $C_{5'}$ -O$\underline{CH_3}$) [See Appendix, Fig.14] $^{13}$C-NMR (100 MHz, $CDCl_3$, 18C); $\delta_c$ 189.67 (1C,

>C=O), 158.35 (1C, $C_4$-OH), 153.15 (2C, C-3′, and C-5′), 144.94 (1C, $C_\beta$), 142.41 (1C, C-4′) 133.78 (1C, C-1′), 130.52 (2C, C-2 and C-6), 127.57 (1C, C-1), 119.34 (1C, $C_\alpha$), 116.07 (2C, C-3 and C-5), 105.95 (2C, C-2′ and C-6′), 60.97 (1C, $C_{4'}$-O<u>C</u>$H_3$), 56.38 (2C, $C_{3'}$ and $C_{5'}$-O<u>C</u>$H_3$) [See Appendix, Fig.15]

### 2.2.7 Synthesis of 2′ - hydroxy - 3′ - C - prenylchalcone (4a) and 2′ - hydroxy - 5′ - C - prenylchalcone (4b):

To a stirred solution of chalcone (2.0 g) in dry dioxane (20 mL) was added gradually borntrifloride-etherate (2.6 mL) at room temperature during the course of 30 minutes this was added a solution of 2-methyl-but-3-en-2- ol (2.50 mL) in dry dioxane (2.50 mL) and the solution stirred for 6 hrs., kept at room temperature overnight and diluted with moist ether (150 mL). The ethearal layer was washed with water and dried over anhydrous $NaSO_2$. It was evaporated to dryness and the residue on column chromatography over silica gel and elution successively with n-hexane or a mixture of n-hexane-acetone (3:1). The n- hexane fraction was a light yellow colour gummy substance, 2′ - hydroxy - 3′ - C - prenylchalcone (**4a**).




Scheme 13: Synthesis of 2′ - hydroxy - 3′ - C - prenylchalcone (**4a**) and 2′ - hydroxy - 5′ - C - prenylchalcone (**4b**) by prenylation of 2′ -hydroxychalcone (**3a**)

$C_{20}H_{20}O_2$, gummy substance light, yellow, yield 62%; $R_f$ 0.90, UV: $\lambda_{max}$ ($CH_3OH$): 197 nm, $^1H$ NMR, $\delta_H$ (500 MHz, $CDCl_3$, 20 H), 7.935 (dd, $J$ = 8.0 Hz, 1H, $C_{6'}$-H), 7.830 (d, $J$ = 16.0 Hz, 1H, $C_\beta$-H), 7.680 (d, $J$ =16.0 Hz, 1H, $C_\alpha$-H), 7.670-7.650 (m, 1H, $C_{5'}$ -H), 7.460-7.420 (m, 2H, $C_2$ and $C_5$ –H), 7.200-7.160 (m, 3H, $C_3$, $C_4$ and $C_5$-H), 7.030 (d, $J$ = 8.0 Hz, 1H, $C_{3'}$-H), 5.500 (s, 1H, OH), 4.050-3.900 [m, 1H, -$CH_2$-C<u>H</u>=$C(CH_3)_2$], 3.40-3.20 [m, 2H, (-C<u>$H_2$</u>-CH=$C(CH_3)_2$], 1.80 (s, $CH_3$, 6H) [See Appendix, Fig.16], $^{13}$C-NMR (125 MHz, $CDCl_3$, 20 C); $\delta_c$ 192.80 (1C, >C=O), 161.50 (1C, $C_{2'}$-OH), 145.10 (1C, $C_\beta$), 138.20 (1C, C-4′), 135.20 (1C, C-1), 131.80 [1C, -$CH_2$-CH=<u>C</u>$(CH_3)_2$], 129.90 (1C, C-3′), 129.06 (2C, C-2 and C-6), 128.68 (3C, C-3, C-4 and C-5), 123.50 (1C, C-6′), 123.10 [1C, -$CH_2$-<u>C</u>H=$C(CH_3)_2$], 122.50 (1C, C-1′), 121.70 (1C, C-5′), 118.70 (1C, Cα), 24.60 (1C, -$CH_2$-CH=C(<u>$CH_3$</u>)($CH_3$)), 22.60 [-<u>C</u>$H_2$-CH=$C(CH_3)_2$], 18.60(1C, -$CH_2$-CH=C(<u>$CH_3$</u>)($CH_3$)) [See Appendix, Fig .17]

The n- hexane: acetone (3:1) fraction was a yellow colored gummy substance, 2′ - hydroxy - 5′ - C - prenylchalcone (**4b**).

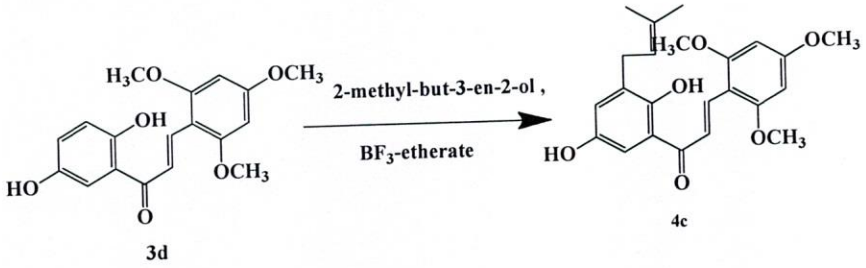
$C_{20}H_{20}O_2$, Gummy and yellow, yield 62% , $R_f$ 0.90, UV: $\lambda_{max}$ ($CH_3OH$): 196 and 241 nm $^1H$ NMR, $\delta_H$ (500 MHz, $CDCl_3$, 20 H), 7.930 (d, $J$ = 15 Hz, 1H, $C_\beta$-H), 7.720-7.700 (m, 1H, $C_{6'}$-H), 7.640 (d, $J$ =10 Hz, 2H, $C_2$ and $C_6$), 7.550 (d, $J$ =15 Hz, 1H, Cα-H), 7.450 (bs, 3H, $C_3$, $C_4$ and $C_5$-H), 7.190-7.130 (m, 1H, $C_{4'}$-H), 6.980-6.960 (m, 1H, $C_{3'}$-H), 5.500 (s, 1H, OH), 5.200 [s, 1H, -$CH_2$-C<u>H</u>=$C(CH_3)_2$], 3.700-3.600 [m, 2H, -C<u>$H_2$</u>-CH=$C(CH_3)_2$], 1.820 (3H, -$CH_2$-CH=C(<u>$CH_3$</u>)($CH_3$)), 1.740 (3H, -$CH_2$-CH=C(<u>$CH_3$</u>)($CH_3$)) [See Appendix, Fig.18], $^{13}$C-NMR (125 MHz, $CDCl_3$, 20 C); $\delta_c$ 192.80 (1C, >C=O), 160.60 (1C, $C_{2'}$-OH), 145.10 (1C, $C_\beta$), 136.20 (1C, C-4′), 135.20 (1C, C-1), 131.80 [1C, -$CH_2$-CH=<u>C</u>$(CH_3)_2$], 131.50 (1C, C-6′), 130.50 (1C, C-5′), 128.60 (2C, C-3 and C-5), 128.50 (2C, C-2 and C-6), 127.90 (1C, C-4), 123.10 [1C, -$CH_2$-<u>C</u>H=$C(CH_3)_2$], 120.90 (1C, C-1′), 117.40 (1C, C-3′), 118.70 (1C, $C_\alpha$), 34.00

[-$\underline{C}H_2$-CH=C$(CH_3)_2$], 24.60 (1C, -$CH_2$-CH=C$\begin{matrix}\underline{C}H_3\\ \underline{C}H_3\end{matrix}$), 18.60 ( 1C, -$CH_2$-CH=C$\begin{matrix}\underline{C}H_3\\ CH_3\end{matrix}$)

[See Appendix, Fig.19]

## 2.2.8 Synthesis of 2′, 5′ - dihydroxy -2, 4, 6 - trimethoxy - 3′- C - prenylchalcone (4c):

To a stirred solution of chalcone (2.0 g) in dry dioxane (20 mL) was added gradually borntrifloride-etherate (2.6 mL) at room temperature during the course of 30 minutes this was added a solution of 2-methyl-but-3-en-2- ol (2.50 mL) in dry dioxane (2.50 mL) and the solution stirred for 6 hrs., kept at room temperature overnight and diluted with moist ether (150 mL). The ethearal layer was washed with water and dried over anhydrous $NaSO_2$. It was evaporated to dryness and the residue on column chromatography over silica gel and elution successively with n-hexane-acetone (3:1).




Scheme 14: Synthesis of 2′, 5′ - dihydroxy - 2, 4, 6 - trimethoxy - 3′ - C - prenylchalcone (**4c**) by prenylation of 2′, 5′ - dihydroxy - 2, 4, 6 - trimethoxychalcone (**3d**)

$C_{23}H_{26}O_6$, wine red semi solid, yield 62%; $R_f$ 0.91, UV: $\lambda_{max}$ ($CH_3OH$): 196 nm, $^1$H NMR, $\delta_H$ (400 MHz, $CDCl_3$, 26 H), 8.330 (d, $J$ = 16.0 Hz, 1H, $C_\beta$-H), 7.420 (d, $J$ =16.0 Hz, 1H, $C_\alpha$-H), 7.080 ($J$ = 8.0 Hz, 1H, $C_{4'}$-H), 7.040 (dd, $J$ = 8.0 Hz, 1H, $C_{6'}$-H), 7.670-7.650 (m, 1H, $C_{5'}$ -H), 6.090 (2H, $C_3$ and $C_5$ -H), 5.750 [1H, -$CH_2$-C$\underline{H}$=C$(CH_3)_2$], 5.350 (2H, $C_{2'}$ and $C_5'$-OH), 3.830 (9H, $C_2$ and $C_4$ and $C_6$-$OCH_3$), 3.210 [-C$\underline{H}_2$-CH=C$(CH_3)_2$], 1.820 (3H, -$CH_2$-CH=C$\begin{matrix}CH_3\\ \underline{C}H_3\end{matrix}$),

1.700 (3H, -$CH_2$-CH=C(<u>$CH_3$</u>)($CH_3$)) [See Appendix, Fig.20] $^{13}$C-NMR (100 MHz, $CDCl_3$,20 C); $\delta_c$ 192.80 (1C, >C=O), 160.60 (1C, C-4), 159.60 (2C, C-2 and C-6), 154.10 ($C_{2'}$-OH), 149.60 (1C, C-5′), 134.70 (1C, C-3′), 131.80 (1C, -$CH_2$-CH=<u>C</u>$(CH_3)_2$), 123.90 (1C, C-1′), 123.10 [1C, -$CH_2$-<u>C</u>H=C$(CH_3)_2$], 122.50 (1C, $C_\beta$), 121.30 (1C, $C_\alpha$), 115.40 (1C, C-4′), 107.00 (1C, C-1), 104.20 (1C, C-6′), 90.90 (2C, C-3 and C-5), 56.20 (2C,$C_2$ and $C_6$-$OCH_3$), 55.8 (1C, C-4), 28.10 (-<u>C</u>$H_2$-CH=C$(CH_3)_2$), 24.60 (1C, -$CH_2$-CH=C(<u>$CH_3$</u>)($CH_3$)), 18.60 (1C, -$CH_2$-CH=C(<u>$CH_3$</u>)($CH_3$)) [See Appendix, Fig.21]

# CHAPTER THREE

# BIOLOGICAL ACTIVITY SCREENING

# CHAPTER THREE

# Biological Activity Screening

## 3.1 Section A; General discussion on Antimicrobial Activity [5, 11, 21, 22]

Over the past 25 years, the incidence of systemic bacterial infections has been rising dramatically due to an increase in the number of immunocompromised hosts. The search is oriented to find new antibacterial drugs, which may selectively attack the bacteria without inhibiting any biochemical system of the host. [23] After the discovery of penicillin and other antibiotics have significantly reduced the complications and mortality rate of infectious diseases. With the wide use of antibiotics in the treatment of bacterial infection has lead to the emergence and spared of resistance strains. The continuous spread of multi drug resistance pathogens have become a serious threat to public health and a major concern for infection control practitioners worldwide.

Bangladesh is predominantly an agricultural country depending mainly on crop plants, agricultural, medicinal and forest product for its economic development. Although crops play a vital role in the economy of the country and agro ecological conditions are favorable for the production of various crops, the yields of crops is often poor. Among the various factors responsible for poor yield of crops, plant disease caused by various microorganisms play a significant role. Gradually men gathered sufficient knowledge of chemistry to inhibit or to kill the microorganism i.e., only inhibit the microbial growth are called 'statis'. But the chemicals which have ability to kill microorganisms are called 'cadal'. Various pesticides are classified as fungicides, viricides, bactericides etc.

Many fatal diseases caused by microorganism viz. bacterial, fungal and viral attacks are known. The treatment of diseases due to bacterial, viral and fungal invasion by chemical compounds were studied and used successfully without affecting the tissues of the host and any other side effects. Many compounds e.g. formaldehyde, iodine, phenol etc. are also active in destroying bacteria.

Antibiotics are the chemical substance obtained from certain non-pathogenic microorganisms (bacteria and fungi) and are used for either killing or inhibiting the growth of pathogenic micro organism without affecting the host tissue.

The words bactericide has originated from Latin words: bacteria. Thus literally speaking a bactericide would be any agencies which have the ability to kill bacteria. By common usage the word is restricted to chemicals. Hence the words bactericide means a chemical capable of killing bacteria. It is not enough that a chemical has high bactericidal activity. Such as chemicals may have no utility unless it stands out in the tests and gives proof of significant control of diseases under varied field conditions. There are several factors which influence the performance of a bactericide under different field conditions. They may be either physical or chemical in nature. The number of chemicals available that shows antibacterial activity runs into hundreds although all are not equally safe, effective and popular. Also different types of organic, aromatic, inorganic and heterocyclic compounds are employed as antibacterial agents.

A biochemical screening to identify the proportions in the clinical use is long overdue. With better understanding of the molecular basis of resistance, it can be envisaged the new diagnostic tools will be developed to allow rapid and appropriate changes in treatment. The use of combinations of bactericides is increasing in medicine and should be a route of choice for

overcoming resistance. [23] Chalcones and their heterocyclic analogs are potential therapeutic agents in bacterial diseases. Chalcones with antibacterial properties have been known since the 1940s. For antibacterial activity, the presence of the enone aggregate in the molecule is important.

New chalcone derivatives are under consideration, and in clinical trial, and will be central to therapy in the medium term. The emergence of resistance to these agents can be predicted and it needs advance assessment to provide assistance for their proper integration into drug therapy.

Compounds with electron releasing groups such as hydroxyl and methoxy showed better antibacterial activity than the others not having such groups. [24] From the above discussion it is clear that the chalcone compounds show antimicrobial activity and widely used as drugs. So, we deliberately synthesized a number of hydroxymethoxy chalcone and finally antibacterial activities of these chalcone were determined. These chalcone were used for valuation of antibacterial activities and syntheses of these compounds are already discussed in the experimental section of the dissertation. The result obtained by *in vitro* antibacterial activity against both Gram-positive and Gram-negative microorganisms of these compounds is discussed in this section.

### 3.1.1 Methods of Antimicrobial activity determination

In order to detect the antimicrobial activity of a new compound for the development as potential new antibiotic or chemotherapeutic agent *in vitro* antimicrobial screening is a useful technique. In general antimicrobial screening is undertaken in two phases; a primary qualitative assay [24, 25] to detect the presence or absence of activity and secondary assay [26, 27]

which quantifies the relative potency expressed as minimum inhibitory concentration (MIC) value of a pure active compound.

*In vitro* antibacterial activities of the test chemicals were studied against four pathogenic bacteria. The primary assay can be performed *in vitro* by a number of methods one of which is disc diffusion technique [25, 26] by this method we could classify the organism as susceptible as well as resistance towards particular compounds. The secondary assay is the serial broth dilution assay [11, 24] which quantifies the antimicrobial activity of pure compound by providing the MIC value of the compound for specific susceptible organism. This is an important consideration for the screening of a new antibiotic substance.

### 3.1.2 Disk Diffusion Technique

The disc diffusion technique [11, 24] is widely accepted for preliminary investigations of materials which are suspected to possess antimicrobial properties. Diffusion procedure is normally used in susceptible intermediate of resistant categories.

Diffusion assays are based on the ability of antibiotics to diffuse from a confined source through a PDA gel and create a concentration gradient. If the agar is seeded or streaked with sensitive organism a zone of inhibition will result where the antibiotic concentration exceeds the minimum inhibitory concentration for that particular organism.

In the disc diffusion technique dried filter paper discs containing known amount of test material are placed on agar plates seeded with test organisms. These plates are kept at low temperature (4 °C) for 24 hrs. Initially the dried discs absorb water from the surrounding test medium and the drug is dissolved. The drug migrates through the adjacent test medium

by concentration gradient of the drug according to physical law that governs diffusion of molecules through an agar gel.[27] As a result there is a gradual change of drug concentration in the agar surrounding each discs. Then the plates are incubated in an incubator at 37.5 °C for 24 hrs.

As the antibiotic diffusion progresses microbial multiplication also proceeds. At that point of time fungal multiplication proceeds more rapidly than the drug can diffuse and fungal cell which are not inhibited by the antimicrobial agents will continue to multiply until a law of growth can be visualized. No growth will appear in the area where drug is present in inhibitory concentrations.

Generally more susceptible the test organism the larger is the zone of inhibition. Antimicrobial activities of the test compounds are expressed by measuring the zone of inhibition observed around the area. The diameter of the inhibition zone is usually measured to understand the extent of inhibition in different concentration.

The size of the inhibitory zones depends principally in the following factors:

(a) Intrinsic antimicrobial sensitivity of the test compounds.

(b) Growth rate of the test microorganisms.

(c) Diffusion rate of the drug, which is related to its water solubility.

(d) Number of concentration of inoculated test organisms.

(e) Concentration / amount of the test sample.

(f) Thickness of the test medium in the Petridishes.

### 3.1.3 Chemicals used:

Eight synthesized chalcones were used as the test chemicals. The chemicals were synthesized, isolated and characterization in the organic research laboratory, Department of chemistry, University of Rajshahi. The names of the tested chemical are listed in Table 2.

Table 2: List of synthesized chalcones used for antibacterial activities:

| Compound no. | Test compounds | Molecular formula |
|---|---|---|
| **3a** | 2′-hydroxy chalcone | $C_{15}H_{12}O_2$ |
| **3b** | 2′-hydroxy-4 -methoxy chalcone | $C_{16}H_{14}O_3$ |
| **3c** | 2′-hydroxy-2, 4, 5-trimethoxy chalcone | $C_{18}H_{18}O_5$ |
| **3d** | 2′, 5′-dihydroxy-2,4,6-trimethoxychalcone | $C_{18}H_{18}O_6$ |
| **3e** | 4-hydroxy-3′, 4′, 5′ - trimethoxy chalcone | $C_{18}H_{18}O_5$ |
| **4a** | 2′-hydroxy-3′-C-prenyl Chalcone | $C_{20}H_{20}O_2$ |
| **4b** | 2′-hydroxy-5′-C-prenyl Chalcone | $C_{20}H_{20}O_2$ |
| **4c** | 2′,5′-dihydroxy-2,4,6-trimethoxy-3′-C-prenylchalcalcone | $C_{23}H_{26}O_6$ |

*Ciprofloxacin-50 (*C-50, 50µg/disc) was used as standard.

### 3.1.4 Test organism:

In the present study of screen the antibacterial activities of chemicals (Table 2) and a number of bacterial strains were used as test organisms. Among these four pathogens were tested (two Gram-positive and two Gram-negative).

### 3.1.5 Collection of organism:

The test tubes cultures of the bacterial pathogens were collected from the pharmaceutical Microbiological laboratory, Department of pharmacy, University of Rajshahi, Rajshahi Bangladesh. Types of test organism have been studied are listed below (Table 3).

Table 3: List of the test microorganisms (bacteria)

| Label | Name of microorganism | Gram-positive ($G^+$) / negative ($G^-$) |
|---|---|---|
| $B_1$ | *Bacillus caerius* | ($G^+$) |
| $B_2$ | *Staphyllococcus aureus* | ($G^+$) |
| $B_3$ | *Escherichia coli* | ($G^-$) |
| $B_4$ | *Agrobacterium Species* | ($G^-$) |

### 3.1.6 Description of procedure:

#### 3.1.6.1 Sterilization procedure:

Antimicrobial screening was carried out in Laminar air flow unit and all types of precaution were highly maintained to avoid any contamination during the test. UV light has switched on before working in laminar hood for 1 hour to avoid any accidental contamination. Petri dishes and other glass

wares were sterilized by autoclaving at a temperature of 121 °C and a pressure of 15 Ibs/sq inch for 20 minutes. Micropipette tips culture media; cotton forceps blank discs etc. were also sterilized.

### 3.1.6.2 Culture media:

The following media were used to demonstrate the antimicrobial activity and for subculture of the test organism.

i) Nutrient agar medium

ii) Nutrient broth medium

Among these, the first one is most frequently used and we also used the same for the antibacterial screening.

### 3.1.6.3 Preparation of nutrient agar:

The constituents of nutrient agar was accurately weighed and dispersed in waters. It was then placed in a water bath to dissolve the ingredients until a transparent solution was obtained.

### 3.1.6.4 Preparation of medium:

The instant Nutrient Agar (NA) media as weighed and then reconstituted with distilled water in a conical flask according to specification (2.8% w/v). It was then heated in a water bath to dissolve the agar until a transparent solution was obtained and it was then autoclaved at 121 °C for 15 minutes at 15 lbs / sq. inch pressure.

### 3.1.6.5 Preparation of fresh culture:

The media prepared in the above section was dispensed in 5.0 mL amount of clear test tubes to prepare slants. The test tubes were plugged with cotton and sterilized in an autoclave at 121 °C for 15 minutes at 15 Ibs/sq. inch pressure. After sterilization the test tubes were kept in an inclined position for solidification. Finally the slants streaked with pure culture of the test organisms under laminar air flow and incubated for 24 hrs.

### 3.1.6.6 Preparation of test plates:

i) 15 mL of Nutrient Agar medium which prepared the previous section was poured in clean test tubes and then plugged with cotton.

ii) The test tubes were sterilized and allowed to cool at about 45 °C to 50 °C.

iii) The medium in the test tubes were inoculated with fresh culture of the test bacteria by means of a sterile loop and agitated to ensure uniform dispersion of bacteria in the medium.

iv) Finally, the medium was poured into sterile petridishes and agitated and clockwise anticlockwise right to left and left to right. Thus plates were ready for sensitivity test.

### 3.1.6.7 Preparation of discs containing sample:

#### 3.1.6.7.1 Sample discs:

i) Solution of the synthetic compounds was prepared in acetone so that 170 µL contained 1mg of the compounds.

ii) Filter paper discs were taken in a petridish and sterilized by oven at 110 °C for 1 hour.

iii) 40 µL of the solution of each compound was placed in a particular disc with the help of a micropipette.

iv) These discs were then air dried.

### 3.1.6.7.2 Standard discs:

These were used as positive control to ensure the activity of standard antibiotic against the test organism as well as for comparison of the response produced by the known antibacterial agent with that produced by test samples. In our investigation, commercially available Ciprofloxacin-50 containing 50 µg/disc of this antibiotic was used as standard disc.

### 3.1.6.8 Placement of the discs and incubation:

i) The sample impregnated discs and standard antibiotic discs were placed gently on the solidified agar plates with the help of a sterile forceps to ensure contact to the medium.

ii) The plates were then kept in a refrigerator at 4 °C for overnight so that the materials that absorbed the discs could get sufficient time to diffuse into the medium.

iii) Finally the plates were incubated at 37.5 °C for 24 hrs.

### 3.1.6.9 Precaution:

The discs were placed in such a way that they were not closer than 15 mm to the edge of the plate and for enough apart to prevent overlapping the zones of inhibition.

### 3.1.6.10 Determination of antibacterial activity of the test agents by measuring the zone of inhibition:

After 24 hrs. incubation the antibacterial activity was carried out by measuring the zone of inhibition in millimeter (mm) by a transparent scale. The zones made by the samples were compared with that of the standard disc. The results are presented in tabular form in Chapter 4.

## 3.2 Section B; General Discussion on Antioxidant activity [11, 12]

In living systems, free radicals are generated as part of the body's normal metabolic process and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result in increased radical activity and damage. Free radicalsor oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long-term complication of diabetes. Antioxidants may be defined as radical scavengers which protect the human body against free

radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegenertion, Parkinson's diseases, mongolism, aging process and perhaps dementias. [28]

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological system from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.

Oxidation is the transfer of electrons from one atom to another. It represents an essential part of metabolism and aerobic life in general, since oxygen is the ultimate electron acceptor in the electron flow systems that transport energy in the form of ATP. [29] Problems may arise, however, when the electron flow generates free radicals, such as $O_2^-$ centered free radicals, known as reactive oxygen species (ROS), and including superoxide ($O_2^{\cdot -}$), peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$), hydroxyl ($HO^{\cdot}$), nitric oxide ($NO^{\cdot}$) radicals. The contribution of free radical–mediated processes to the pathogenesis of human disease is indicated by biomarkers of oxidative damage to lipids, protein and DNA. Such markers have been identified in patients with atherosclerosis, certain cancer, neurodegenerative diseases and lung disorders, especially those with an inflammatory component to their etiology. A range of oxygen species (ROS) and reactive nitrogen species (RNS) have been implicated in the mechanisms of damage associated with disease development, including superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide ($HOO^{\cdot}$), lipid alkoxyl ($RO^{\cdot}$), and peroxyl radicals ($ROO^{\cdot}$), peroxynitrite ($ONOO^{\cdot}$), nitric oxide ($NO^{\cdot}$) and nitrogen dioxide radical ($NOO^{\cdot}$).

Antioxidants are compounds capable of preventing and even counteracting the damage caused in human tissue by the normal effects of physiological oxidation. A lot of research has shown that antioxidant can play a role in preventing the development of some chronic diseases. In addition to those mentioned previously, diseases such as atherosclerosis, emphysema, iron overload, malaria, muscular dystrophy, retinal degeneration and rheumatoid arthritis are but a few examples where research has shown the likelihood of direct links and the possibility of positive dietary and perhaps even nutraceutical intervention. Antioxidant activity and nutritional labeling data including vitamins, fiber, and minerals will aid in the interpretation of clinical results obtained as various food products are tested in biological models for chronic disease. It is reasonable to expect that high antioxidant foods have greater potential to reduce free radicals in the body than do low antioxidant foods. Thus it is important to know the antioxidant content of foods, in addition to knowing the basic nutritional information such as the protein, fiber, fat, mineral and vitamin contents.

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occuring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as mono-phenols are weak antioxidants.

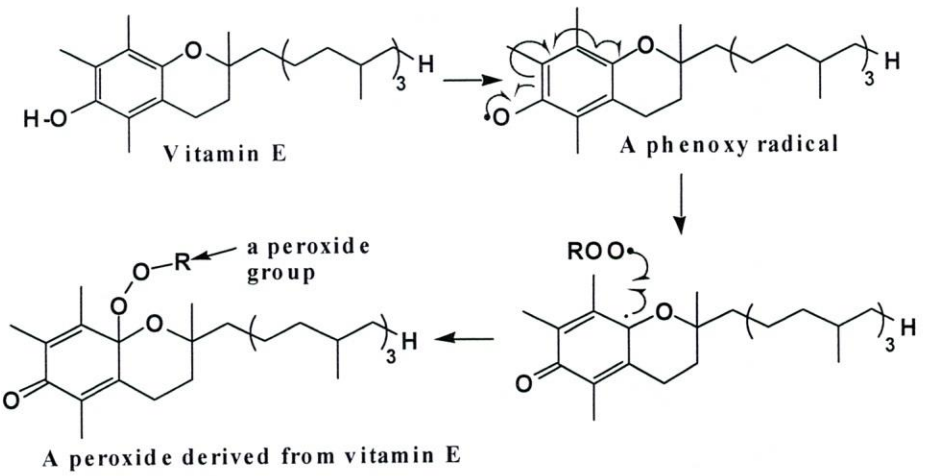
Table 4: List of some foods containing high levels of antioxidants[30]

| Food | Antioxidant activity (TE / 100 gm) |
|---|---|
| Red Grapes | 1350 |
| Red Cabbage | 1000 |
| Broccoli Flowers | 500 |
| Spinach | 500 |
| Green Grapes | 400 |
| Tomato | 300 |
| Green Bean | 175 |
| Red Bean | 11459 |
| Green Cabbage | 150 |
| Lima Beans | 1055 |

Table 5: List of some naturally occuring antioxidant [11]

| Antioxidant compounds | Food containing high levels of these antioxidant |
|---|---|
| Vitamin C (ascorbic acid) | Fruits and vegetables |
| Vitamin E (tocopherols, tocotrienols) | Vegetable oils |
| Polyphenolic antioxidants (resveratrol, flavonoids) | Tea, coffee, soy, fruit, olive oil, chocolate, cinnamon, oregano and red wine |
| Carotenoids (lycopene, carotene) | Fruit, vegetables and eggs |

Vitamin E is very often used and takes as anti aging antioxidant. It functions in the following way through the formation of phenoxy radical combine with other unwanted radical in living cell.




Scheme 15: Free radical scavenging mechanism of vitamin E

It is known that the antioxidant properties of chalcones are quite dependant on the two aryl structures, that is, the substitution pattern on the two aryl rings of the chalcone moiety. Especially, the hydroxyl substituent is one of the key groups that enhance greatly the antioxidant activity of chalcone mainly due to its easy conversion to phenoxy radicals through the hydrogen atom transfer mechanism. This phenoxy radical formation may be crucial to the antioxidant properties, which are assessed primarily as radical scavenging potential of phenolic chalcones. In fact, the hydroxyl substituent is common among chalcones from natural sources.

Chalcones basic structure includes two aromatic ring bound by α, β-unsaturated carbonyl group, a unique templates associated with diverse application. Due to the presence of the reactive keto, vinylinic group, chalcones and their analogues have been reported to be antioxidant. Hydroxyl, methoxy and prenyl substituents are associated with antioxidant properties.

### 3.2.1 Study of antioxidant property by some common methods: [11, 30]

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. In recent years, oxygen radical absorbance capacity assay and enhanced chemiluminescence assay have been used to evaluate antioxidant activity of foods, serum and other biological fluids. These methods require special equipment and technical skills for the analysis. The different types of methods published in the literature for the determinations of antioxidant activity of food involve electron spin resonance (ESR) and chemiluminescence methods. These analytical methods measure the radical scavenging activity of antioxidants against free radicals like the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, the superoxide anion ($O_2^{\cdot -}$), the hydroxyl radical ($HO^{\cdot}$) or peroxyl radicals ($ROO^{\cdot}$). The various methods used to measure antioxidant activity of food products can give varying results depending on the specific free radical being used as a reactant. There are other methods which determine the resistance of lipid or lipid emulsions to oxidation in the presence of antioxidant being tested. The malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) assay have been used extensively science the 1950's to estimate the peroxidantion of lipids in membrane and biological system. These methods can be time consuming because they depend on oxidation of a substrate which is influenced by temperature, pressure, matrix etc. and may not be practical when large numbers of samples are involved. Antioxidant activity methods using free radical traps are relatively straightforward to perform. The ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid )] radical cation has been used to screen the relative radical- scavenging abilities of flavonoids and phenolics have used the Oxygen Radical Absorbance Capacity (ORAC) produce to determine antioxidant capacities of fruits and vegetables.[34] In the ORAC method, a sample is added to the peroxyl radical generator, 2, 2′-

azinobis (2- aminopropane) dihydrochloride (AAPH) and inhibition of the free radical action is measured using the fluorescent compound, B-phycoerythrin necessary to react with one half of the DPPH is expressed in terms of micromole equivalents of Trolox (TE) per 100 grams of sample, or simply Trolox units per 100 gm or TE/100g.

Table 6: List of some commonly used antioxidant used as standard

| **Standard antioxidants** | **Antioxidant activity (TE/100 Grams)** |
|---|---|
| Ascorbate | 442,000 |
| Trolox | 400,000 |
| Vitamin E | 201,000 |
| BHT | 395,000 |

### 3.2.1.1 DPPH Radical Scavenging Activity

The radical scavenging activity was measured using the method of Hatano *et al.* with some modifications. Summarily, 0.2 mL solution of DPPH in methanol was prepared and 1.5 mL of this solution was added to the equal volume of each test samples dissolved in methanol at different concentrations. After shaking, the mixture was maintained in dark for 30 mins. Then, the absorbance was measured at 517 nm against a blank. Ascorbic acid and butylated hydroxyanisole (BHA) were used as standard references. The scavenging activity was calculated using the formula:

Scavenging Activity (%) = [($A_{517}$ of control – $A_{517}$ of sample) / $A_{517}$ of control] × 100.

### 3.2.1.2 Ferrous ion Chelating Activity:

Ferrous iron-ferrozine complex method with some modification was used for determination of chelating activity of samples for ferrous ions $Fe^{2+}$. Briefly, 25µL of $FeCl_2$ solution (2 mM) was added to a mixture containing 1.5 mL of $H_2O$ and 2 mL of the test samples in methanol at different concentrations. The reaction was preceded by adding 50 µL ferrozine solution (5mM) after 30 seconds. The mixture was shaken well and incubated for 10 mins. at room temperature. Absorbance of the solution was then measured at 562 nm Quercetin was used as positive control. The ability of the extracts and fractions to chelate ferrous ion was calculated using the equation described above for DPPH.

### 3.2.1.3 Inhibition of β-carotene Bleaching:

Antioxidant activity of the samples was determined according to a slightly modified version of the β-carotene bleaching method. In this study 5mg of β-carotene was dissolved in 10 mL of chloroform. 33 µL of linoleic acid, 750 µL of β-carotene solution and 225 mg of Tween 40 were mixed. The solvent was completely removed using a rotary evaporator. Then 75 mL of oxygenated distilled water was added and the mixture was emulsified for 15 mins. in a sonicator to form an emulsion A. Aliquots of 3.5 mL of this were transferred into a series of stopper test tubes containing 1 mL of samples dissolved in ethanol in various concentrations. Optical density (OD) at 470 nm was determined for all samples immediately (t=0) and at the end of the time period (t=120). A second emulsion was also prepared and used as blank to zero the spectrophotometer. This emulsion consisted of 50 mL of oxygenated water, 22 µL of linoleic acid and 150 mg of Tween 40. The percentage inhibition was calculated according to the following formula:

Inhibition (%) = [(AA (120) – AC (120)) / (AC (0) – AC (120))] × 10

In which AA (120) is the absorbance of the sample at t = 120 mins. AC (120) is the absorbance of the control at t = 120 mins. and AC (0) is the absorbance of the control at t = 0 mins.

### 3.2.1.4 Trolox Equivalent Antioxidant Capacity (TEAC):

Antioxidant capacity of the samples were measured with some modifications . In this procedure ABTS (54.2 mg) was dissolved in phosphate buffer (pH = 7) and activated to ABTS + radical by addition of 100 mg $MnO_2$ with stirring for 30 mins. Then, the solution was centrifuged (5 min, 10000 rpm), filtered (0.22μm) and diluted with phosphate buffer so that $A_0 = 0.7$. Then, 2 mL of ABTS + radical was added to test tubes containing 1 mL of samples dissolved in ethanol in various concentrations. Time of reaction was 20 mins. Absorbance of the solution were measured at a wavelength of 734 nm and antioxidant capacity of the samples were calculated according to the following formula:

Inhibition (%) = $[(A_0 - A_1)/A_0] \times 100$.

Where $A_0$ = the absorbance of the control, $A_1$ = the absorbance of the sample. Trolox was used as standard.

## 3.3 Radical Scavenging Activity Determination of the Present Study by DPPH Method:

DPPH assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration. When DPPH radicals encounter a proton donating substance such as an antioxidant,

it would be scavenged and the absorbance is reduced. Thus, the DPPH radicals were widely used to investigate the scavenging activity of some natural compounds.

### 3.3.1 Principle:

The 1, 1-diphenyl -2- picrylhydrazyl radical has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in mucous or methanol solution. With this method it was possible to determine die antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form this molecule had an absorbance at 517 nm which disappeared alter acceptance of ail electron or hydrogen radical form an antioxidant compound to become a stable diamagnetic mole

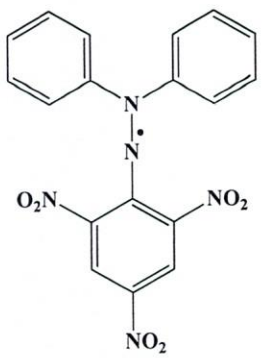



Figure 3: Structure of 1, 1-diphenyl-2-picrylhydrazyl

Strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1660 when the odd electron of DPPH

radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization of stoichiometric with respect to number of electrons captured.

### 3.3.2 Materials:

#### i) Apparatus used:

- ✓ Micropipette (1-10 mL)
- ✓ UV spectrophotometer
- ✓ Electronic balance
- ✓ Test tube
- ✓ Beaker

#### ii) Chemical used:

- ✓ Ascorbic acid
- ✓ DPPH (1, 1-diphenyl-2-picrylhydrazyl)
- ✓ Methanol

### 3.3.3 Experimental procedure:

Firstly, 5 test tubes were taken to make aliquots of five kinds (2, 5, 10, 15, 20 μg/mL) of concentrated solution. Test sample and ascorbic acid were weighed 3 times and dissolved in methanol to make the required concentration by dilution technique. DPPH was weighed and dissolved in methanol to make .02 % solution. To dissolve homogeneously, a magnetic stirrer was used. After making the desired concentration 2 μL of DPPH solution was applied on each test tube by pipette. DPPH was also applied on the blank test tube at the same time where only methanol was taken as blank. The test tube was incubated at room temperature for 30 mins. in dark place to

complete the reaction. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer against blank. Ascorbic acid was used as positive control.

The percentage (%) of scavenging was calculated from the following equation.

$$\% \text{ of scavenging} = [(A_0 - A_1) / A_0] \times 100.$$

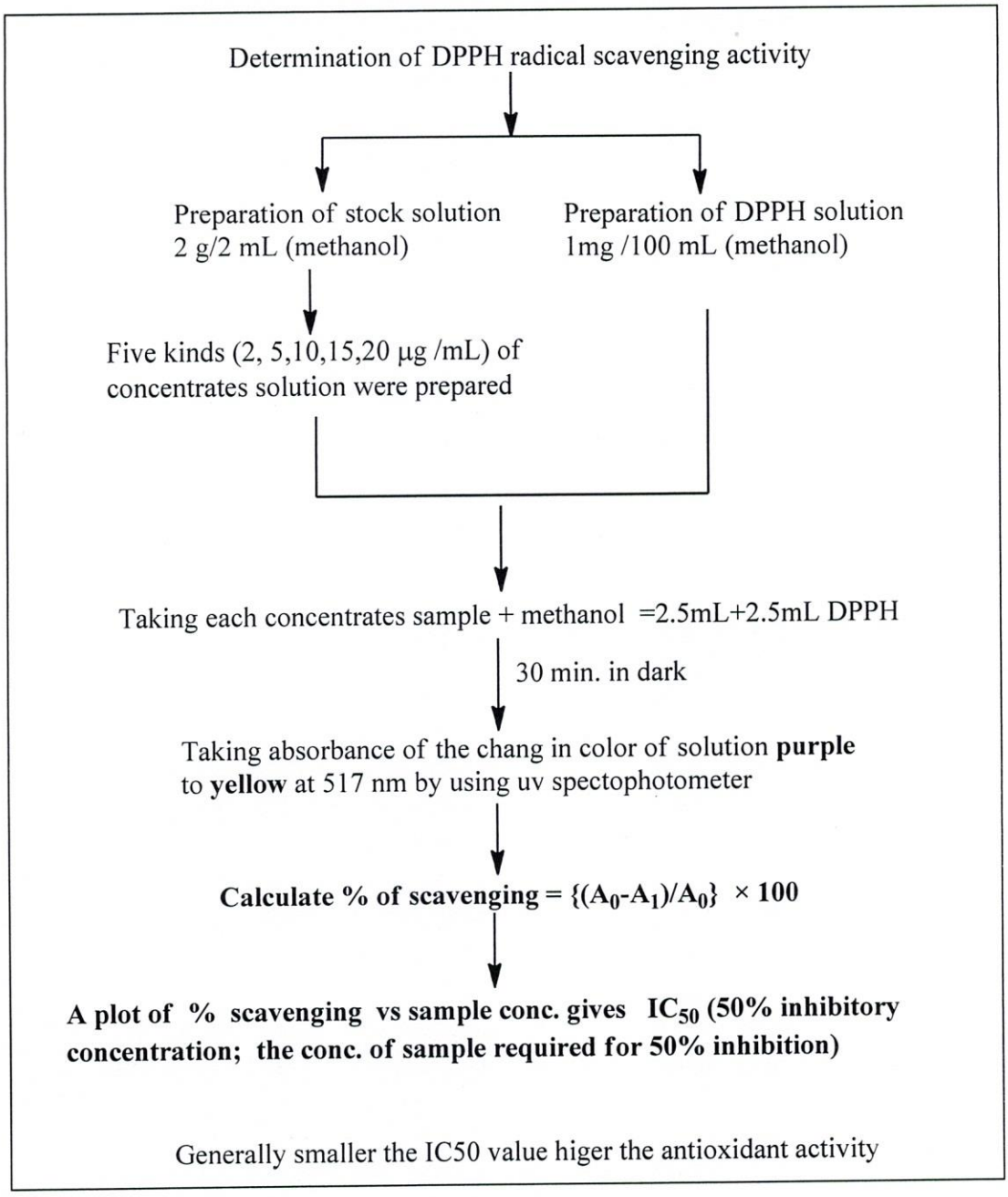
Where,

$A_0$ = the absorbance of the control, and

$A_1$ = the absorbance of the sample.

Then percent of scavenging were plotted against concentration using MS Excel software. Inhibitory concentration $IC_{50}$ value was calculated from the graph which indicates value conc. (µg/mL) at 50% inhibition. Percent inhibition, $IC_{50}$ values are presented in tabular form in Chapter 4 and activity is discussed.

A Flowchart of Determination of DPPH radical scavenging activity

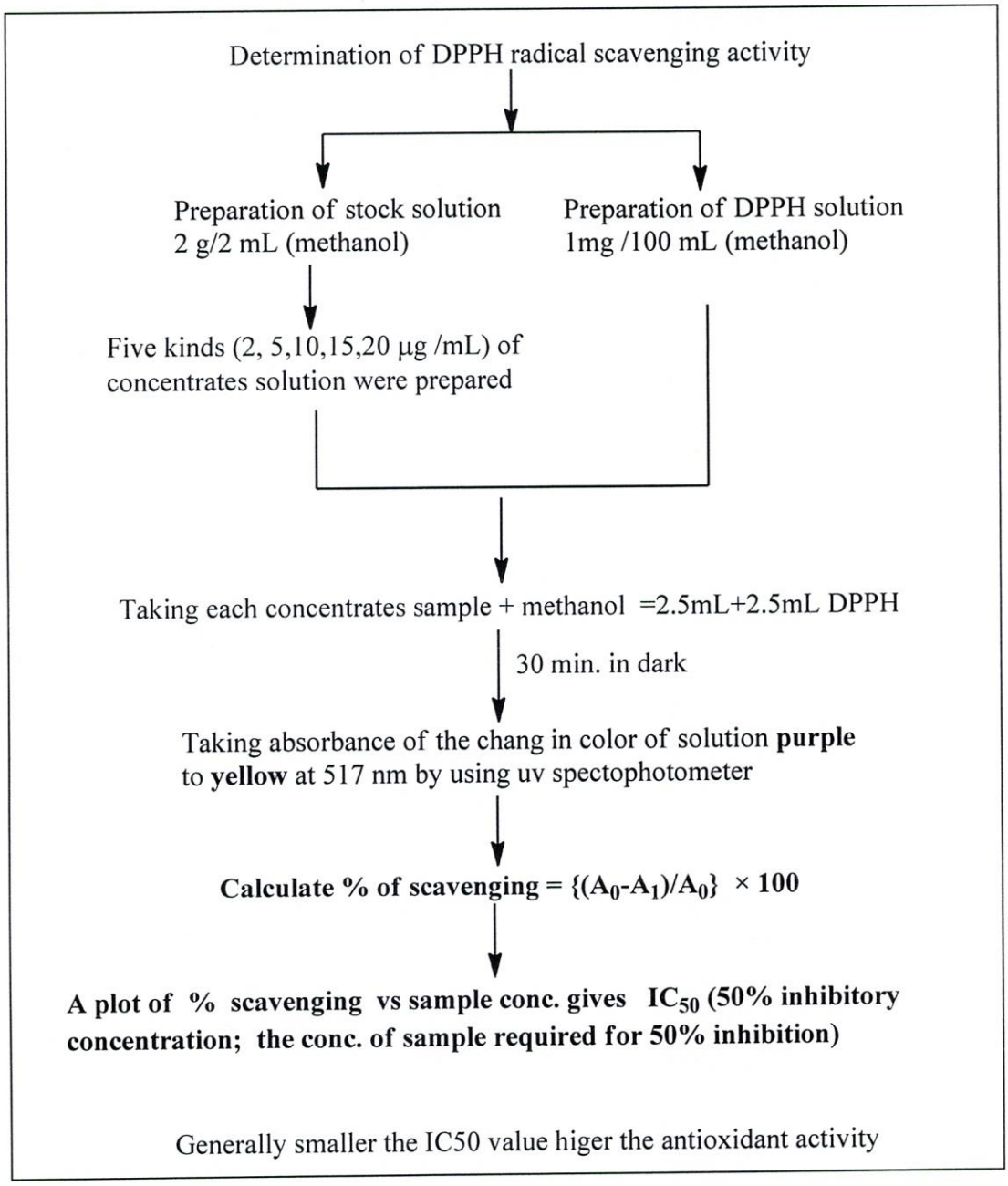



Scheme 16: Determination of DPPH radical scavenging activity

## 3.4 Reducing power capacity:

The reducing power of chalcones was determined by Oyaizu method. [31] It has been reported that there is a direct correlation between antioxidant activities and reducing power of certain compounds. The reducing properties

A Flowchart of Determination of DPPH radical scavenging activity




Scheme 16: Determination of DPPH radical scavenging activity

### 3.4 Reducing power capacity:

The reducing power of chalcones was determined by Oyaizu method. [31] It has been reported that there is a direct correlation between antioxidant activities and reducing power of certain compounds. The reducing properties

are generally associated with the presence of reductants, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. The presence of reductants such as antioxidant substances in the samples causes the reduction of the $Fe^{2+}$ - ferricyanide complex to the ferrous form by donating an electron. The amount of $Fe^{2+}$ complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

$$Fe^{3+}\text{- ferricyanide} + e^{-} = Fe^{2+}\text{ - ferricyanide}$$

### 3.4.1 Reagents

i. Potassum ferricyanide (MERCK, Germany)
ii. Trichloro acetic acid (MERCK, Germany)
iii. Ferric Chloride (sigma chemical company, USA)
iv. Ascorbic acid (sigma chemical company, USA)
v. Phosphate Buffer (sigma chemical company, USA)

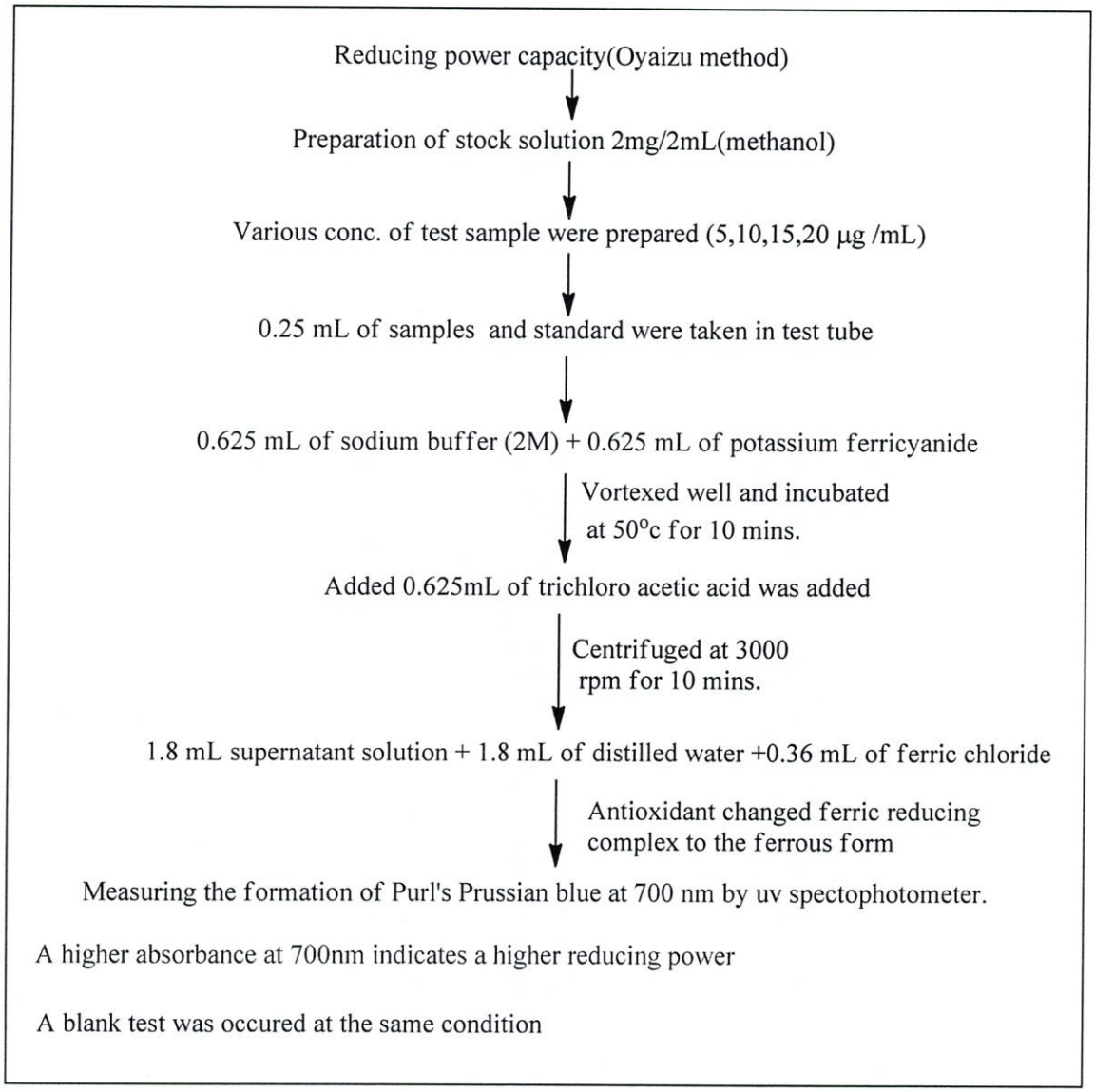
### 3.4.2 Procedure

i. 0.25 mL of samples and standard of different concentrations solution were taken in a test tube.
ii. 0.625 mL of sodium buffer (2M) and 0.625 mL of potassium ferricyanide were added to them and vortexed well.
iii. The reaction mixture was incubated at 50 °C for 10 mins. and 0.625 mL of trichloro acetic acid was added.
iv. The reaction mixture was centrifuged at 3000 rpm for 10 mins.
v. 1.8 mL supernatant solution was withdrawn and mixed with 1.8 mL of distilled water.
vi. 0.36 mL of ferric chloride was added to dilute the reaction mixture.

vii. Then the absorbance of the solution was measured at 700 nm using a spectrophotometer against a blank.

viii. A typical blank solution containing the same solution mixture without sample or standard was prepared and incubated under the same condition as the rest of the sample solution.

A Flowchart of Determination Reducing power capacity



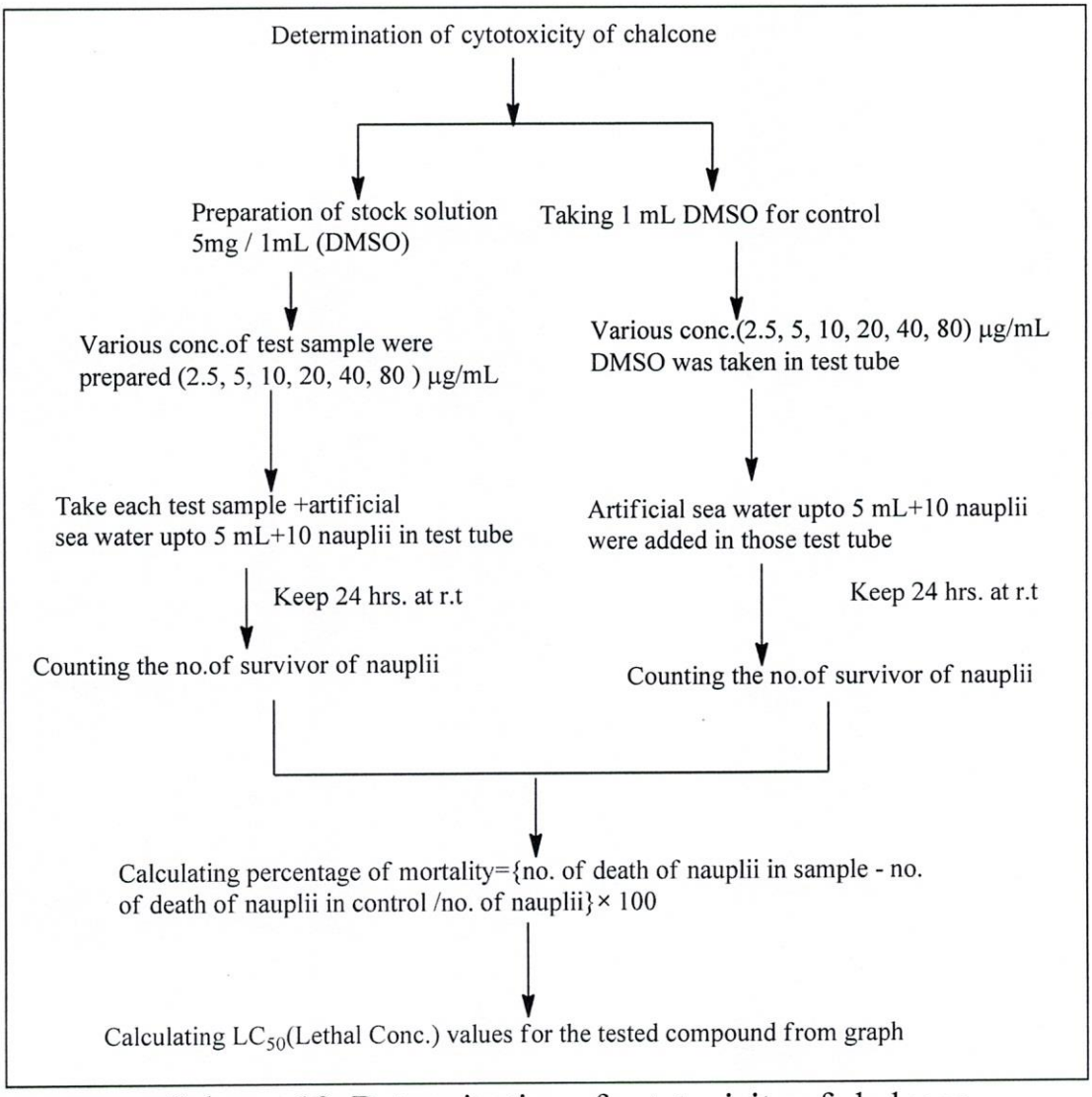

Scheme 17: Determination of Reducing power capacity

## 3.5 Cytotoxicity Bioassay [32]

*In vivo* lethality assay in a simple zoological organism, such as Brine shrimp lethality test (BST) has been applied as a simple and useful tool for preliminary screening[35, 36] of toxicity of physiologically active plant extract or synthesized compounds, detection of fungal toxins, heavy metal, pesticides and also cytotoxicity testing of dental materials. [35, 37] This general bioassay is rapid, reliable and has been used for over thirty years in toxicological studies. However, it has been demonstrated that a positive relationship exists between brine shrimp lethality and human carcinoma. Thus, BST can also be extrapolated for cell line toxicity and anti tumer activity. Principal of this method was based on the ability of certain compounds to kill laboratory cultured *Artemia* nauplii brine shrimp. BST is one of the simplest biological responses to monitor is lethality, since there is only one criterion: either dead or alive. It has been shown that *Artemia* is highly vulnerable to toxins at early development stages and assumed to exhibit their greatest sensitivity to test compounds. [35] Subsequently, in this study we used 24 hrs. nauplii as object experimental. Brine shrimp lethality bioassay was carried out to investigate cytotoxicity of the synthesized chalcones. Here *in vivo* lethality test were carried out using brine shrimp nauplii eggs *(Artemia salina Lech)*. Eggs were placed in a small tank containing 3.8% NaCl solution for hatching. After 48 hours of hatching period the nauplii were ready for the experiment. After hatching, active nauplii free from egg shells were collected with Pasteur pipette from tank and ready to be used for the assay. Then 5mg of each synthesized compound was accurately measured and dissolved in 1 mL of DMSO to get a concentration of 5mg/mL. From the stock solutions, a variety of solution conc. were prepared as 2.5, 5, 10, 20, 40 and 80 µg/mL (ppm) respectively. 10 Brine shrimp nauplii were then placed in each test tube using Pasteur pipette. For the control test of each test tube containing the same

volume of DMSO plus sea water up to 5 mL was used. After 24 hrs. of incubation the test tubes were observed using a magnifying glass and the number of survivors in each test tube were counted, noted and calculating percentages of death. Larvae were considered dead if they did not show any movement during several seconds of observation. [35] Lethal dose 50% ($LC_{50}$) values for the tested compounds were calculated from the graph. $LC_{50}$ value greater than 1000 ppm for plant extracts was considered inactive, whereas $LC_{50}$ values less than 30 ppm for pure compounds were considered toxic.

A Flowchart of Determination of cytotoxicity of chalcone




Scheme 18: Determination of cytotoxicity of chalcone

# CHAPTER FOUR

# RESULT AND DISCUSSION

# CHAPTER FOUR

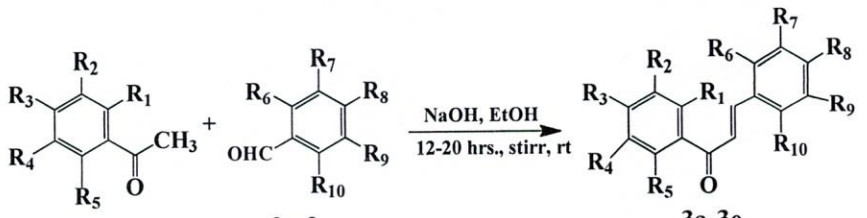
## *Results and Discussion*

### 4.1 Section A; Synthesis and Structure Elucidation

Part- I of this dissertation is concerned with the synthesis and structural elucidation of some hydroxyl, methoxy and prenyl chalcones e.g. 2′ - hydroxychalcone **(3a)**, 2′ - hydroxy - 4 - methoxychalcone **(3b)**, 2′ - hydroxy - 2, 4, 5 - trimethoxychalcone **(3c)**, 2′, 5′ - dihydroxy - 2, 4, 6 - trimethoxy chalcone **(3d)**, 4 - hydroxy -3′, 4′, 5′- trimethoxychalcone **(3e)**, 2′- hydroxy - 3′ - C - prenylchalcone **(4a)**, 2′- hydroxy - 5′ - C - prenylchalcone **(4b)**, 2′, 5′- dihydroxy - 2, 4, 6 - trimethoxy - 3′ - C - prenylchalcone **(4c).**

One of the starting materials β-resacetophenone **(1b)** has been prepared and confirmed by literature melting point, $R_f$ value and IR spectrum.

Among the above 2′ - hydroxychalcones **3a-3e** were synthesized by Claisen-Schmidt condensation according to scheme 7 using substituted acetophenone (**1a -1c**) and substituted benzaldehyde **(2a-2e).** The completion of reaction was monitored by using silica gel thin layer chromatography (TLC). The compounds were purified by recrystylation, PTLC or column cromatography.




1a-1c
acetophenone

2a-2e
benzaldehyde

3a-3e
chalcone

1a) $R_1$=-OH, $R_2$=$R_3$=$R_4$ = $R_5$=H
1b) $R_1$=$R_4$=-OH, $R_2$= $R_3$=$R_5$=H
1c)$R_1$=$R_5$=H,$R_2$=$R_3$= $R_4$=-$OCH_3$

2a) $R_6$=$R_7$= $R_8$= $R_9$=$R_{10}$=H
2b) $R_6$=$R_7$= $R_9$=$R_{10}$= H $R_8$= -$OCH_3$
2c) $R_6$= $R_8$=$R_9$=-$OCH_3$, $R_7$= $R_{10}$=H
2d) $R_6$=$R_8$=$R_{10}$=-$OCH_3$, $R_7$=$R_9$=H
2e)$R_6$=$R_7$=$R_9$=$R_{10}$=H $R_8$=-OH

3a) $R_1$= -OH, $R_2$=$R_3$=$R_4$=$R_5$=$R_6$= $R_7$=$R_8$=$R_9$=$R_{10}$=H
3b) $R_1$= -OH, $R_2$=$R_3$=$R_4$= $R_5$ =$R_6$=$R_7$=$R_9$=$R_{10}$=H,$R_8$=-$OCH_3$
3c) $R_1$= -OH, $R_2$=$R_3$=$R_4$=$R_5$= $R_7$ =$R_{10}$=H, $R_6$=$R_8$=$R_9$=-$OCH_3$
3d) $R_1$=$R_4$=-OH, $R_2$=$R_3$=$R_5$ =$R_7$=$R_9$ = H,$R_6$=$R_8$=$R_{10}$=-$OCH_3$
3e) $R_1$=$R_5$=$R_6$=$R_7$=$R_9$=$R_{10}$=H, $R_2$=$R_3$=$R_4$=-$OCH_3$, $R_8$=-OH

Scheme 7: Synthetic route for five synthesized chalcones **(3a-3e)** from their corresponding acetophenone **(1a-1c)** and aldehyde **(2a-2e)**

**3a** and **3d** were also undertaken by prenylation to produce their prenylated derivatives according to scheme 13 and scheme 14

Chalcones, a group of compounds with two aromatic rings connected by a keto-vinyl chain, -CO-CH=CH- so these have some unique structural features in spectroscopy.

The oxygenated chalcones usually possess UV absorption maxima in the range of 340-390 nm and chalcones lacking B-ring oxygenation may have their absorption at considerably shorter wavelengths and a minor peak usually appears in the range of 220-270 nm.

The infrared spectra of chalcones show usually a band near 1625-1650 $cm^{-1}$, characteristic of an α, β- unsaturated carbonyl group.

The α-H and β-H of chalcones resonate at δ 6.7 -7.4 and δ 7.3 -7.7 as two doublets ($J$ = 16-17 Hz) respectively in the $^1$H NMR spectra. This large $J$ value shows that the olifinic bond has *trans* geometry. In the $^{13}$C NMR

spectra of chalcones, the carbonyl carbon appears between δ 188.6 and 194.6. The α and β carbon atoms give rise to signals in between δ 116.1-128.1 and δ 136.9-145.4 respectively and can be readily identified by their characteristic appearance as a six line multiplet in the off resonance. The important spectral values are presented in tabular form (Table 7) and characteristics features were noticed.

Table 7: List of spectral data for some important functionality for compounds **(3a-3e, 4a-4c)**

| Comp no. | UV $\lambda_{max}$ ($CH_3OH$) nm | IR (KBr, $cm^{-1}$) | $^1H$ and $^{13}C$ NMR (δ ppm) |
|---|---|---|---|
| 3a | 196, 233 | 3414.06 (-OH), 3046.91 (C=C-H, olifinic, str.), 1640.01 (C=O conj. keto), 1618.00 (C=C, arm.) | 12.800 (s, 1H, $C_2OH$), 7.920 (d, $J$ = 14.8 Hz, 1H, $C_\beta$-H), 7.660 (d, $J$=14.8 Hz, 1H, $C_\alpha$-H) 193.77 (1C, >C=O), 145.49 (1C, $C_\beta$), 120.17 (1C, $C_\alpha$) |
| 3b | 197 | 3414.07 (-OH), 3024.51 (C=C-H, str.), 1640.50 (C=O), 1609.00 (C=C, arm. str.) | 12.800 (s, 1H, $C_2'$-OH), 7.910 (d, $J$=16 Hz, 1H, $C_\beta$-H ), 7.540 (d, $J$=16 Hz, 1H, $C_\alpha$-H), 3.870 (s, 3H, $C_4$-O$\underline{C}H_3$) 192.80 (1C, >C=O), 145.10 (1C, $C_\beta$), 118.70 (1C, $C_\alpha$), 55.80 (1C, $C_4$-O$\underline{C}H_3$) |
| 3c | 196, 259, 307 | 3416.30 (-OH), 3009.49 (C=C-H str.), 1660.01 (C=O), 1609.36 (C=C, arm.) | 13.080 (s, 1H, $C_{2'}$-OH), 8.170 (d, $J$=16 Hz, 1H, $C_\beta$-H ), 7.390 (d, $J$=16 Hz, 1H, $C_\alpha$-H), 3.730 (s, 9H, $C_2$, $C_4$, $C_5$-O$\underline{C}H_3$) 187.01 (1C, >C=O), 142.80 (1C, $C_\beta$), 121.61 (1C, $C_\alpha$), 56.40 ($C_2$- O$\underline{C}H_3$), 56.30 (2C, $C_4$ and $C_5$-O$\underline{C}H_3$) |

| | | | |
|---|---|---|---|
| 3d | 196, 242, 384 | 3415.26 (-OH), 3000.50 (-C=C-H, str.), 1605.56 (C=O), 1548.81 (C=C, arm.) | 12.770 (s, 2H, $C_{2'}$ and $C_{5'}$-OH), 8.360 (d, $J$ =15.6 Hz, 1H, $C_\beta$-H), 7.910 (d, $J$ =16 Hz, 1H, $C_\alpha$- H), 3.920 (s, 6H, $C_2$ and $C_6$-O$\underline{C}H_3$), 3.860 (s, 3H, $C_4$-O$\underline{C}H_3$)<br>194.89 (1C, >C=O), 159.00 (1C, $C_{2'}$-OH), 157.69 (1C, $C_{5'}$-OH), 147.18 (1C, $C_\beta$), 123.93 (1C, Cα), 55.85 (2C, $C_2$ and $C_6$-O$\underline{C}H_3$), 55.40 (1C, $C_4$-O$\underline{C}H_3$) |
| 3e | 197 | 3413.88 (-OH), 3232.28 (-C=C-H, olifinic str.), 1640.58 (C=O, conj. keto), 1610.44 (C=C, arm.) | 9.850 (s,1H, $C_4$–OH), 7.770(d, $J$ = 14.8 Hz, 1H, $C_\beta$-H), 7.340 (d, $J$ =15.6 Hz, 1H, $C_\alpha$- H), 3.90 0(s, 9H, $C_{3'}$ $C_{4'}$ and $C_{5'}$ -O$\underline{C}H_3$)<br>189.67 (1C, >C=O), 158.35 (1C, $C_4$-OH),144.94 (1C, $C_\beta$),119.34 (1C, $C_\alpha$), 60.97(1C, $C_{4'}$-O$\underline{C}H_3$), 56.38 (2C,$C_{3'}$ and $C_{5'}$-O$\underline{C}H_3$) |
| 4a | 197 | | 7.830 (d, J =16.0 Hz, 1H, $C_\beta$-H), 7.680 (d, J =16.0 Hz, 1H, Cα-H), 5.500 (s, 1H, OH), 4.050-3.900 (m, 1H, $CH_2$-C$\underline{H}$), 3.400-3.200 (m, 2H, $CH_2$) 1.800 (s, $CH_3$,6H)<br>192.80(1C,>C=O),161.50(1C,$C_{2'}$OH),145 .10 (1C, $C_\beta$),118.70 (1C, $C_\alpha$),<br>24.60 (1C, -$CH_2$-CH=C($CH_3$)($\underline{C}H_3$) )<br>22.60 (1C, $\underline{C}H_2$-CH)<br>18.60 (1C, -$CH_2$-CH=C($\underline{C}H_3$)($CH_3$)) |

| | | | |
|---|---|---|---|
| 4b | 196, 241 | | 7.930 (d, $J$ =15 Hz, 1H, $C_\beta$-H), 7.550 (d, $J$ =15 Hz, 1H, Cα-H), 5.500 (s, 1H, OH), 5.200 (s, 1H, $CH_2$-C<u>H</u>), 3.700-3.600 (m, 2H, C<u>H</u>$_2$), 1.800 (s, 3H, -$CH_2$-CH=C($CH_3$)(<u>C</u>$H_3$)), 1.740 (3H, -$CH_2$-CH=C(<u>C</u>$H_3$)($CH_3$))<br>192.80(1C,>C=O),160.60(1C,$C_2$·OH),145.10(1C,$C_\beta$),123.00($CH_2$-<u>C</u>H), 118.700 (1C,$C_\alpha$),<br>24.60 (1C, -$CH_2$-CH=C($CH_3$)(<u>C</u>$H_3$))<br>22.60(1C, <u>C</u>$H_2$),<br>18.60(1C, -$CH_2$-CH=C(<u>C</u>$H_3$)($CH_3$)) |
| 4c | 196 | | 8.330 (d, $J$ =16.0 Hz, 1H, $C_\beta$-H), 7.420 (d, $J$ =16.0 Hz, 1H, $C_\alpha$-H), 5.350 (s, 2H, $C_{2'}$ and $C_{5'}$-OH), 5.750 (1H, $CH_2$-C<u>H</u>), 3.210 (2H, C<u>H</u>$_2$), 1.820 (s, $CH_3$,6H), 1.820 (s, 3H, -$CH_2$-CH=C($CH_3$)(<u>C</u>$H_3$)), 1.700 (s, 3H, -$CH_2$-CH=C(<u>C</u>$H_3$)($CH_3$))<br>192.80(1C,>C=O),154.10(1C,$C_2$·OH), 122.50(1C,$C_\beta$),123.00($CH_2$-<u>C</u>H), 121.30 (1C, $C_\alpha$),<br>24.60 (1C, -$CH_2$-CH=C($CH_3$)(<u>C</u>$H_3$))<br>28.10(1C, <u>C</u>$H_2$),<br>18.60(1C, -$CH_2$-CH=C(<u>C</u>$H_3$)($CH_3$)) |

## 4.2 Section B; Results for Antimicrobial Screening

Chalcones are a group of natural products found in fruits, vegetables, nuts, seeds and flowers as well as in teas and wines are important constituents of human diet. They have been demonstrated to possess many biological and pharmacological activities such as antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antimutagenic and antiallergic activities and inhibitory activities on several enzymes. [5,33]

In the present study to screen the antibacterial activities all the compounds **(3a-3e, 4a-4c)** were employed as test against four bacteria *Bacillus caerius* ($G^+$, $B_1$), *Staphyllococcus aureus* ($G^+$, $B_2$), *Escherichia coli* ($G^-$, $B_3$), *Agrobacterium Species* ($G^-$, $B_4$). The primary assay was performed by disc diffusion technique to classify the microorganism susceptible as well as resistant towards particular compounds. The bioactivity is expressed by the diameter of zone of inhibition in mm. Ciprofloxacin is used as standard drug. The result of Antibacterial screening is presented as tabular form in Table 8

Table 8: Result of the antibacterial activity of the compounds (**3a-3e, 4a-4c**) against *Bacillus caerius.* ($G^+$, $B_1$) at 250 ($\mu g\ disc^{-1}$)

| Comp. no | Molecular formula | Diameter of inhibition zone (mm) | *C-50 |
|---|---|---|---|
| 3a | $C_{15}H_{12}O_2$ | 24 | 40 |
| 3b | $C_{16}H_{14}O_3$ | 28 | |
| 3c | $C_{18}H_{18}O_5$ | 30 | |
| 3d | $C_{18}H_{18}O_6$ | **38** | |
| 3e | $C_{18}H_{18}O_5$ | 12 | |
| 4a | $C_{20}H_{20}O_2$ | 08 | |
| 4b | $C_{20}H_{20}O_2$ | 12 | |
| 4c | $C_{23}H_{26}O_6$ | 20 | |

* Ciprofloxacin-50

The trend for antibacterial (*Bacillus caerius, $G^+$, $B_1$)* activity at conc. 250µg $disc^{-1}$ **C-50 (40 mm) > 3d (38 mm) > 3c (30 mm) > 3b (28 mm) > 3a (24 mm) > 4c (20 mm) >3e, 4b (12 mm) > 4a (08 mm)**

*a) Bacillus caerius.* ($G^+$, $B_1$) [Figure-4]

The inhibition zones of *Bacillus caerius* due to the treatment of different compounds at the different concentrations are presented in Table 8. All the compounds **(3a-3e, 4a-4c)** showed inhibition zones at concentration 250 (µg $disc^{-1}$). It was found that the inhibition zones of compound **3c** and **3d** were more effective than that of the other compounds and similar to the standard.

Table 9: Results of the antibacterial activity of the compounds against *Staphyllococcus aureus* ($G^+$, $B_2$) at 250 (µg $disc^{-1}$)

| Comp. no | Molecular formula | Diameter of inhibition zone (mm) | *C-50 |
|---|---|---|---|
| 3a | $C_{15}H_{12}O_2$ | 20 | 42 |
| 3b | $C_{16}H_{14}O_3$ | **40** | |
| 3c | $C_{18}H_{18}O_5$ | 20 | |
| 3d | $C_{18}H_{18}O_6$ | 25 | |
| 3e | $C_{18}H_{18}O_5$ | 12 | |
| 4a | $C_{20}H_{20}O_2$ | 08 | |
| 4b | $C_{20}H_{20}O_2$ | 12 | |
| 4c | $C_{23}H_{26}O_6$ | 30 | |

* Ciprofloxacin-50

The trend for antibacterial (*Staphylococcus aureus,* $G^+$, $B_2$) activity at conc. 250 μg $disc^{-1}$

**C-50 (42 mm) > 3b (40 mm) > 4c (30 mm) > 3d (25 mm) > 3a, 3c (20 mm) > 3e, 4b (12 mm) > 4a (08 mm)**

b) *Staphyllococcus aureus* ($G^+$, $B_2$) [Figure-5]

The inhibition zones of *Staphyllococcus aureus* due to the treatment of different compounds at the different concentrations are presented in Table 9. It was found that the inhibition zone of compound **3b** was extremely high 40 mm as compared to that of standard (42 mm) compound. Other compounds show significant antibacterial activity.

Table 10: Results of the antibacterial activity of the compounds against *Escherichia coli.* ($G^-$, $B_3$) at 250 (μg $disc^{-1}$)

| Comp. no | Molecular formula | Diameter of inhibition zone (mm) | *C-50 |
|---|---|---|---|
| 3a | $C_{15}H_{12}O_2$ | 18 | 40 |
| 3b | $C_{16}H_{14}O_3$ | **30** | |
| 3c | $C_{18}H_{18}O_5$ | **30** | |
| 3d | $C_{18}H_{18}O_6$ | 20 | |
| 3e | $C_{18}H_{18}O_5$ | 06 | |
| 4a | $C_{20}H_{20}O_2$ | 06 | |
| 4b | $C_{20}H_{20}O_2$ | 06 | |
| 4c | $C_{23}H_{26}O_6$ | 26 | |

* Ciprofloxacin-50

The trend for antibacterial (***Escherichia coli***, $G^-$, $B_3$) activity at conc. 250 µg $disc^{-1}$

**C-50 (40 mm) > 3b, 3c (30 mm) > 4c (26 mm) > 3d (20 mm) > 3a (18 mm) > 3e, 4a, 4b (06 mm)**

c) *Escherichia coli.* ($G^-$, $B_3$) [Figure-6]

The inhibition zones of *Escherichia coli* due to the treatment of different compounds at the different concentrations are presented in Table 10. It was found that the inhibition zone of compound **3b, 3c** (30 mm) show fairly good activity as compared to that of standard (40 mm) compound. Other compounds relatively less susceptible towards this microorganism.

Table 11: Results of the antibacterial activity of the compounds against *Agrobacterium Species ($G^-$, $B_4$)* at 250 (µg $disc^{-1}$)

| Comp. no | Molecular formula | Diameter of inhibition zone (mm) | *C-50 |
|---|---|---|---|
| 3a | $C_{15}H_{12}O_2$ | 15 | 42 |
| 3b | $C_{16}H_{14}O_3$ | 20 | |
| 3c | $C_{18}H_{18}O_5$ | 15 | |
| 3d | $C_{18}H_{18}O_6$ | 24 | |
| 3e | $C_{18}H_{18}O_5$ | 07 | |
| 4a | $C_{20}H_{20}O_2$ | 08 | |
| 4b | $C_{20}H_{20}O_2$ | 08 | |
| 4c | $C_{23}H_{26}O_6$ | **40** | |

* Ciprofloxacin-50

The trend for antibacterial (*Agrobacterium Species, $G^-$, $B_4$)* activity at conc. 250 μg $disc^{-1}$

**C-50 (42 mm) > 4c (40 mm) > 3d (24 mm) > 3b (20 mm) > 3a, 3c (15 mm) > 4a, 4b (08 mm) > 3e (07 mm)**

d) *Agrobacterium Species ($G^-$, $B_4$)* [Figure-7]

The inhibition zones of *Agrobacterium Species* due to the treatment of different compounds at the different concentrations are presented in Table 11. All the compounds show better activity specially **4c** (40 mm) against the microorganism.

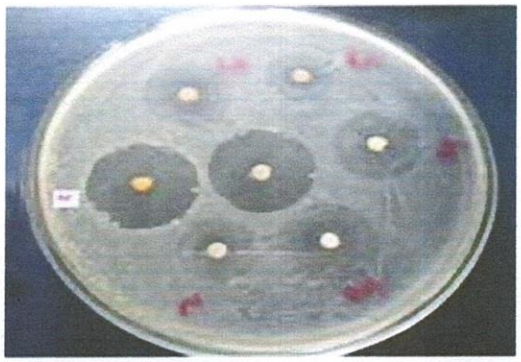


Figure 4: Photographic representation of the zone of inhibition at the concentration of 250 μg $disc^{-1}$ against *Bacillus caerius* ($G^+$, $B_1$)

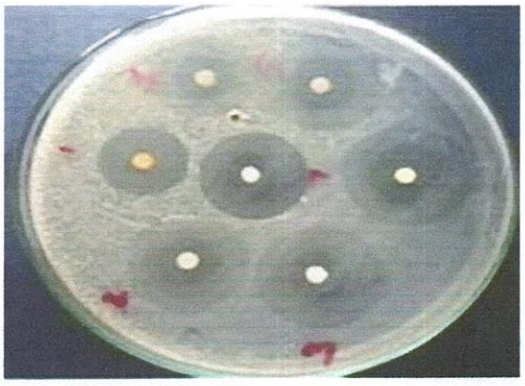


Figure 5: Photographic representation of the zone of inhibition at the concentration of 250 μg $disc^{-1}$ against *Staphyllococcus aureus* ($G^+$, $B_2$)

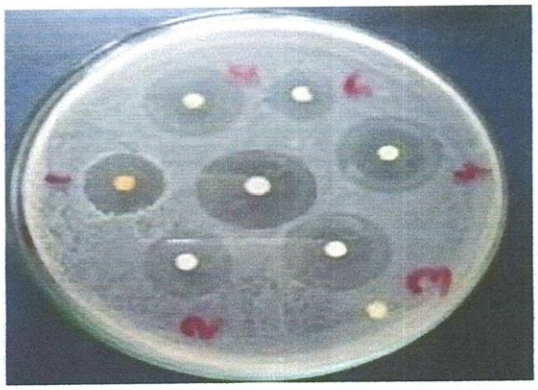


Figure 6: Photographic representation of the zone of inhibition at the concentration of 250 μg $disc^{-1}$ against *Escherichia coli* ($G^-$, $B_3$).

Figure 7: Photographic representation of the zone of inhibition at the concentration of 250 μg $disc^{-1}$ against *Agrobacterium Species* ($G^-$, $B_4$)

**3b, 3c** and **3d** exhibited fairly good potentialities against both $G^+$ and $G^-$ bacteria and in some cases about near to that of the standard drugs. Prenyl derivative of compound **3d**, compound **4c** shows very high activity against ($G^-$) pathogen than other prenyl derivatives. This led us to conclude that the presence of electron releasing hydroxyl (-OH) and methoxy groups ($-OCH_3$) are responsible for the better antimicrobial effects. **4c** contains lipophilic prenyl group and enhance cell membrane permeability thus more susceptible to ($G^-$). In **3e** three methoxy groups are occupied ring A and in three adjacent carbons. So steric effect may be a cause of poor activity.

## 4.3 Antioxidant Activity Screening by DPPH method

A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 1, 1-diphenyl-2 picrylhydrazyl (DPPH) radical. The electron in the DPPH free radical gives a strong absorption maximum at 517 nm and purple in color in methanolic solution. The color turns purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to no. of electrons captured. The activity is measured in terms of % inhibition and minimum inhibitory concentration $IC_{50}$ from graph. The % scavenging and $IC_{50}$ values are shown in Table 12.

Table 12: DPPH radical scavenging data of synthesized chalcones **(3a-3e, 4a, 4c)** and their corresponding $IC_{50}$ values

| **Compound no.** | **Conc. µg /mL** | **Absorbance at 517 nm** | **% Inhibition** | ***$IC_{50}$** |
|---|---|---|---|---|
| 3a | 2 | 0.210 | 23.36 | 54.94 |
| | 5 | 0.203 | 25.91 | |
| | 10 | 0.191 | 30.29 | |
| | 15 | 0.189 | 31.02 | |
| | 20 | 0.186 | 32.12 | |
| 3b | 2 | 0.184 | 32.85 | 36.80 |
| | 5 | 0.180 | 34.31 | |
| | 10 | 0.178 | 35.04 | |
| | 15 | 0.165 | 39.78 | |
| | 20 | 0.160 | 41.61 | |

| | | | | |
|---|---|---|---|---|
| 3c | 2 | 0.109 | 10.60 | 74.73 |
| | 5 | 0.233 | 14.96 | |
| | 10 | 0.223 | 18.61 | |
| | 15 | 0.216 | 21.17 | |
| | 20 | 0.220 | 19.71 | |
| 3d | 2 | 0.120 | 56.20 | **0.923** |
| | 5 | 0.110 | 59.65 | |
| | 10 | 0.093 | 66.06 | |
| | 15 | 0.082 | 70.07 | |
| | 20 | 0.076 | 72.26 | |
| 3e | 2 | 0.222 | 18.98 | 93.01 |
| | 5 | 0.219 | 20.07 | |
| | 10 | 0.216 | 21.17 | |
| | 15 | 0.210 | 23.36 | |
| | 20 | 0.205 | 25.18 | |
| 4a | 2 | 0.221 | 19.34 | 65.67 |
| | 5 | 0.213 | 22.26 | |
| | 10 | 0.208 | 24.08 | |
| | 15 | 0.201 | 26.64 | |
| | 20 | 0.197 | 28.10 | |
| 4c | 2 | 0.252 | 8.030 | 103.31 |
| | 5 | 0.240 | 12.41 | |
| | 10 | 0.235 | 14.23 | |
| | 15 | 0.232 | 15.33 | |
| | 20 | 0.230 | 16.06 | |

*Ascorbic acid is the standard and $IC_{50}$ 0.08 (µg /mL). The Table excludes the value of **4b**.

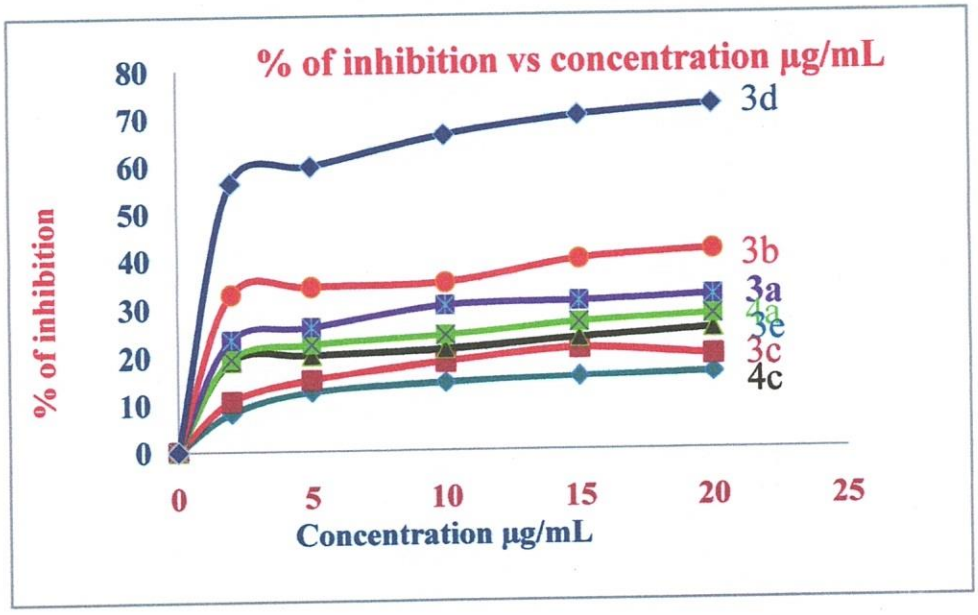



Figure 8: Graph showing DPPH radical scavenging of synthesized chalcones **(3a-3e, 4a, 4c)** without standard Ascorbic acid.

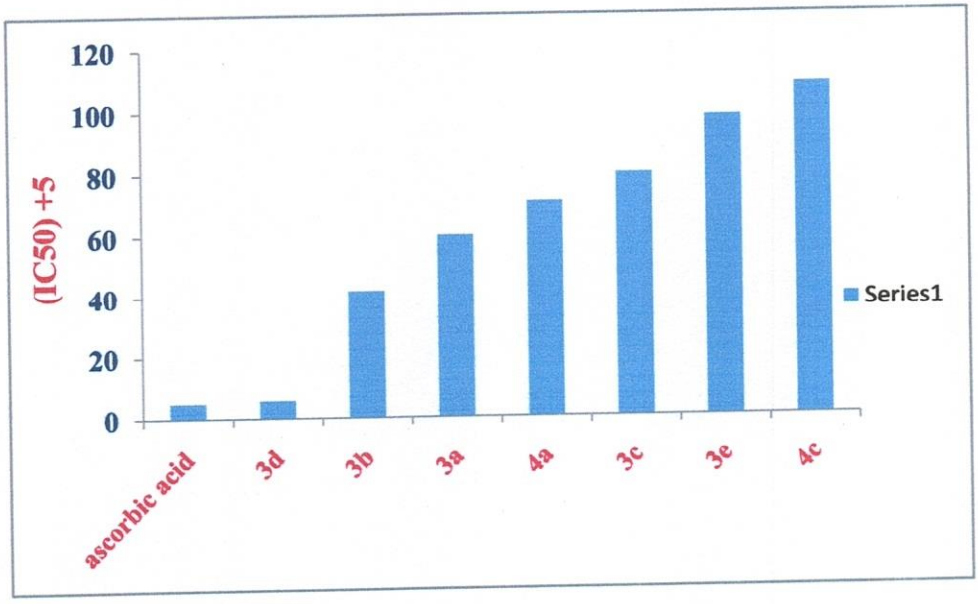



Figure 9: Comparison of **($IC_{50}$ + 5)** value of Ascorbic acid and synthesized chalcones **(3a-3e, 4a, 4c).** 5 is added with original $IC_{50}$ to display the smaller values in the graph.

Generally smaller the $IC_{50}$ value higer the antioxidant activity thus the compound **3d** showed highest activity **(0.923)**. As it contains two Phenolic -OH group and suppose to produce phenoxide free radical easily and stabilized by electronic group methoxy occupied *ortho* and *para* position of Ring B. Presence of prenyl group reduce this activity.

## 4.4 Reducing power capacity

It has been reported that there is a direct correlation between antioxidant activities and reducing power of chalcones. The reducing properties are generally associated with the presence of reductants, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. In this test the yellow color of the test solution changed to various shades of green and blue, depending on the reducing power of each sample. The presence of reductants i.e.-antioxidants caused the concentration of the $Fe^{3+}$/ferric reducing complex to the ferrous form. Therefore, by measuring the formation of Purl's Prussian blue at 700 nm we can monitor the $Fe^{2+}$ concentration; a higher absorbance at 700 nm indicates a higher reducing power. The result is shown in Table 13. The reducing power of all the samples increased gradually with the increase in concentrations of the samples.

Table 13: Reducing power of chalcones **(3a-3e, 4a-4c)** with standard Ascorbic acid

| Compound no. | Conc.(µg/mL) | Absorbance |
|---|---|---|
| 3a | 5 | 0.008 |
| | 10 | 0.014 |
| | 15 | 0.063 |
| | 20 | 0.078 |
| 3b | 5 | 0.079 |
| | 10 | 0.088 |
| | 15 | 0.099 |
| | 20 | 0.136 |
| 3c | 5 | 0.290 |
| | 10 | 0.561 |
| | 15 | 0.869 |
| | 20 | 1.106 |
| 3d | 5 | 0.044 |
| | 10 | 0.059 |
| | 15 | 0.063 |
| | 20 | 0.065 |
| 3e | 5 | 0.016 |
| | 10 | 0.060 |
| | 15 | 0.129 |
| | 20 | 0.155 |
| 4a | 5 | 0.004 |
| | 10 | 0.017 |
| | 15 | 0.037 |
| | 20 | 0.048 |

| | | |
|---|---|---|
| 4b | 5 | 0.200 |
| | 10 | 0.304 |
| | 15 | 0.342 |
| | 20 | 0.355 |
| 4c | 5 | 0.070 |
| | 10 | 0.219 |
| | 15 | 0.247 |
| | 20 | 0.260 |
| Ascorbic acid | 5 | 0.659 |
| | 10 | 0.914 |
| | 15 | 2.232 |
| | 20 | 2.395 |

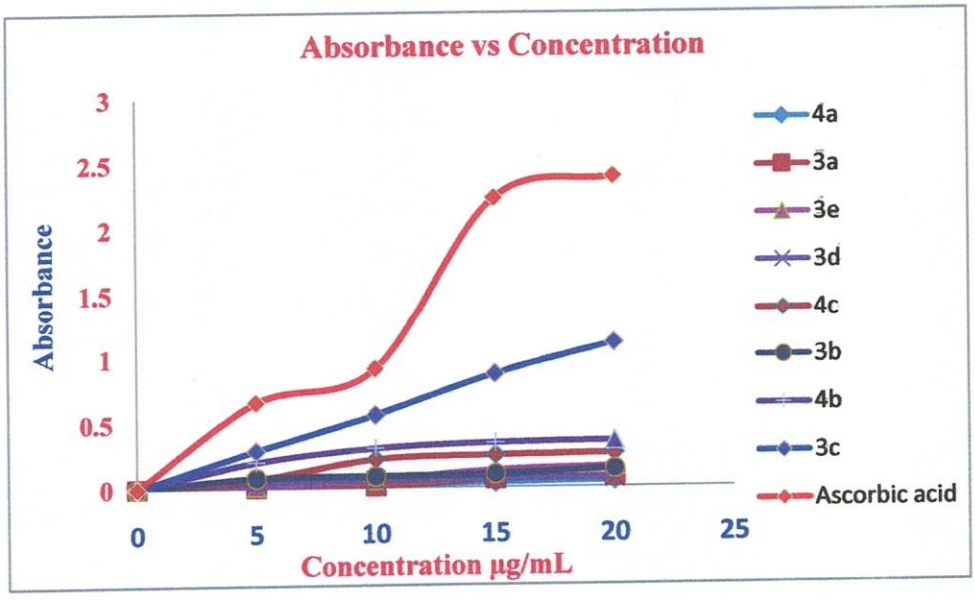



Figure 10: Reducing power of chalcones **(3a-3e, 4a-4c)** with standard Ascorbic acid

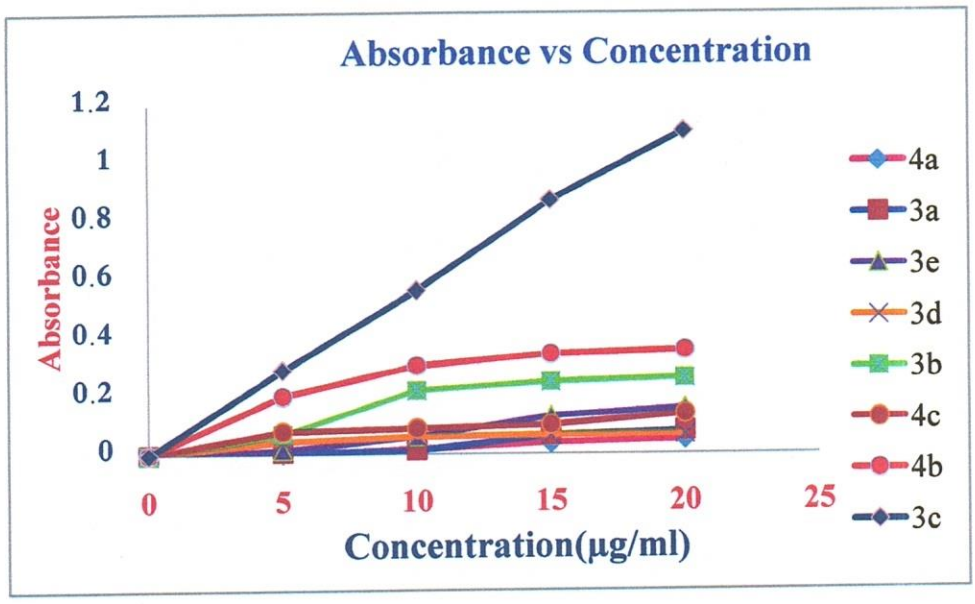



Figure 11: Reducing power of chalcones **(3a-3e, 4a-4c)** without standard

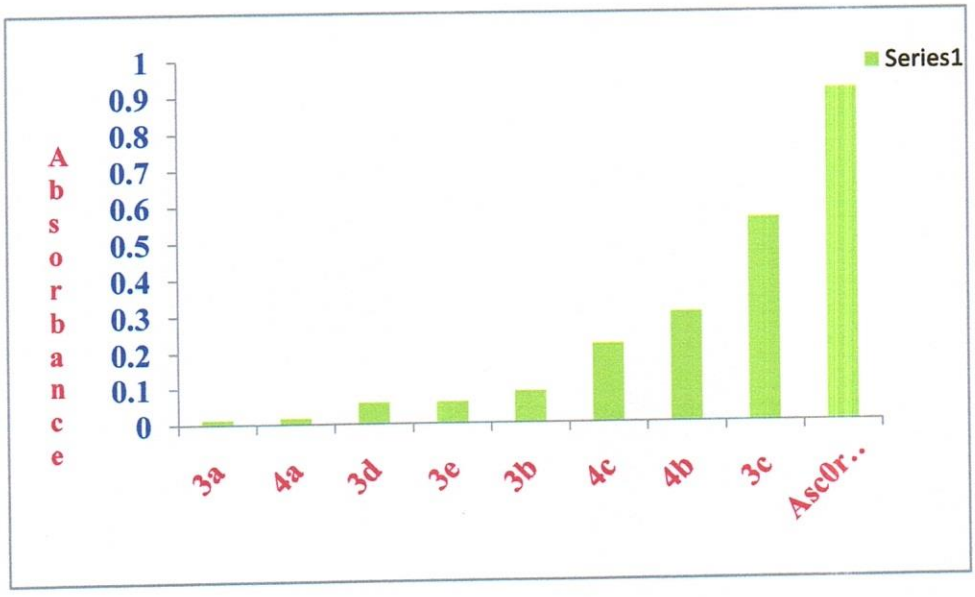



Figure 12: Reducing power of chalcones **(3a-3e, 4a-4c)** compare to standard Ascorbic acid in 10 µg/mL concentration

All the chalcones show appreciable reducing activity. Insertion of prenyl functionality increase reducing capacity.

## 4.5 Cytotoxic activity [6]

Brine shrimp lethality test (BST) has been applied as a simple and useful tool for preliminary screening of toxicity of physiologically active plant extract or synthesized compounds, detection of fungal toxins, heavy metal, pesticides and also cytotoxicity testing of dental materials. This general bioassay is rapid, reliable and has been used for over thirty years in toxicological studies. However, it has been demonstrated that a positive relationship exists between brine shrimp lethality and human carcinoma. Thus, BST can also be extrapolated for cell line toxicity and anti tumor activity. Principal of this method was based on the ability of certain compounds to kill laboratory cultured *Artemia nauplii* brine shrimp. BST is one of the simplest biological responses to monitor is lethality, since there is only one criterion: either dead or alive. It has been shown that Artemia is highly vulnerable to toxins at the early developmental stages and assumed to exhibit their greatest sensitivity to test compounds. Subsequently, in this study we used nauplii as object experimental.

Table 14: Brine Shrimp Lethality Assay of Chalcones **(3c-3e, 4a-4c)** in DMSO

| **Comp. no** | **Conc. μg /mL** | **% of mortality** | ***$LC_{50}$** |
|---|---|---|---|
| 3c | 2.5 | 11.11 | 54.22 |
| | 5 | 12.50 | |

| | | | |
|---|---|---|---|
| 3d | 2.5 | 11.11 | 71.75 |
| | 5 | 12.50 | |
| | 10 | 14.28 | |
| | 20 | 14.28 | |
| | 40 | 28.57 | |
| | 80 | 57.14 | |
| 3e | 2.5 | 33.33 | 10.10 |
| | 5 | 37.50 | |
| | 10 | 62.50 | |
| | 20 | 71.43 | |
| | 40 | 71.43 | |
| 4a | 2.5 | 55.55 | 0.27 |
| | 5 | 62.50 | |
| | 10 | 75.00 | |
| 4b | 2.5 | 44.44 | 1.61 |
| | 5 | 50.50 | |
| | 10 | 57.14 | |
| | 20 | 57.14 | |
| | 40 | 85.71 | |
| | 80 | 85.71 | |
| 4c | 2.5 | 33.33 | 29.38 |
| | 5 | 37.50 | |
| | 10 | 37.50 | |
| | 20 | 42.86 | |
| | 40 | 57.14 | |

*__3a__ and __3b__ do not show any presentable data and excluded from this table. Only those conc. of __3c-4c__ are included at which nauplii remain alive.

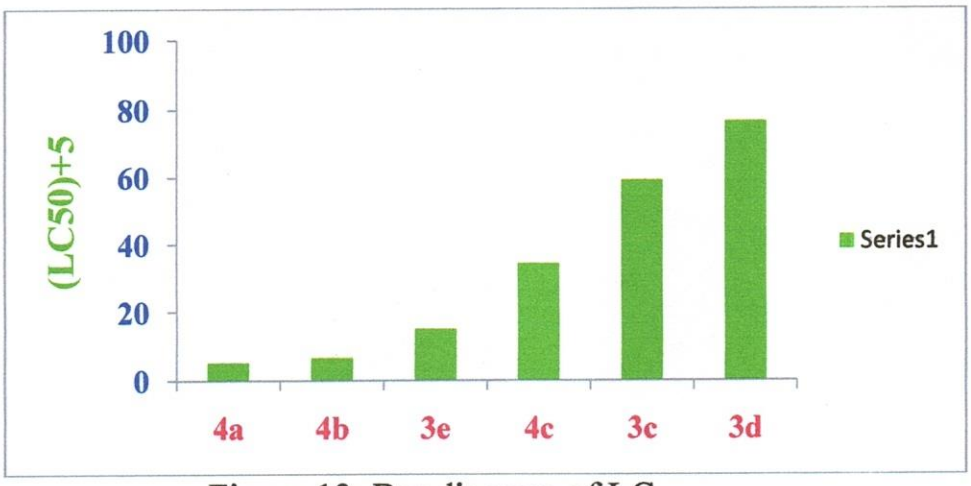



Figure 13: Bar diagram of $LC_{50}$

From literature review [6] it is known $LC_{50}$ value greater than 1000 ppm for plant extracts was considered inactive, whereas $LC_{50}$ values less than 30 ppm for pure compounds were considered toxic. All compounds **(3c-3e, 4a-4c)** showed a dose dependent cytotoxic activity at the tested concentrations. The $LC_{50}$ results of chalcone derivatives evaluated in this screening are listed in Table 14. Compound **3d** was the most active than other compounds, presenting the highest $LC_{50}$ of 71.75 ppm. **3c** also showed better activity $LC_{50}$ value 54.22 ppm. Whereas from the result **3e, 4a, 4b, 4c** showed poor activity. It is also known from the literature [6] chalcone containing *o*-methoxy substitution in ring-B reducing its ability to kill *Artemia nauplii* due to steric hindrance to the receptor of specimen. In **3c** and **3d** there are three methoxy functionality in ring B and in **3d** both the *ortho* potion are occupied by methoxy group so **3d** is least toxic. In **3c** only one *ortho* potion is occupied by methoxy group so it has lower $LC_{50}$ value than that of **3d** and slightly toxic compared to **3d. 4a, 4b, 4c** these three compounds contain lipophillc prenyl functionality which enhance cell membrane permeability and increase toxicity. Among these three prenylated chalcones **4c** contain *ortho, para* methoxy functionality and having the higher $LC_{50}$ value 29.38 ppm. The present study also revealed that **3a, 3b, 3e, 4a, 4b** synthesized compounds are toxic against *Artemia sp.* Therefore, these compounds should be studied furthermore for getting antitumor compounds.

# CHAPTER FIVE

# CONCLUSION

# CHAPTER FIVE

## *Conclusion*

Bangladesh is predominantly an agricultural country depending mainly on crop plants, agricultural, medicinal and forest product for its economic development. Although crops play a vital role in the economy of the country and agro ecological conditions are favorable for the production of various crops, the yields of crops is often poor. Among the various factors responsible for poor yield of crops, plant disease caused by various microorganisms play a significant role. Gradually men gathered sufficient knowledge of chemistry to inhibit or to kill the microorganism i.e., only inhibit the microbial growth are called 'statis'. But the chemicals which have ability to kill microorganisms are called 'cadal'. Various pesticides are classified as fungicides, viricides, bactericides etc.

Our previous study on synthesis of chalcone, hydroxychalcone, chalcone epoxide, flavone and oxygenated flavones which exhibiting a good antimicrobial activity have been reported for long. [21, 22] In the present study we are interested to extend our studies by the introduction of hydroxyl, methoxy and prenyl group at different position of benzene nucleus moiety of chalcone (Figure 1) which may give an important information about positional effect of these substituents and its role as biofuctionality.

Chalcones are the important constituent of many natural sources and have variety of biological activities. The synthetic technique is based on Claisen-Smith base catalyzed condensation of substituted acetophenone (**1a-1c**) with substituted benzaldehyde (**2a-2e**) shown in scheme 7. Thus we have successfully synthesized feight substituted chalcones. The parent chalcone is 2′ - hydroxychalcone **(3a)** and its methoxy derivatives ;2′ - hydroxy - 4 - methoxychalcone **(3b)**, 2′ - hydroxy - 2, 4, 5 - trimethoxychalcone **(3c)**, 2′, 5′

- dihydroxy - 2, 4, 6 - trimethoxychalcone **(3d)**, 4 - hydroxy -3′, 4′, 5′- trimethoxychalcone **(3e)**. **3a** and **3d** were also undertaken by prenylation to produce their prenylated derivatives; 2′- hydroxy - 3′ - C - prenylchalcone (**4a**), 2′- hydroxy - 5′ - C - prenylchalcone (**4b**), 2′, 5′- dihydroxy - 2, 4, 6 - trimethoxy - 3′ - C - prenylchalcone **(4c)**. The compounds were purified by recrystylation, PTLC or column cromatography. All the compounds provided a single spot in TLC analysis and possess a very sharp melting range, therefore it can be concluded that the synthesized compounds were pure. The structures of the synthesized compounds were confirmed by UV, IR, $^{1}H$ and $^{13}C$ NMR spectroscopic data. Chalcones, a group of compounds with two aromatic rings connected by a keto-vinyl chain, -CO-CH=CH- so these have some unique structural features which were noticed by analysis and important spectral values are presented in tabular form in Table 7.

Once the structure of a biological active compound is known the medicinal chemist is ready to move on to study the structure activity relationship by varying slightly from the original molecule and studying what effect this has on biological activity. The present study includes design a suitable method for the synthesis of chalcones, with varying hydroxy, methoxy and prenyl substituents and to provide SAR through antibacterial , antioxidant and cytotoxicity screening.

Compounds with electron releasing groups such as hydroxyl and methoxy showed better antibacterial activity than the others not having such groups. All the compounds were employed as test chemicals for *in vitro* antibacterial potentiality test against four pathogenic bacteria viz. *Bacillus caerius* ($G^+$, $B_1$), *Staphylococcus aureus* ($G^+$, $B_2$), *Eschericia coli* ($G^-$, $B_3$), *Agrobacterium Species* ($G^-$, $B_4$). The primary assay was performed by disc diffusion technique to classify the microorganism susceptible as well as resistant towards particular compounds. The bioactivity is expressed by the

diameter of zone of inhibition in mm and presented in Table 8-Table 11. Most of the compounds show moderately good antibacterial activity against both $G^+$ and $G^-$ bacteria.

The trend for antibacterial (*Bacillus caerius, $G^+$, $B_1$)* activity at conc. 250μg $disc^{-1}$

**C-50 (40 mm) > 3d (38 mm) > 3c (30 mm) > 3b (28 mm) > 3a (24 mm)> 4c (20 mm)>3e, 4b (12 mm) >4a (08 mm)**

The trend for antibacterial (*Staphylococcus aureus, $G^+$, $B_2$)* activity at conc. 250 μg $disc^{-1}$

**C-50 (42 mm) > 3b (40 mm) > 4c (30 mm) > 3d (25 mm) > 3a, 3c (20 mm) > 3e, 4b (12 mm) > 4a (08 mm)**

The trend for antibacterial (***Escherichia coli***, $G^-$, $B_3$) activity at conc. 250 μg $disc^{-1}$

**C-50 (42 mm) > 3b, 3c (30mm) > 4c (26 mm) > 3d (20mm)> 3a (18 mm)>3e, 4a, 4b (06mm)**

**3b, 3c, 3d** exhibited fairly good potentialities against both bacteria and in some cases about near to that of the standard drugs. Prenyl derivative of compound **3d**, compound **4c** show very high activity against ($G^-$) than other prenyl derivatives. This led us to conclude that the presence of electron releasing hydroxyl (-OH) and methoxy group ($-OCH_3$) are responsible for the antimicrobial effects. **4c** contains lipophilic prenyl group and more susceptible to ($G^-$). In **3e** three methoxy groups are occupied ring A and in three adjacent carbon. So steric effect may be a cause of poor activity.

In addition the synthesized chalcones **(3a-3e, 4a-4c)** were evaluated for *in vitro* antioxidant activity using diphenylpicrylhydrazyl (DPPH) model. Observation for antioxidant activity is expressed in terms of percent

scavenging of DPPH radical and the inhibitory concentration 50% ($IC_{50}$) is lowest for structure **3d**, 0.923 (μg/mL) at DPPH conc. 0.02% indicating highest activity and presented in Table 12. The compound **3d** contains two phenolic -OH group and suppose to produce phenoxide free radical easily and stabilized by electronic group methoxy occupied *ortho* and *para* position of aromatic ring attached to benzaldehyde part. Other structures showed good antioxidant activity as compared to the standard, ascorbic acid, $IC_{50}$ 0.08 (μg/mL). The antioxidant activity was also measured in another method using reducing power capacity. All the chalcones especially prenylated chalcone showed appreciable reducing activity and presented in Table 13, **3c** showed the highest value. Cytotoxic activities of the same compounds were undertaken *in vivo* by brine shrimp lethality test (BST) and expressed by lethal concentration 50%, ($LC_{50}$) and presented in Table 14. The compound **3d** showed the least toxicity having $LC_{50}$ 71.75 (μg/mL) whereas $LC_{50}$ values less than 30 ppm for pure compounds were considered toxic. Increment the no. of methoxy and hydroxy group causes higher $LC_{50}$ values among the compounds **(3a-3d)**. As steric hindrance effect causes better tolerance by reducing their killing ability. On the contrary prenylation causes cell membrane permeability and enhance toxicity. In **3e** three methoxy groups are occupied ring A and in three adjacent carbon. It shows poor activities in any sort of biological screening undertaken. All the examined compounds can be regarded as prosperous materials to introduce a safe, cheap and easy to prepare natural coloring agent for food and beverage industry, textile replacing costly natural dye or harmful azo dyes as well as antioxidant in food preservation and in cosmetic industry.

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# *APPENDIX*

# Appendix-A: List of Spectra

1. **Figure-1**: IR spectrum of 2′ - hydroxychalcone, **3a** in KBr $cm^{-1}$
2. **Figure-2:** $^{1}H$ NMR spectrum of 2′ - hydroxychalcone, **3a**, in $CDCl_3$, 400 MHz
3. **Figure-3:** $^{13}C$ NMR spectrum of 2′ - hydroxychalcone, **3a**, in $CDCl_3$, 100 MHz
4. **Figure-4:** IR spectrum of 2′ - hydroxy - 4 - methoxychalcone, **3b** in KBr $cm^{-1}$
5. **Figure-5:** $^{1}H$ NMR spectrum of 2′ - hydroxy - 4 - methoxychalcone, **3b** in $CDCl_3$, 500 MHz
6. **Figure-6:** $^{13}C$ NMR spectrum of 2′ - hydroxy - 4 - methoxychalcone, **3b** in $CDCl_3$, 125 MHz
7. **Figure-7:** IR spectrum of 2′ - hydroxy - 2, 4, 5 - trimethoxychalcone, **3c** in KBr $cm^{-1}$
8. **Figure-8:** $^{1}H$ NMR spectrum of 2′ - hydroxy - 2, 4, 5 - trimethoxychalcone, **3c** in $CDCl_3$, 400 MHz
9. **Figure-9:** $^{13}C$ NMR spectrum of 2′ - hydroxy - 2, 4, 5 - trimethoxychalcone, **3c** in $CDCl_3$, 100 MHz
10. **Figure-10:** IR spectrum of 2′, 5′ -dihydroxy - 2, 4, 6 - trimethoxychalcone, **3d** in KBr $cm^{-1}$
11. **Figure-11:** $^{1}H$ NMR spectrum of 2′, 5′ -dihydroxy - 2, 4, 6- trimethoxy chalcone, **3d** in $CDCl_3$, 400 MHz
12. **Figure-12:** $^{13}C$ NMR spectrum of 2′, 5′ -dihydroxy - 2, 4, 6- trimethoxy chalcone, **3d** in $CDCl_3$, 100 MHz
13. **Figure-13:** IR spectrum of 4 - hydroxy - 3′, 4′, 5′ - trimethoxychalcone, **3e** in KBr $cm^{-1}$

14. **Figure-14:** $^{1}H$ NMR spectrum of 4 - hydroxy - 3′, 4′, 5′ - trimethoxychalcone, **3e** in $CDCl_3$, 400 MHz
15. **Figure-15:** $^{13}C$ NMR spectrum of 4 - hydroxy - 3′, 4′, 5′ - trimethoxychalcone, **3e** in $CDCl_3$, 100 MHz
16. **Figure-16:** $^{1}H$ NMR spectrum of 2′ - hydroxy - 3′ - C - prenylchalcone, **4a** in $CDCl_3$, 500 MHz
17. **Figure-17:** $^{13}C$ NMR spectrum of 2′ - hydroxy - 3′ - C - prenylchalcone, **4a** in $CDCl_3$, 125 MHz
18. **Figure-18:** $^{1}H$ NMR spectrum of 2′ - hydroxy - 5′ - C - prenylchalcone, **4b** in $CDCl_3$, 500 MHz
19. **Figure-19:** $^{13}C$ NMR spectrum of 2′ - hydroxy - 5′ - C -prenylchalcone, **4b** in $CDCl_3$, 125 MHz
20. **Figure-20:** $^{1}H$ NMR spectrum of 2′, 5′ - dihydroxy -2, 4, 6 - trimethoxy - 3′- C -prenylchalcone, **4c** in $CDCl_3$, 400 MHz
21. **Figure-21:** $^{13}C$ NMR spectrum of 2′, 5′ - dihydroxy -2, 4, 6 - trimethoxy - 3′- C - prenylchalcone, **4c** in $CDCl_3$, 100 MHz

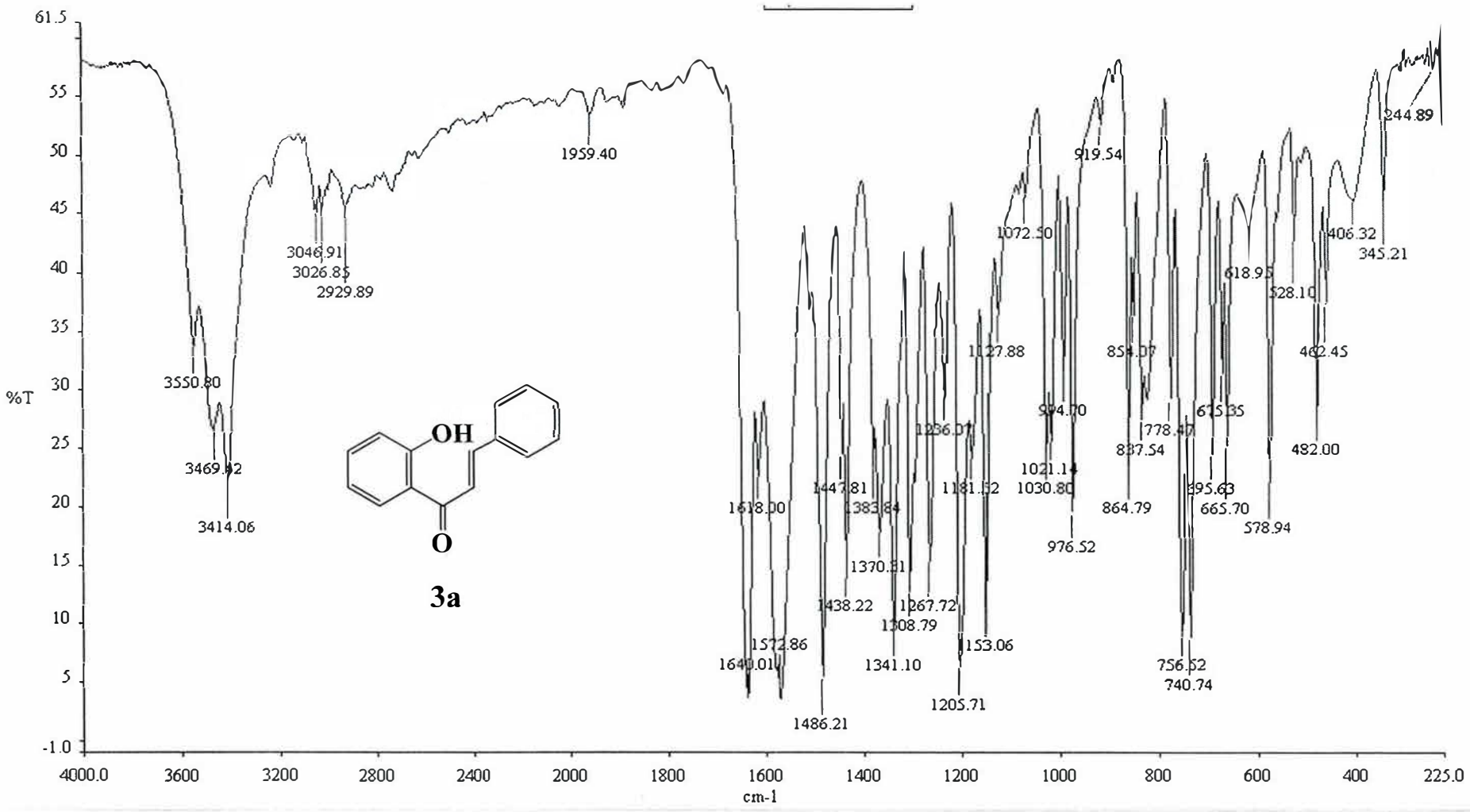


**Figure-1**: IR spectrum of 2′ - hydroxychalcone, **3a** in KBr $cm^{-1}$

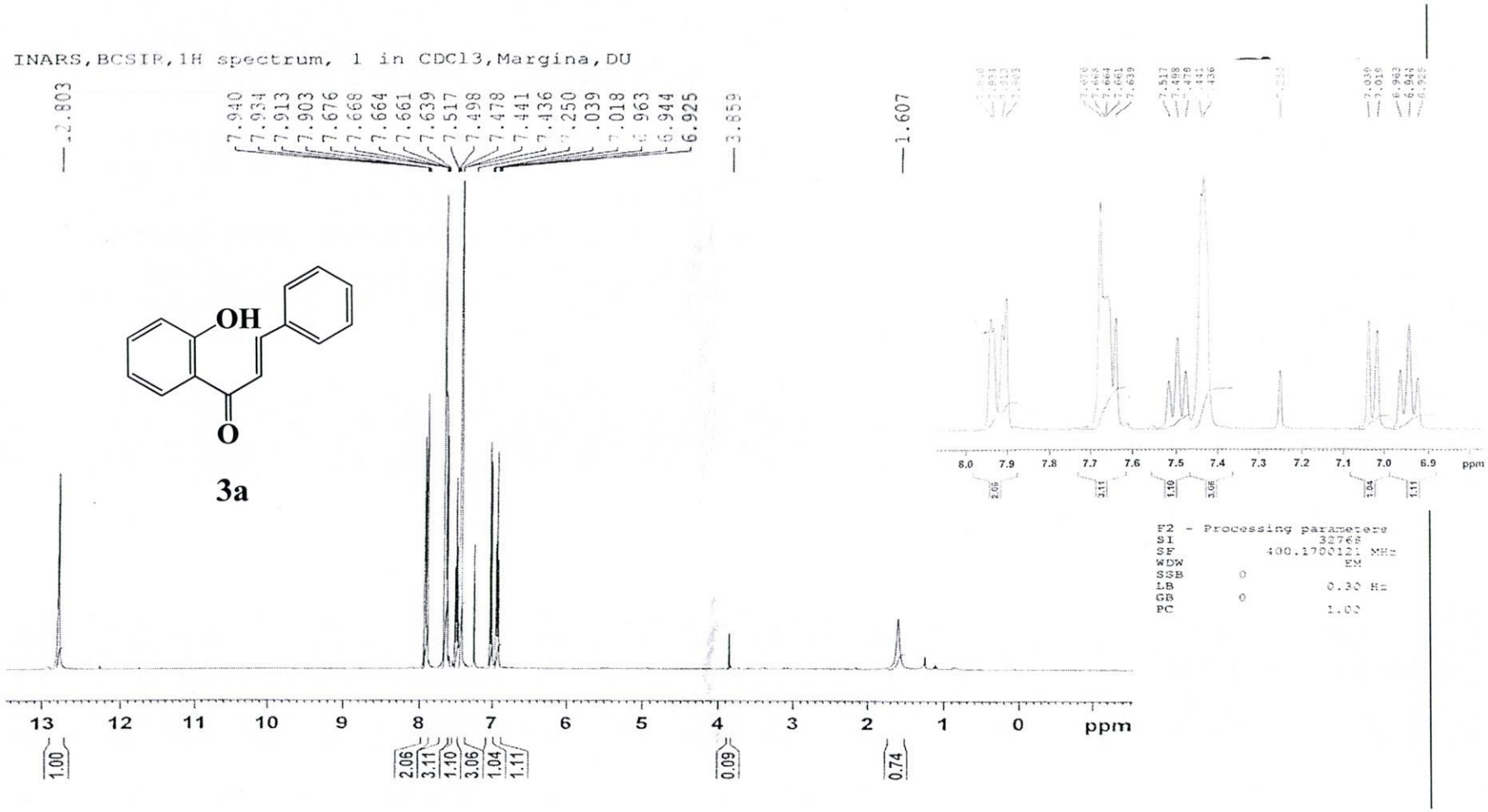


**Figure-2:** $^{1}$H NMR spectrum of 2′ - hydroxychalcone, **3a**, in $CDCl_3$, 400 MHz

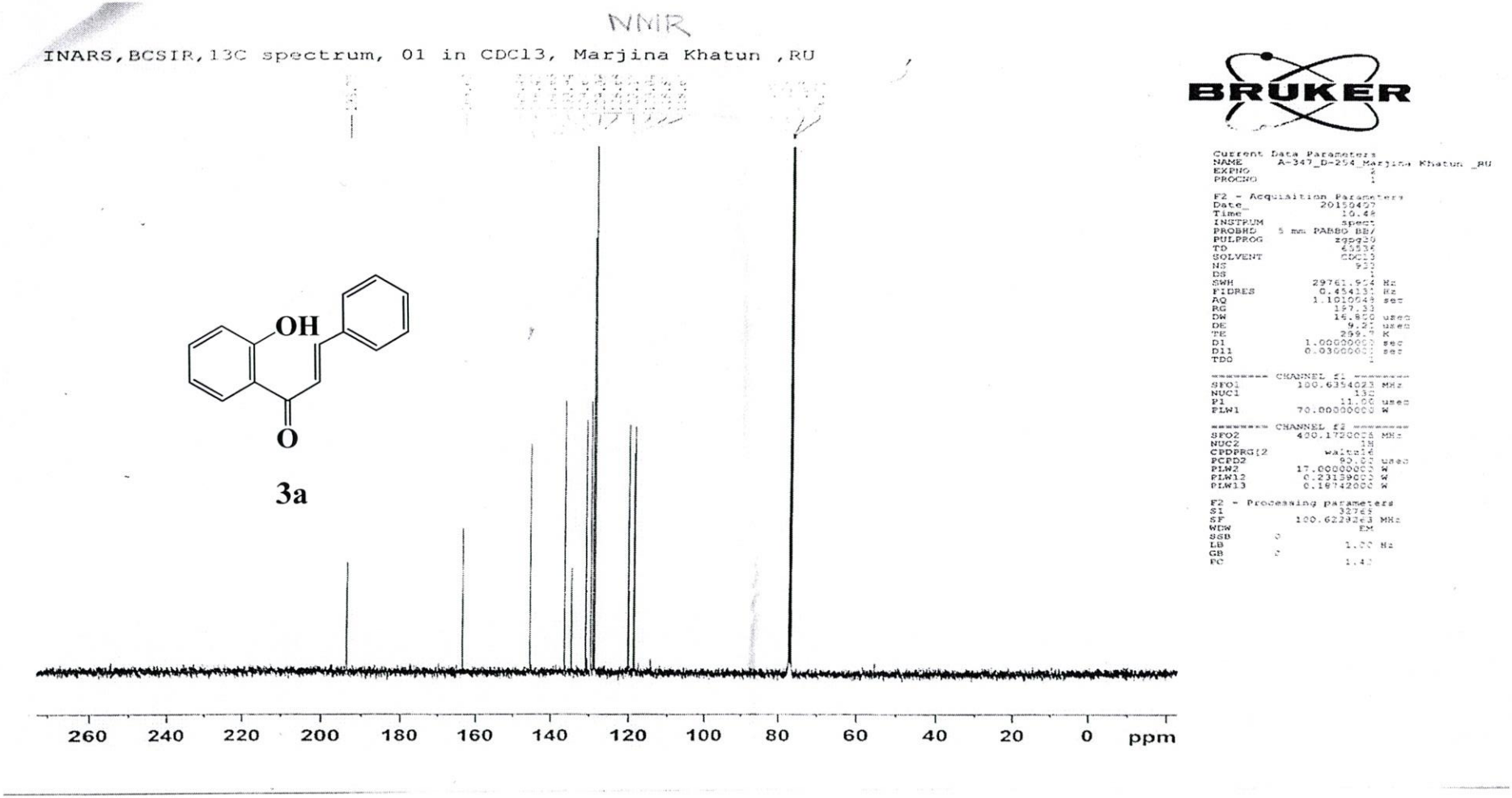


**Figure-3:** $^{13}C$ NMR spectrum of 2′ - hydroxychalcone, **3a**, in $CDCl_3$, 100 MHz

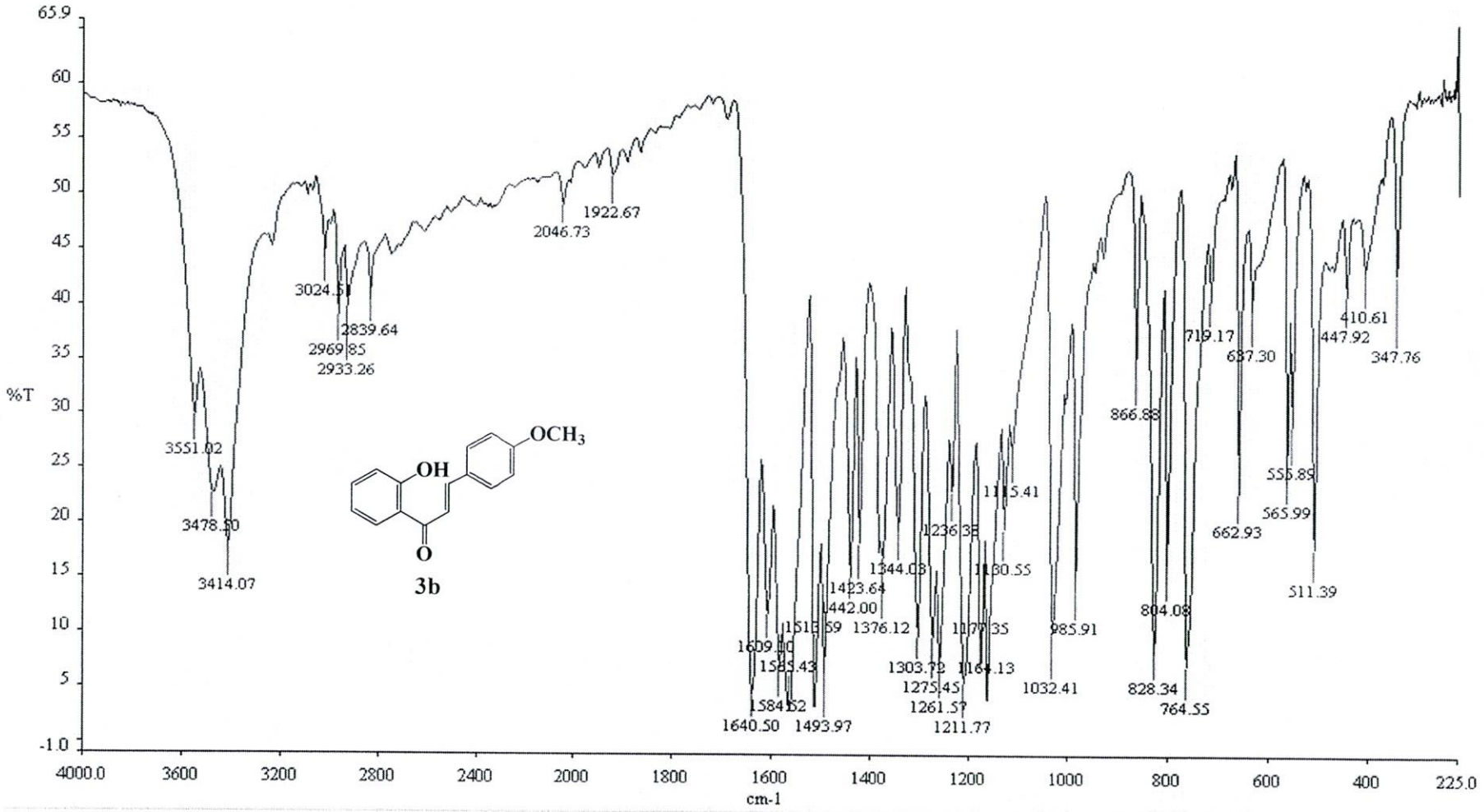


**Figure-4:** IR spectrum of 2′ - hydroxy - 4 - methoxychalcone, **3b** in KBr $cm^{-1}$

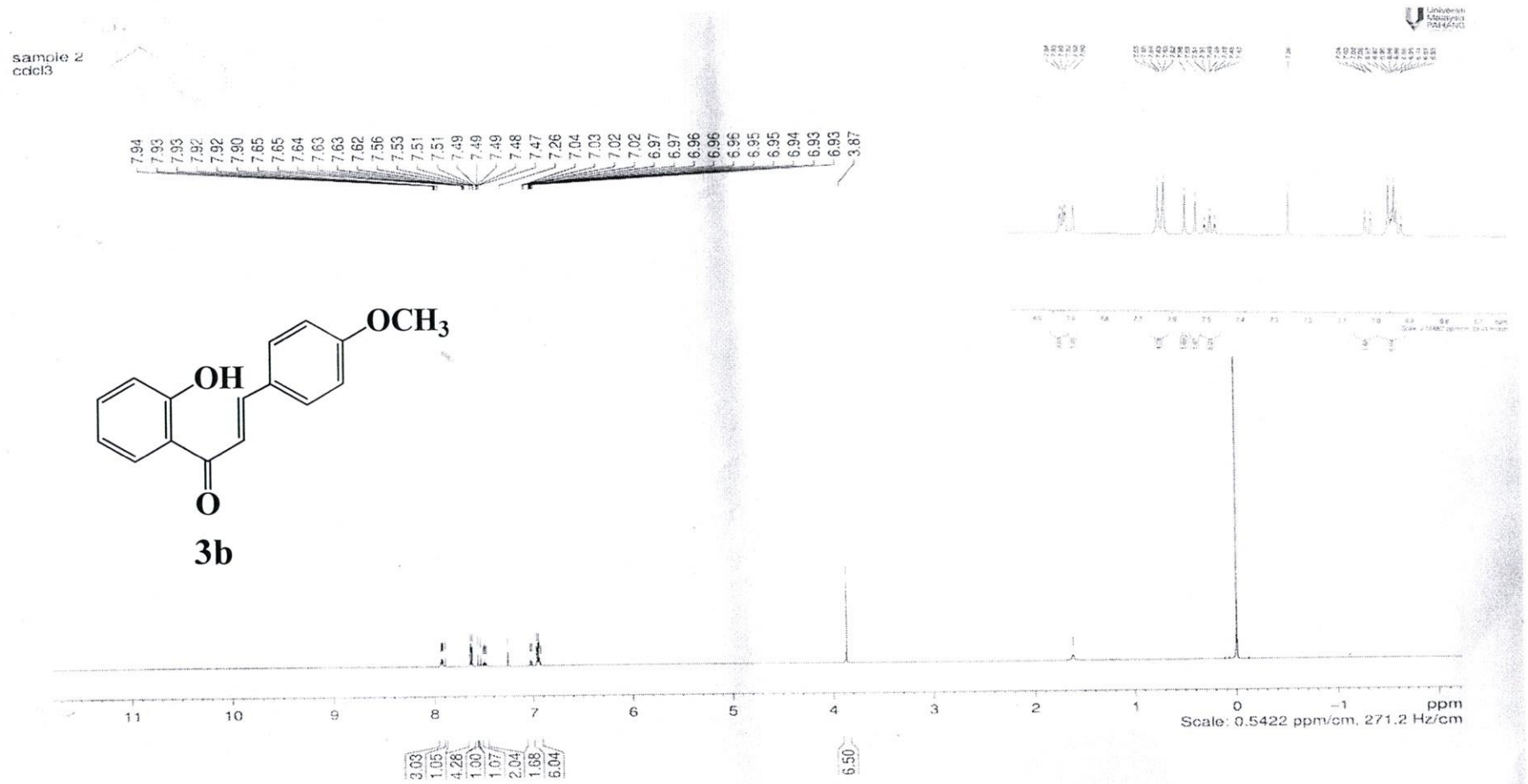


**Figure-5:** $^{1}$H NMR spectrum of 2′ - hydroxy - 4 - methoxychalcone, **3b** in $CDCl_3$, 500 MHz

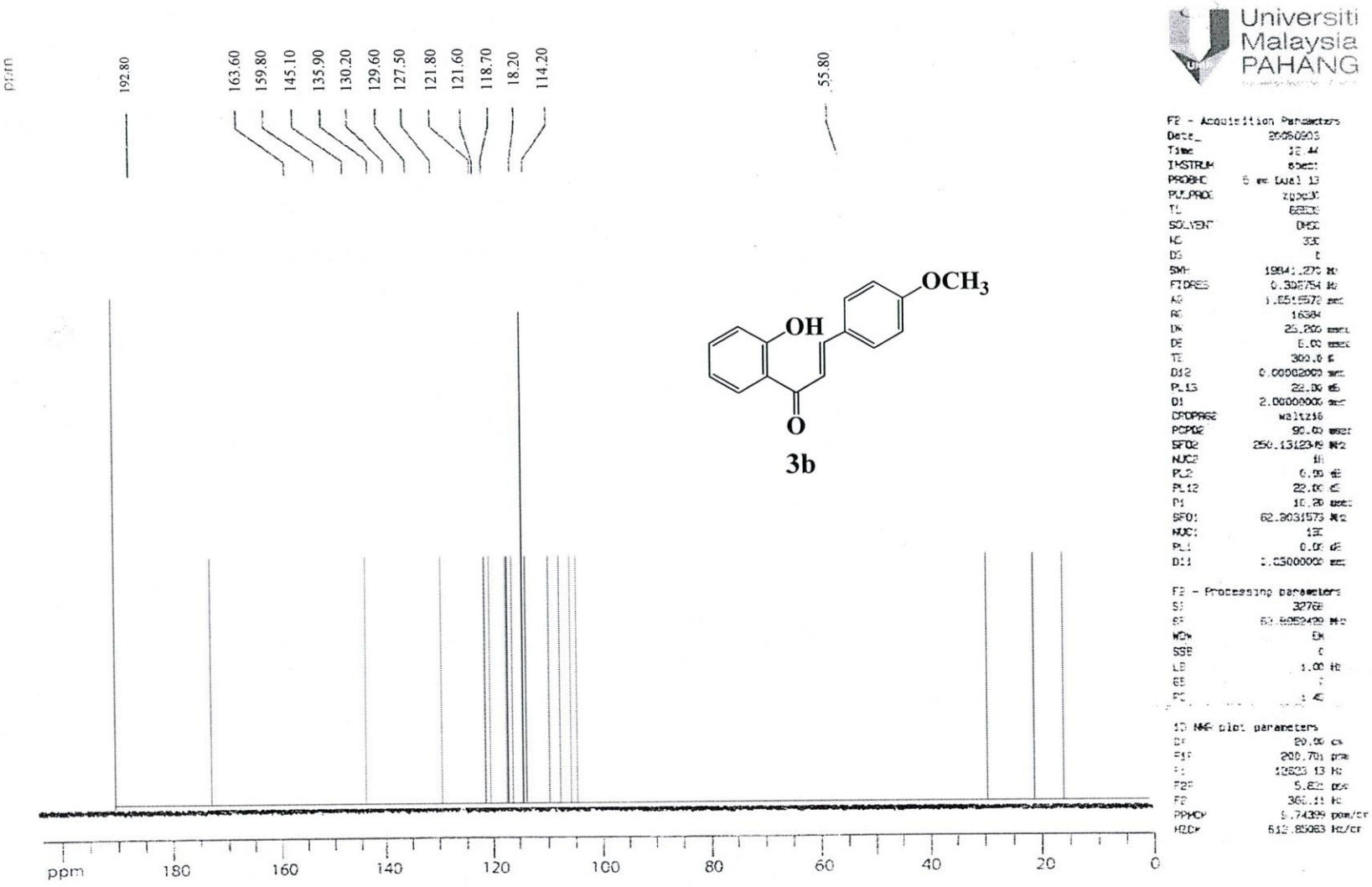


**Figure-6:** $^{13}C$ NMR spectrum of 2′ - hydroxy - 4 - methoxychalcone, **3b** in $CDCl_3$, 125 MHz

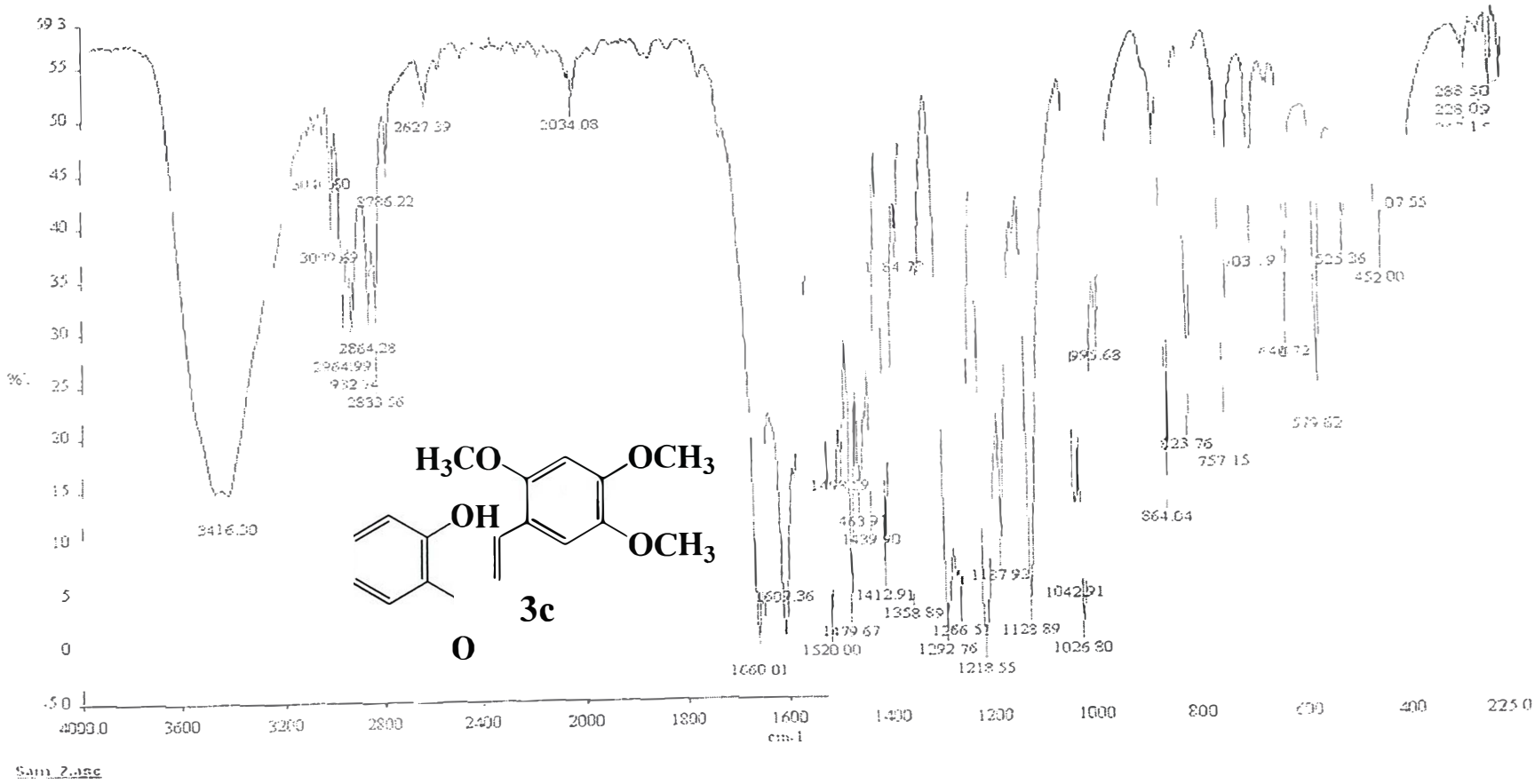


**Figure-7:** IR spectrum of 2′ - hydroxy - 2, 4, 5 - trimethoxychalcone, **3c** in KBr $cm^{-1}$

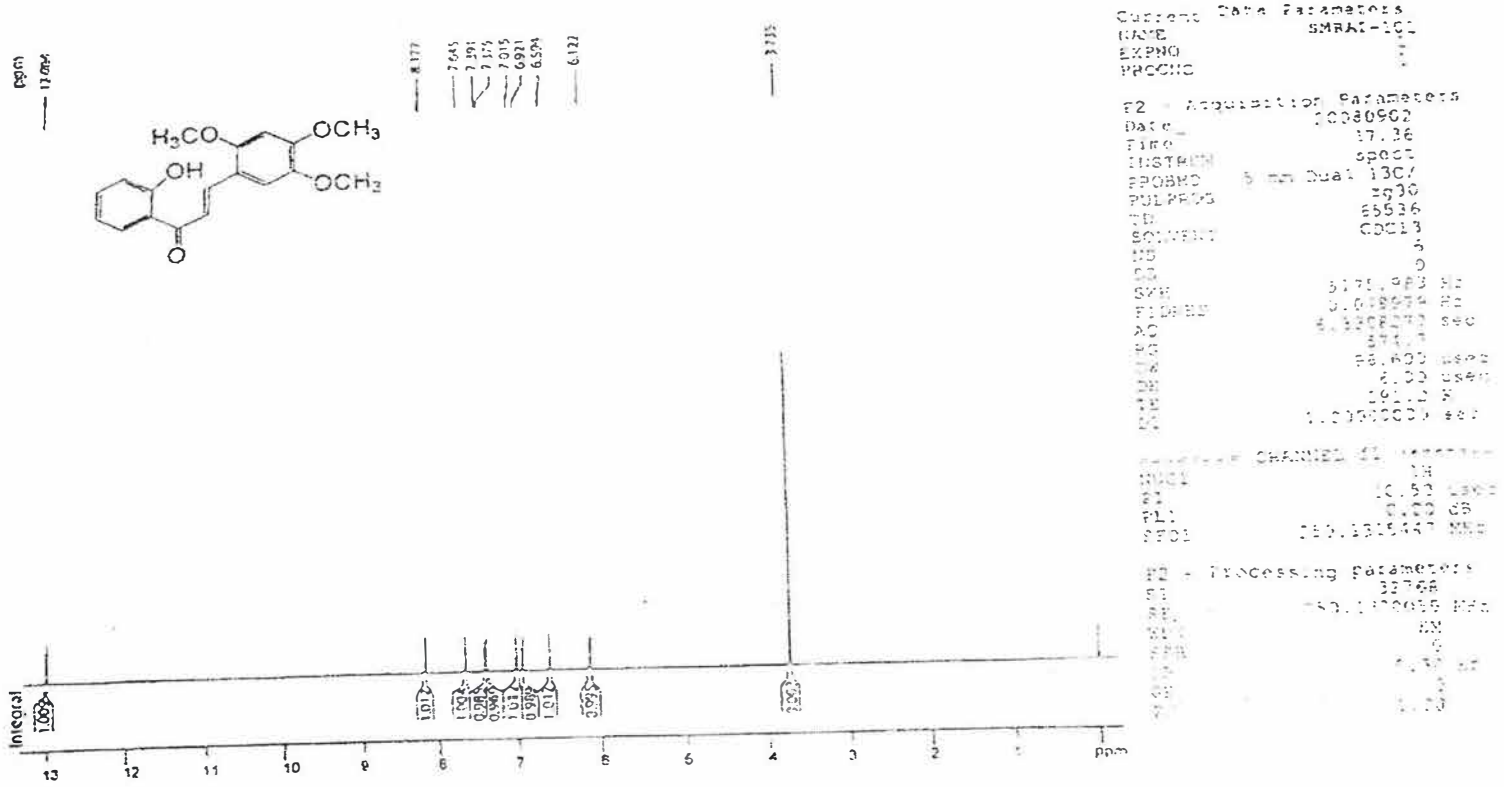


**Figure-8:** $^{1}H$ NMR spectrum of 2′ - hydroxy - 2, 4, 5 - trimethoxychalcone, **3c** in $CDCl_3$, 400 MHz

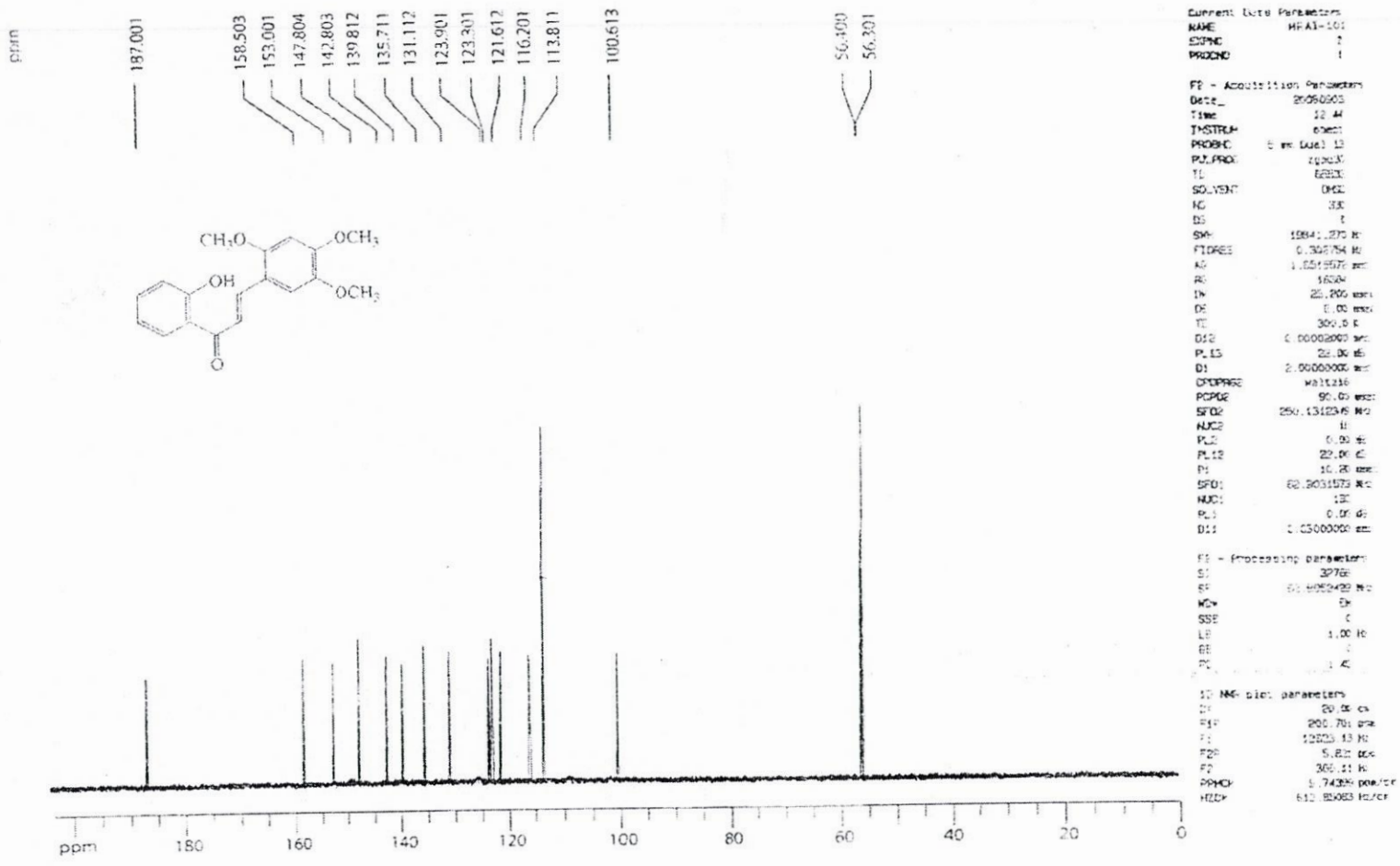


**Figure-9:** $^{13}C$ NMR spectrum of 2′ - hydroxy - 2, 4, 5 - trimethoxychalcone, **3c** in $CDCl_3$, 100 MHz

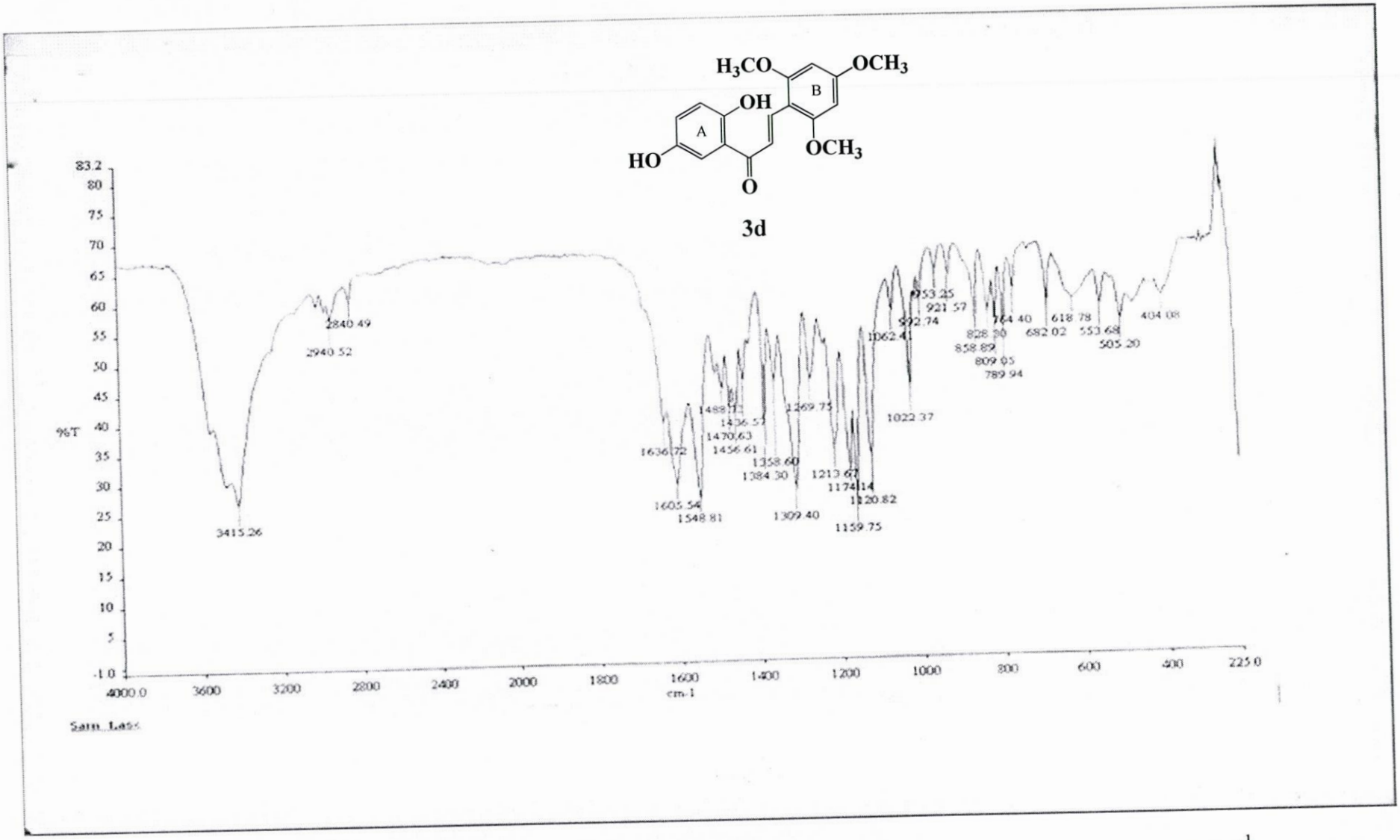


**Figure-10:** IR spectrum of 2′, 5′ -dihydroxy - 2, 4, 6 - trimethoxychalcone, **3d** in KBr $cm^{-1}$

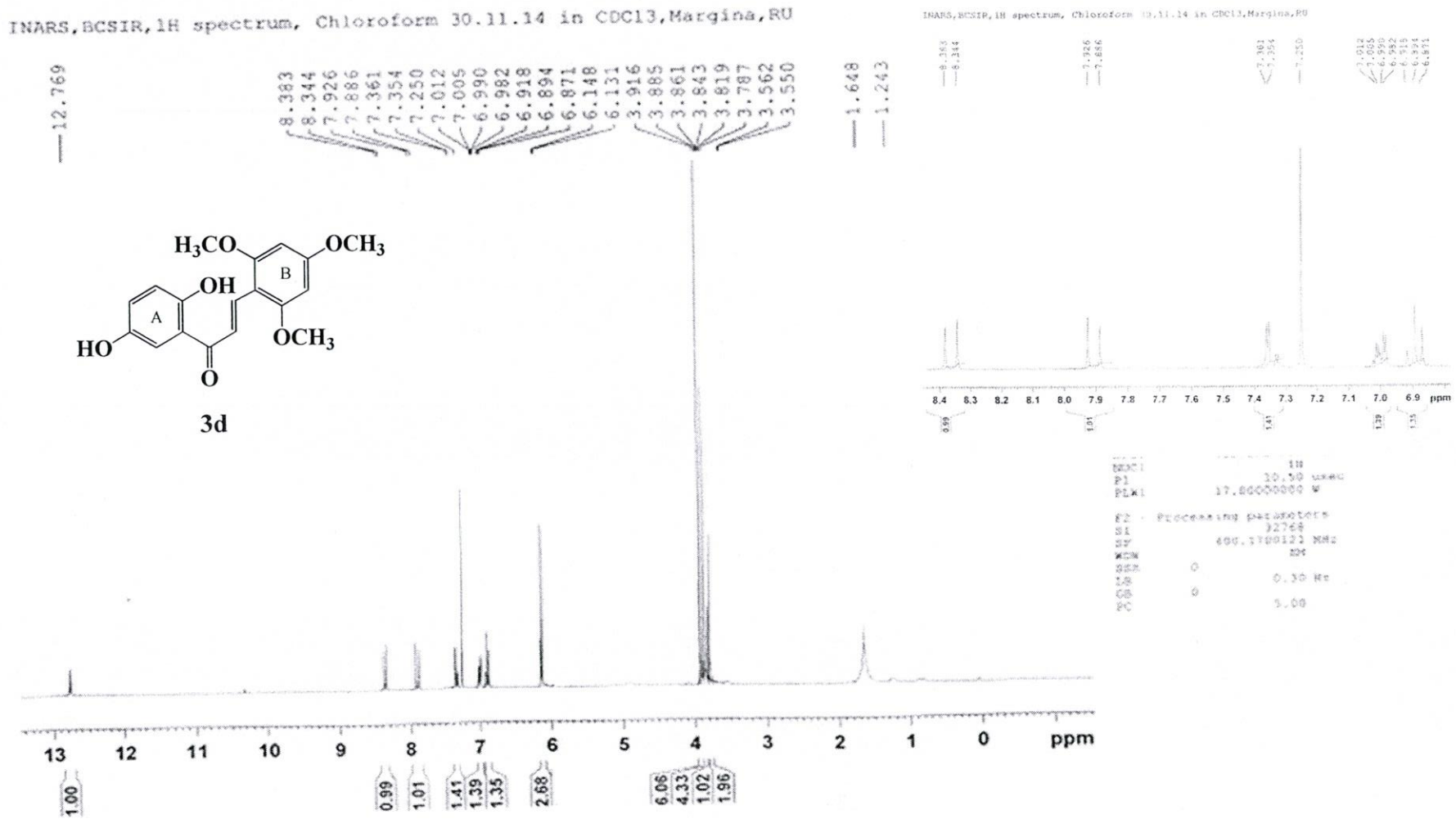


**Figure-11:** $^{1}$H NMR spectrum of 2′, 5′ -dihydroxy - 2, 4, 6- trimethoxy chalcone, **3d** in $CDCl_3$, 400 MHz

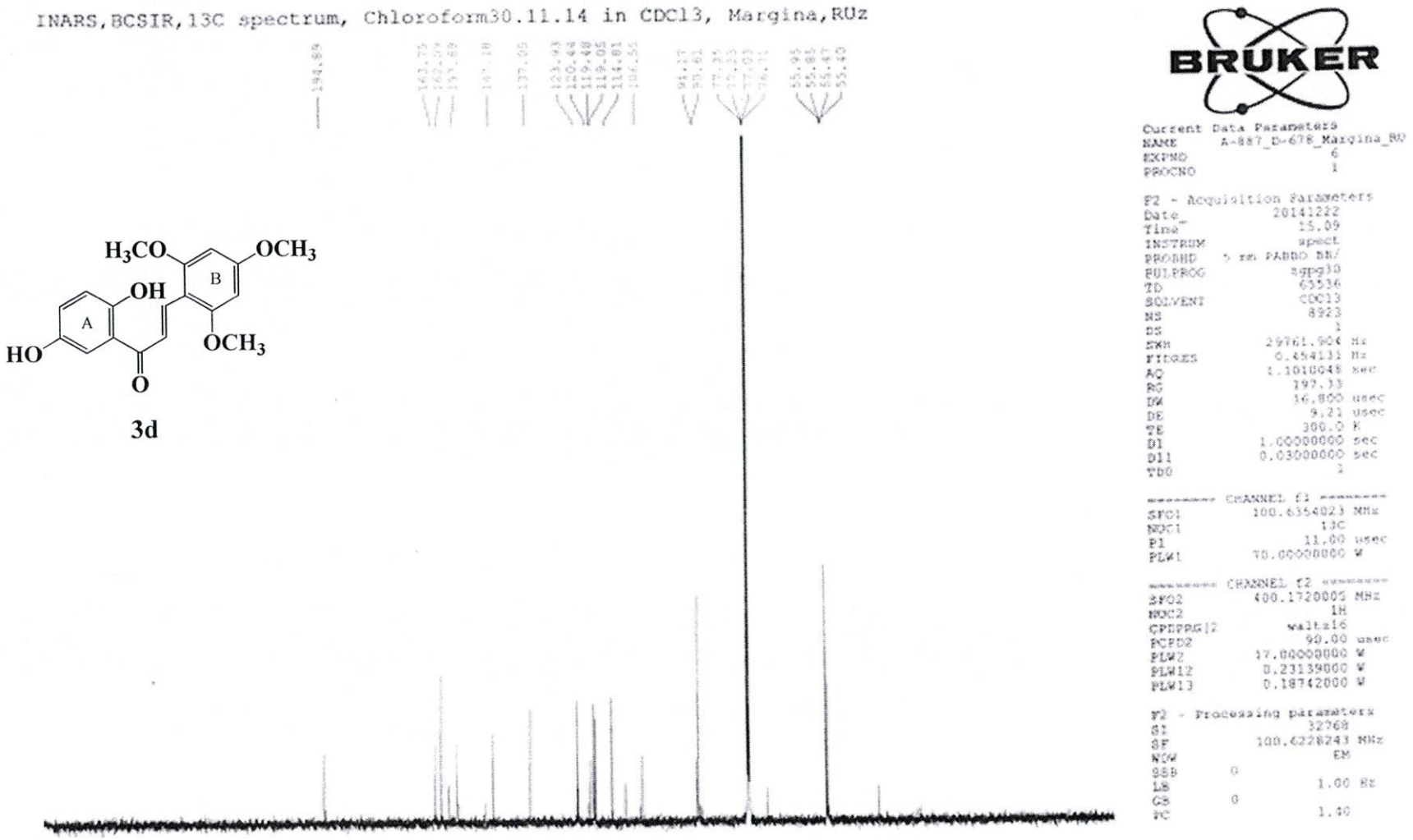


**Figure-12:** $^{13}$C NMR spectrum of 2′, 5′ -dihydroxy - 2, 4, 6- trimethoxy chalcone, **3d** in $CDCl_3$, 100 MHz

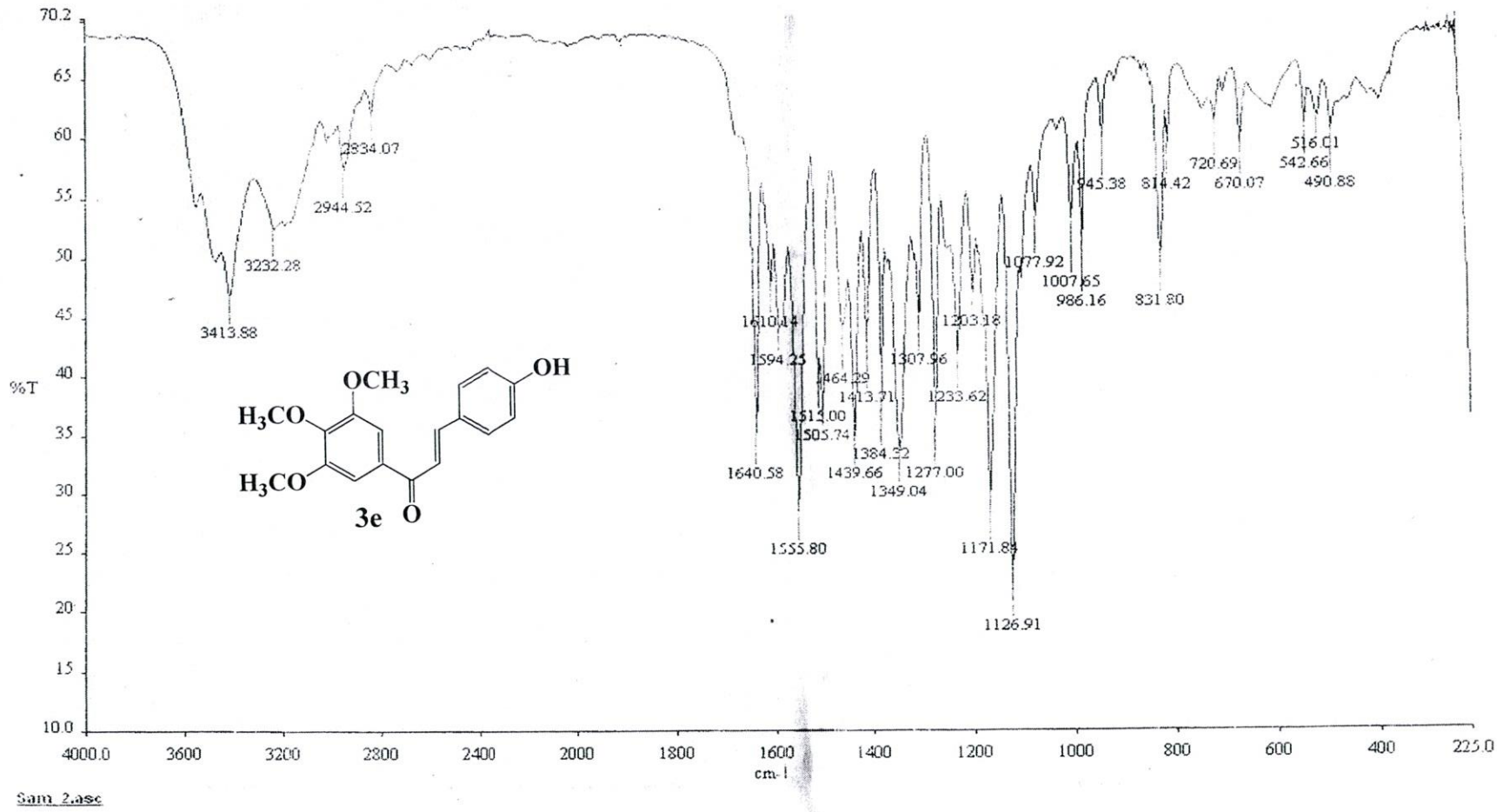


**Figure-13:** IR spectrum of 4 - hydroxy - 3′, 4′, 5′ - trimethoxychalcone, **3e** in KBr $cm^{-1}$

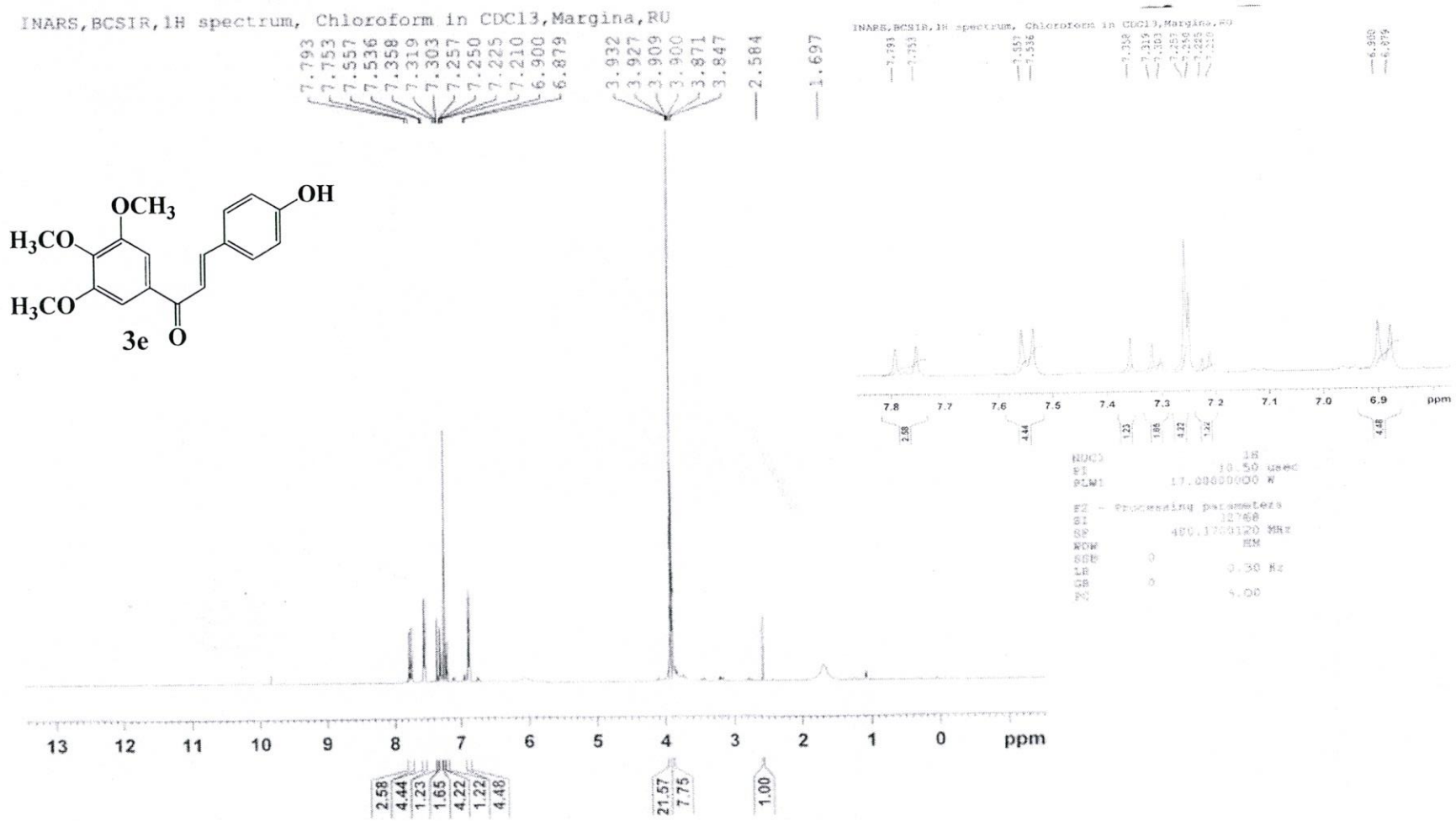


**Figure-14:** $^{1}$H NMR spectrum of 4 - hydroxy - 3′, 4′, 5′ - trimethoxychalcone, **3e** in $CDCl_3$, 400 MHz.

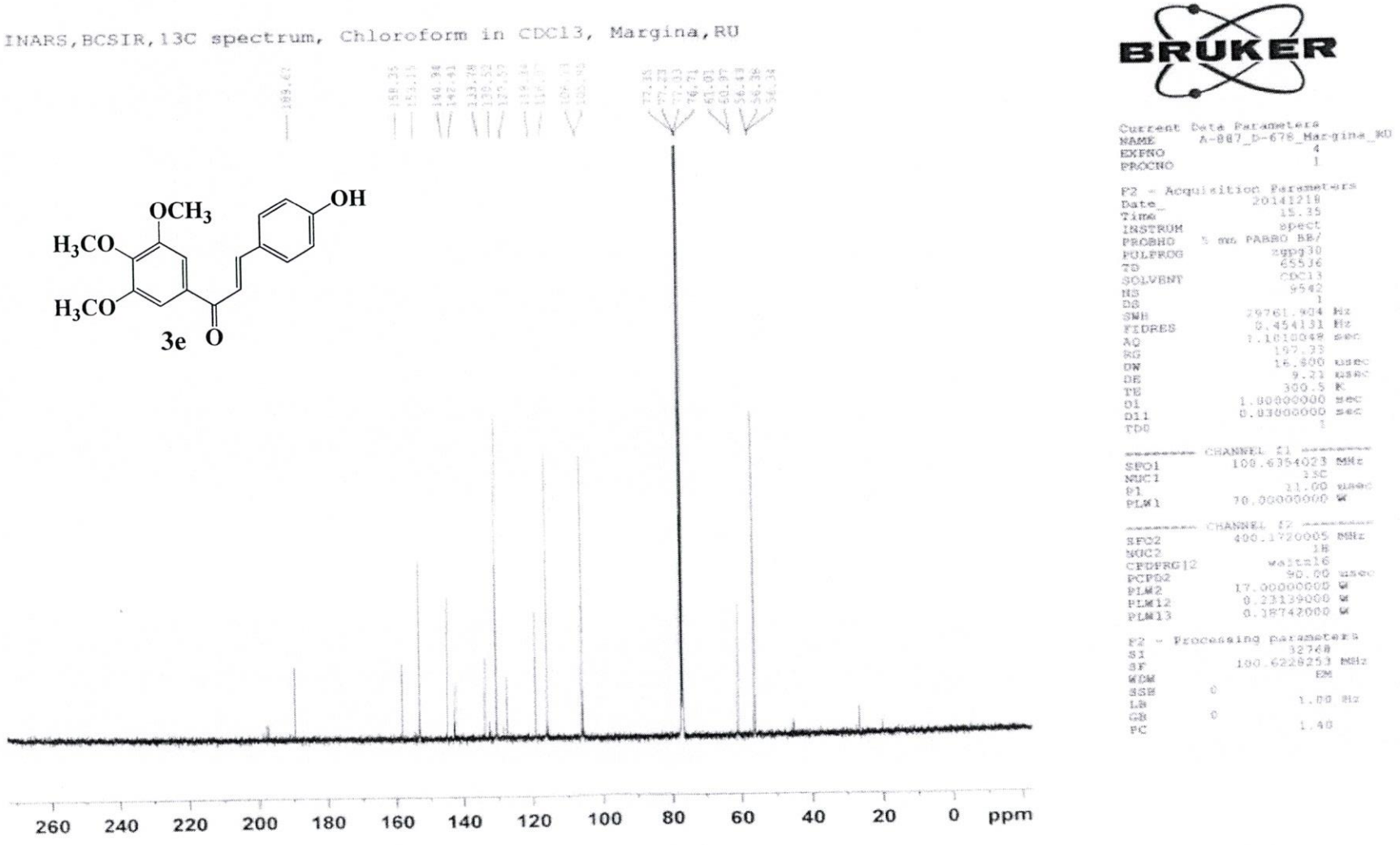


**Figure-15:** $^{13}C$ NMR spectrum of 4 - hydroxy - 3′, 4′, 5′ - trimethoxychalcone, **3e** in $CDCl_3$, 100 MHz

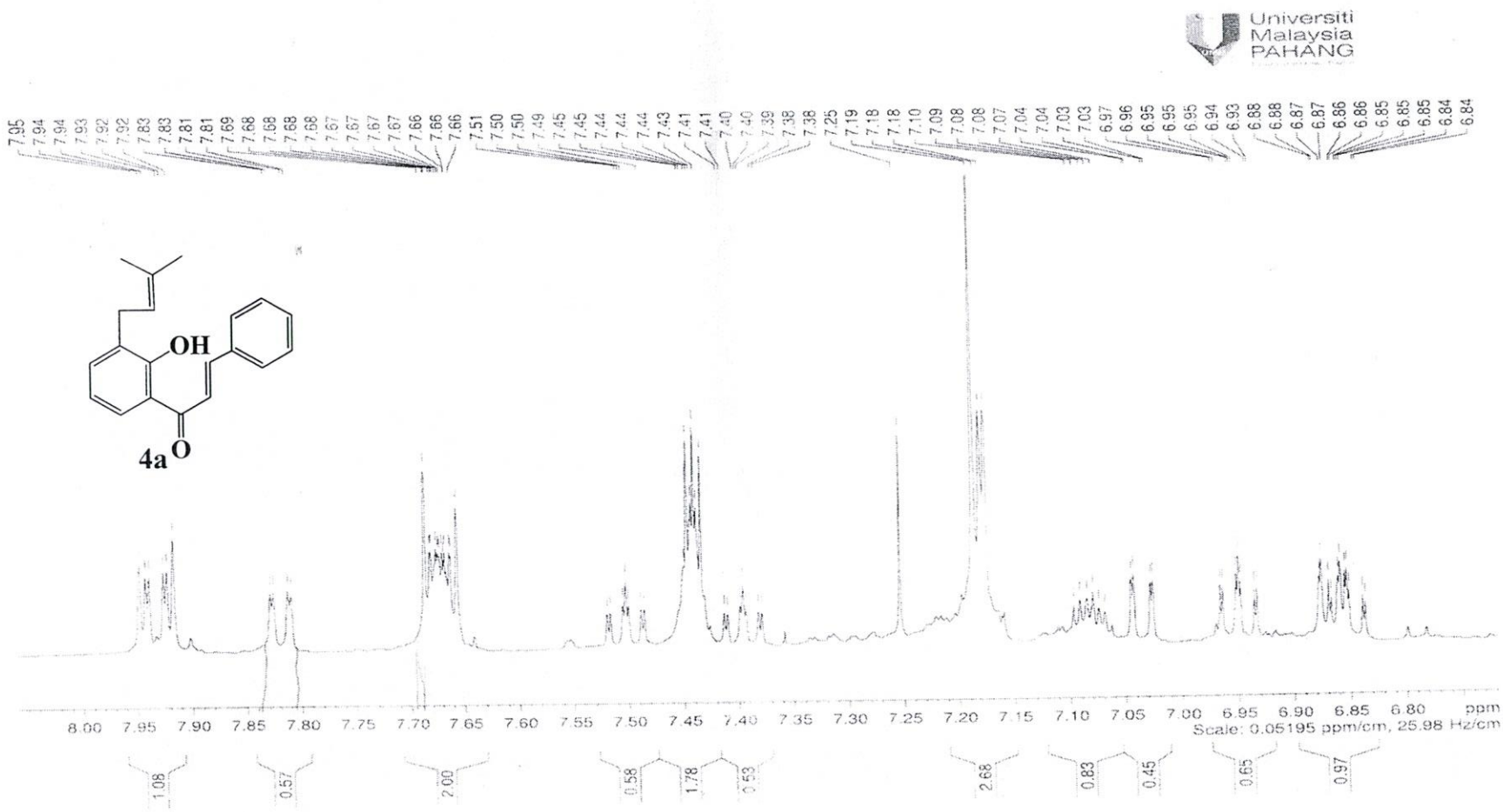


**Figure-16:** $^{1}$H NMR spectrum of 2′ - hydroxy - 3′ - C - prenylchalcone, **4a** in $CDCl_3$, 500 MHz

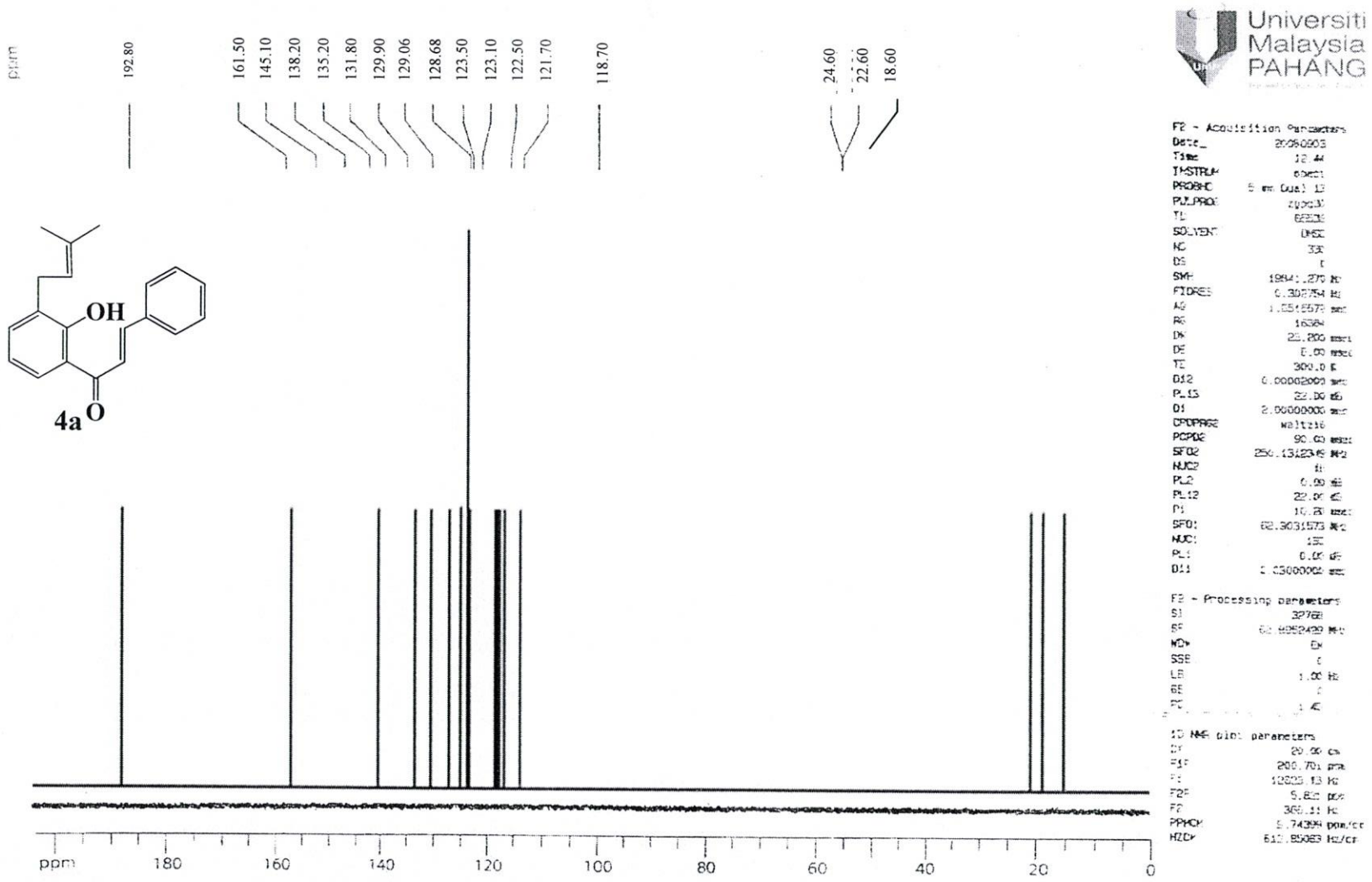





**Figure-17:** $^{13}C$ NMR spectrum of 2′ - hydroxy - 3′ - C - prenylchalcone, **4a** in $CDCl_3$, 125 MHz

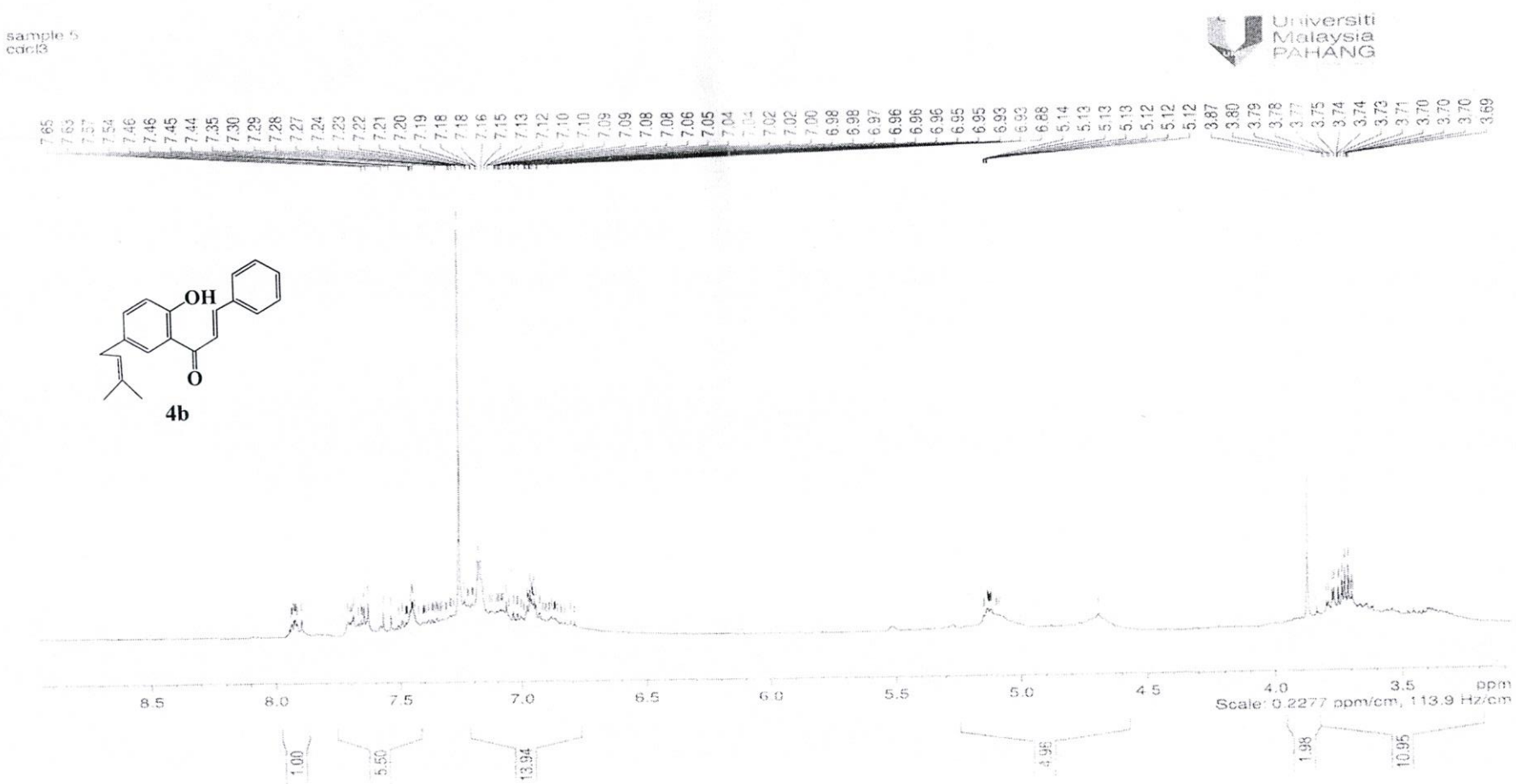


**Figure-18:** $^{1}$H NMR spectrum of 2′ - hydroxy - 5′ - C - prenylchalcone, **4b** in $CDCl_3$, 500 MHz

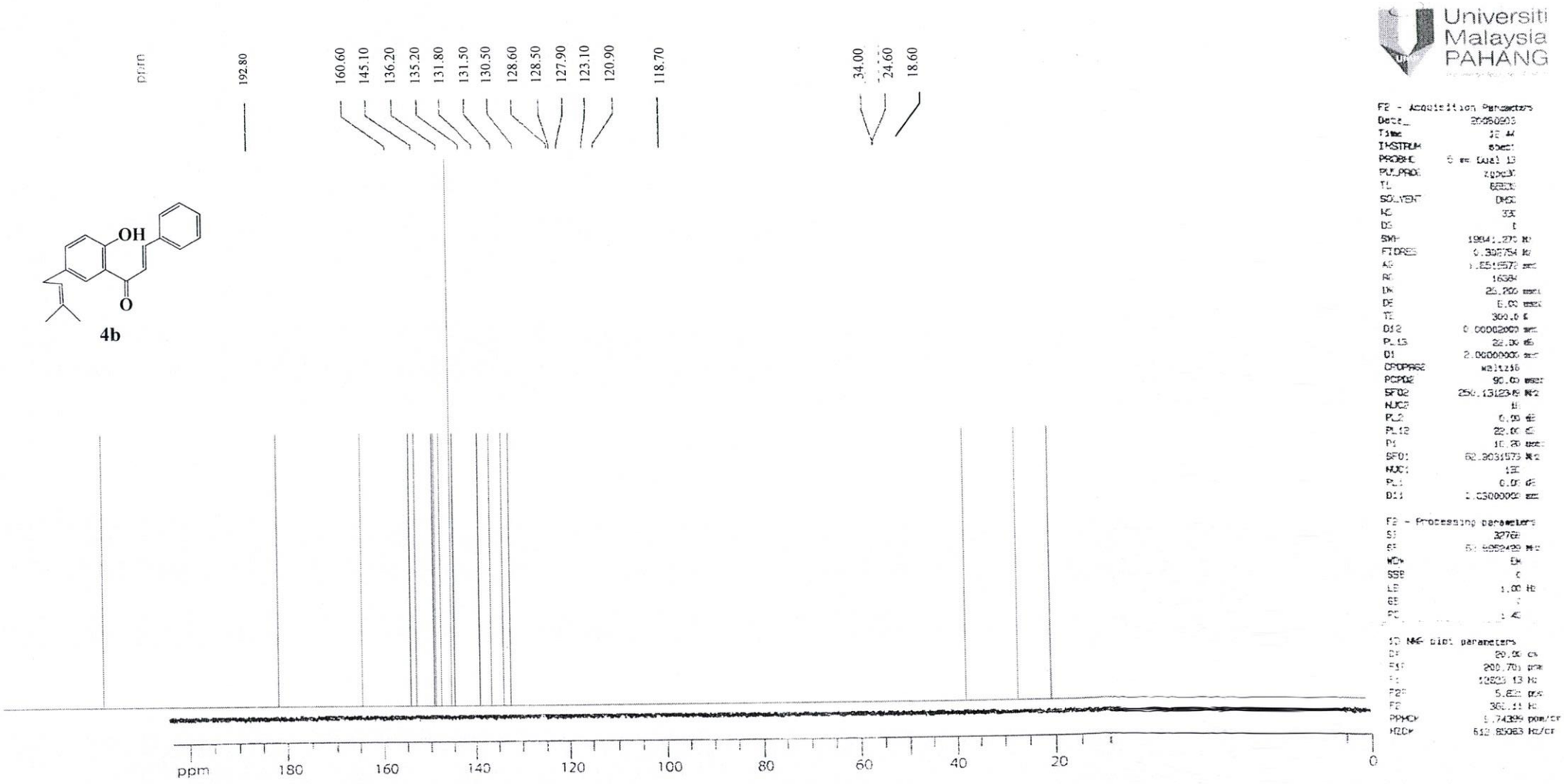





**Figure-19:** $^{13}C$ NMR spectrum of 2′ - hydroxy - 5′ - C -prenylchalcone, **4b** in $CDCl_3$, 125 MHz

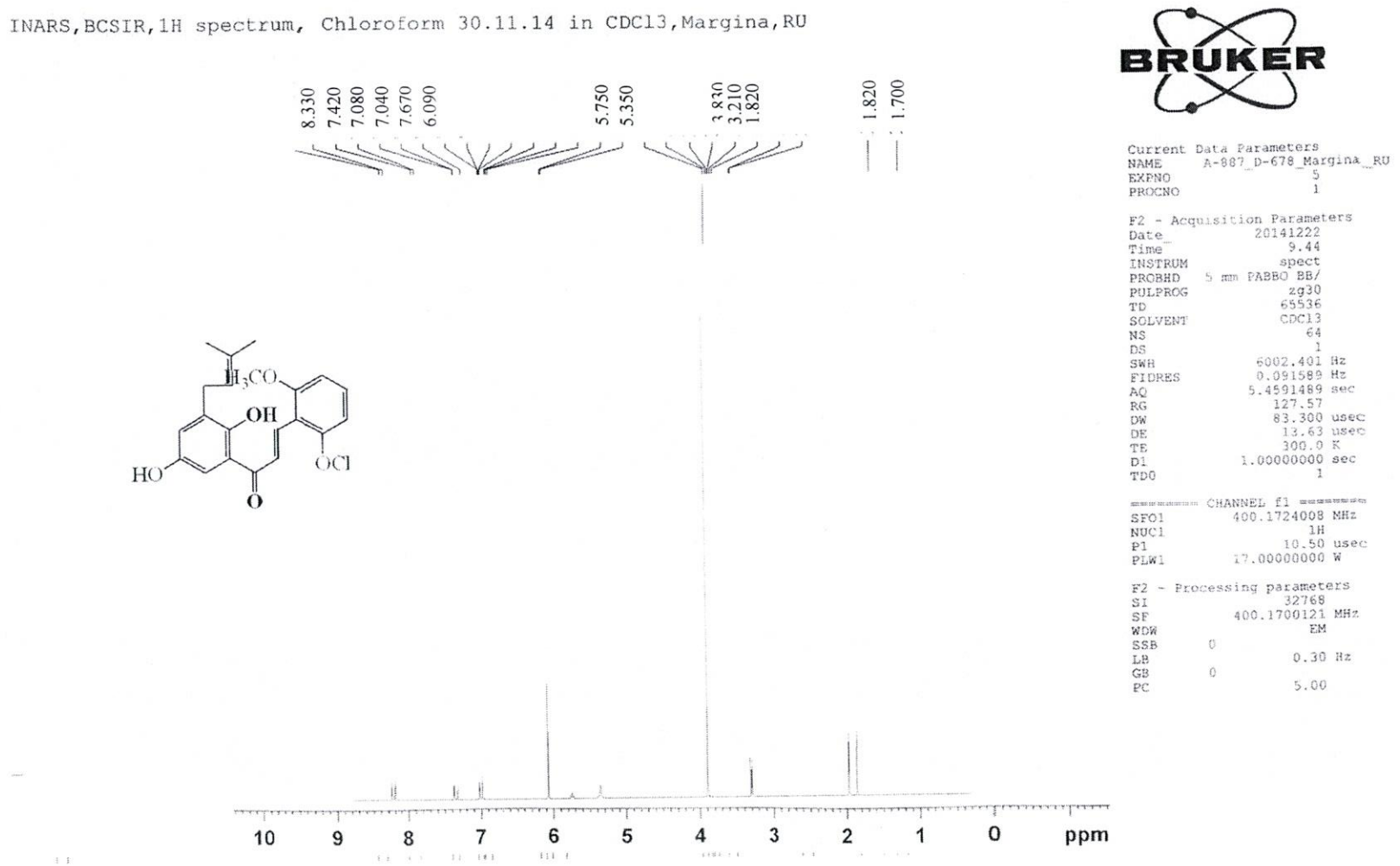


**Figure-20:** $^{1}$H NMR spectrum of 2′, 5′ - dihydroxy -2, 4, 6 - trimethoxy - 3′- C -prenylchalcone, **4c** in $CDCl_3$, 400 MHz

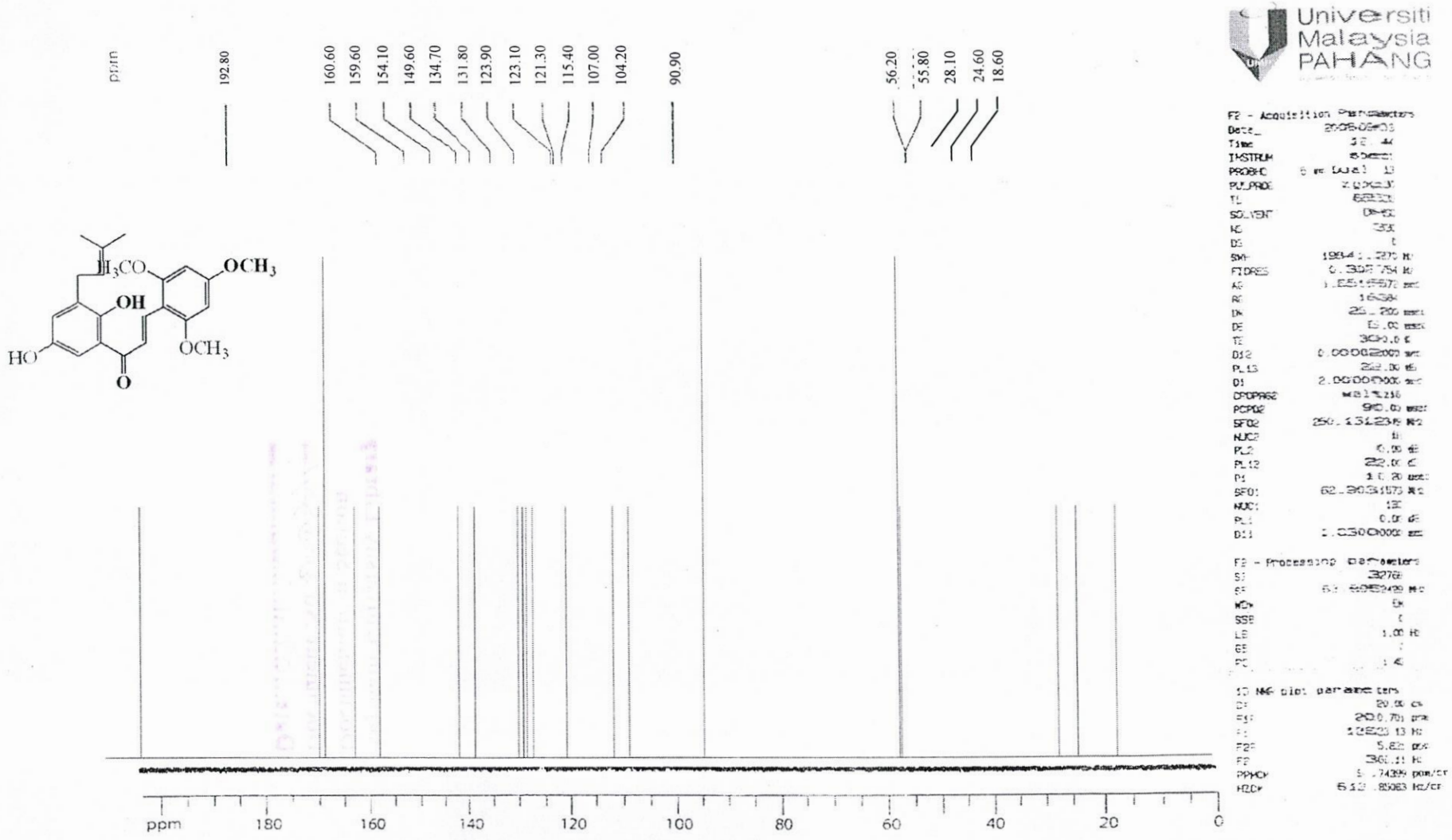


**Figure-21:** $^{13}C$ NMR spectrum of 2′, 5′ - dihydroxy -2, 4, 6 - trimethoxy - 3′- C - prenylchalcone, **4c** in $CDCl_3$, 100 MHz