SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES OF TRANSITION METAL COMPLEXES DERIVED FROM CYCLIC 1,3 DIKETONES

Thesis Submitted to the Faculty of Science,
University of Calicut in partial fulfillment of the requirements for
*the Degree of **Doctor of Philosophy** in Chemistry*

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MARCH 2019

CERTIFICATION OF SUPERVISOR

This is to certify that all the corrections /suggestions from the adjudicators have been incorporated in the thesis entitled **"synthesis, characterization and biological studies of transition metal complexes derived from cyclic 1,3 diketones"** carried out by *Prathibha P.* under my supervision.

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CERTIFICATE

This is to certify that the thesis entitled **"Synthesis, characterization and biological studies of transition metal complexes derived from cyclic1,3diketones"** is an authentic record of the research work carried out by **PRATHIBHA P**, under my supervision in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Chemistry of the University of Calicut, and further that no part thereof has been presented before for any other Degree.

Dr. Mathew Paul Ukken

(Supervising Teacher)

DECLARATION

I, PRATHIBHA P, hereby declare that the thesis, entitled **"Synthesis, characterization and biological studies of transition metal complexes derived from cyclic 1,3 diketones"** submitted to the University of Calicut, in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Chemistry, is an authentic record of the research work carried out by me under the supervision and guidance of **Dr. Mathew Paul Ukken**, Associate Professor, Department of Chemistry, Christ College, Irinjalakuda, Kerala and further that no part thereof has been presented before for any other Degree.

PRATHIBHA P.

ACKNOWLEDGEMENT

PREFACE

Now a days study in the area of coordination chemistry of biologically significant ligands and their synthetic analogues has gained extensive momentum. Metal complexation significantly increases the biological properties of ligands. The chemical component of medicinal plant acts as a good coordinating agent in several cases as they possess various functional groups. Turmeric which contains curcumin is the best example in this way. The wide range of medicinal properties of turmeric is chiefly attributed due to curcuminiods. A pharmacological effect of curcumin that ranges from anti-oxidant to anti tumour properties is due to its inhibitory effects on metabolic enzymes.But these properties have a restricted value in future owning to its low solubility in aqueous medium.The major problem faced in experimental trials with curcumin is its reduced bioavailability in body. Synthetic chemical modifications of curcumin have been studied intensively in an attempt to find a molecule with similar but enhanced properties of curcumin. Here all the efforts were made to synthesis curcuminoid derivatives from the cyclic 1, 3 diketones. Some modified cyclohexanone derivatives of curcumin displayed cytotoxic activity towards human breast cancer cells. It is known that presence of β-diketone moiety is an important factor for the antimicrobial properties. However a structural and biological aspect of these kinds of synthetic ligands and their metal chelates has not received deserved attention. The present investigation is an attempt in this direction.

Cyclic 1,3diketone derivatives and and its biological properties.

Cyclic-1,3-diketonesa class of organic compounds has wide range of applications. The versatile chemistry and ready availability of cyclohexane 1, 3 dione and its derivative make them suitable precursors for the preparation of divergent compounds. Cyclohexanedione derivatives far exceed any other alicyclic system in number. In nature the predominance of cyclohexanedione derivatives over those of other alicyclic systems is amazing. The introduction of second carbonyl group into cyclohexane ring has profound effect on the enolisation of first carbonyl. Cyclohexane1,3 diones are also completely enolised and possess an acidity comparable

to that of carboxylic acid.Curcumin analogues are synthesized by coupling aromatic aldehydes with cyclic ketones . The presence of β- diketone moiety is important for the antimicrobial activity.

Coordination of cyclohexane1,3dione ligand to a metal is proven to alter its chemical and biological activities The development of chelate chemistry of cyclohexane 1,3 diones and its dimethyl derivative named dimedone with transition metals gained considerable attention in textile industry,bacterisides and water purification. It also significantly influence many biological processes and had numerous applications in the field of medicine.Dimedone and its derivative possessed many biological properties such as anticarcinogenic antioxidant antihistaminic and anticoagulant. Modified cyclohexanone derivatives of curcumuin are screened, elicited, increased cytotoxic potency compared to curcumin and other previous studied derivatives.In the recent past; there is increased interest in the synthesis of this class of compounds due to their increased applications.

Biologically active compounds from 1,3diketones using aromatic amines are mentioned earlier. The present situation reports further derivatives with aromatic aldehydes. These compounds have been found to exhibit similar biological activity as their aromatic amine counterparts.

The present study reports fourteen different (4E,6E) bis (aryl) cyclohexane 1,3 diones and their transition metal complexes have been synthesized and characterized using various analytical and spectral techniques. In the (4E,6E) bis (aryl) cyclohexane 1,3 diones , the aryl part include electron releasing ,electron withdrawing, heterocyclic and polynuclear moiety. The thesis is divided into five parts.

Part I.   Introduction

This part highlights the importance of various aspects of coordination chemistry in biological processes giving emphasis to keto-enoltautomerism of 1,3diketones with olefinic linkage as biologically important ligands. In the present investigation transition metal ions such as Cu^{2+},Zn^{2+} and Ni^{2+} arecomplexed with synthetic curcuminoid analogues.Biological activities investigated in the present work include cytotoxic activity, antibacterial activity and antifungal activity of synthesized compounds.

Part II .Literature Review

Literature review emphasizes the history of curcumin and related compounds as biologically significant agents. The activity of curcuminiod analogues in various biological fields is improved by complexation. Importance of present investigation has been hinted at appropriate places.The biological activities of curcuminoids like anti-inflammatory,antioxidant,antiprotozoal,nematocidal, anti-bacterial, antiviral, antitumour activity etc are discussed in this part.

Part III. Materials, Methods and Experimental techniques

Materials,methods and experimental techniques is a general description on various chemicals and methods employed, instruments used and techniques adopted for the study. The present investigation focuses on the structural and biological studies of fourteen different synthetic analogues of curcuminiods and their metal complexes.

Part IV. Synthesis, Characterization and Biochemical activities of (4E,6E) bis (aryl) cyclohexane 1,3 diones.

This part is divided into four chapters .Each chapter is further divided into five sections.

Chapter I. This chapter deals with the Synthesis,Characterization and Biochemical activities of mono and disubstituted(4E,6E)-4,6-bis (aryl) cyclohexane -1,3-diones and their transition metal complexes with Cu(ii), Zn (ii) & Ni(ii).

Section I:The Synthesis & characterization of (4E,6E)-4,6-bis (aryl) cyclohexane -1,3-diones with mono &disubstituted phenyl ring.

Section II:Synthesis and characterisation of transition metal chelates of mono and di substituted (4E,6E)-4,6-bis (aryl) cyclohexane -1,3 diones.

Section III:Invitroantitumour studies .The studies include Invitro cytotoxic study of ligands and their metal complexes [Cu (II),Zn(II) & Ni(II)] by Trypan blue dye exclusion method.*In vivo*antitumour studies are conducted in mice with the ligand (4E, 6E)-4, 6-bis(2, 3-dimethoxy benzylidene)cyclohexane -1, 3-dione (1c) and its Cu (II) complex. The ligands 1d and 1e and their copper complexes are used to find the effect on solid tumour development in mice.

SectionIV:Antibacterial study of mono &disubstituted(4E,6E)-4,6-bis(aryl)cyclohexane -1,3- diones and their transition metal chelates with Cu^{2+}, Zn^{2+} & Ni^{2+}.

Section V: Antifungal studies of (4E, 6E)-4, 6 -bis (2-methyl benzylidene) cyclohexane -1, 3- dione& (4E,6E)-4,6- bis (2,5-dimethyl benzylidene) cyclohexane -1,3- dione and their metal chelates.

Chapter II. The chapter deals with the Synthesis, Characterization and Biochemical activities of chloro substituted (4E,6E)-4,6-bis (aryl) cyclohexane -1,3-diones and their Transition metal chelates.

Section I:Synthesis and characterisation of (4E, 6E)- 4, 6- bis (chloro-aryl) cyclohexane 1, 3 diones.

SectionII:Synthesis and characterisation of transition metal complexes of chlorosubstituted (4E, 6E)-4, 6- bis (chloro-aryl) cyclohexane- 1,3diones.

Section III: Cytotoxic and antitumour activities of (4E,6E)-4,6- bis (chloro aryl) cyclohexane- 1,3- diones and their metal chelates. The studies include Invitro cytotoxic study of ligands and their metal complexes [Cu(II), Zn(II) andNi(II)] by Trypan blue dye exclusion method towards DLA and EAC cell lines. *In vivo*antitumour studies are conducted in mice with the ligands 2a andCu(II)& Zn(II) complexes.

Section IV: Antibacterial studies of (4E, 6E)-4, 6- bis (chloro aryl) cyclohexane- 1, 3 – dione and their metal chelates.

Section V: Antifungal studies of (4E,6E)-4,6- bis (chloro aryl) cyclohexane- 1,3- dione and their metal chelates.

ChapterIII.The chapter deals with the Synthesis, Characterization and Biochemical activities of   heterocyclic based ligands and its metal chelates.

Section I: Synthesis and characterisation of (4E,6E) -4,6-bis (thiophene-2-yl methylidene)cyclohexane -1,3 dione(3a) and (4E,6E) -4,6-bis (4-methyl thiophene-2-yl methylidene)cyclohexane -1,3 dione(3b).

Section II. Synthesis and characterisation of transition metal chelates of (4E,6E) -4,6-bis (thiophene-2-yl methylidene)cyclohexane -1,3 dione and (4E,6E) -4,6-bis (4-methyl thiophene-2-yl methylidene)cyclohexane -1,3 dione.

Section III:Invivo and invitro cytotoxic and antitumour activities of (4E,6E) -4,6-bis (thiophene-2-yl methylidene)cyclohexane -1,3 dione and (4E,6E) -4,6-bis (4-methyl thiophene-2-yl methylidene)cyclohexane -1,3 dione and their transition metal complexes. The studies include In vitro cytotoxic study of ligands and their metal

complexesby Trypan blue dye exclusion method towards DLA and EAC cell lines. *In vivo*antitumour studies and solid tumour studies are conducted in mice with the ligand 3a and 3b their Zn(II)complexes.

Section IV: Antibacterial study of (4E,6E) -4,6-bis (thiophene-2-yl methylidene)cyclohexane -1,3 dione and (4E,6E) -4,6-bis (5-methyl thiophene-2-yl methylidene)cyclohexane -1,3 dione and their transition metal complexes.

Section V: Antifungal study of of (4E,6E) -4,6-bis (thiophene-2-yl methylidene)cyclohexane -1,3 dione and (4E,6E) -4,6-bis (5-methyl thiophene-2-yl methylidene)cyclohexane -1,3 dione and their transition metal complexes.

Chapter IV: The chapter deals with the Synthesis, Characterization and Biochemical activities of curcuminoid analogues with naphthyl , substituted naphthyl and anthracenyl ring and their Transition metal chelates.

Section I: Synthesis and characterization of (4E, 6E)-4, 6-bis (poly nuclear) cyclohexane -1, 3 –diones. They were characterized by UV, IR, ^{1}HNMR, ^{13}CNMR and Mass spectral techniques.

Section II:Synthesis and characterisation of transition metal chelates of (4E, 6E)-4, 6-bis (polynuclear) cyclohexane -1, 3 diones.

Section III:Invitro cytotoxic investigation of (4E, 6E)-4, 6 bis (anyhraceneylmethylidene) cyclohexane 1, 3 dione and metal complexes. In vitro, in vivo and solid tumour studies are conducted by ligands and its metal complexes.

Section IV:Antibacterial examination of curcuminoid equivalents with substituted naphthyl ring and   metal complexes.

Section V:Antifungal examination curcuminoid equivalents with poly nuclear ring and metal complexes.

Part V:Conclusion and References

LIST OF PUBLICATION

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ABBREVIATIONS

ROS   —   Reactive Oxygen Species

COX-2   —   Cyclooxygenase 2

LOX   —   Lipoxygenase

NF-κB   —   Nuclear Factor kappa B

cPLA   —   Cytosolic Phospholipase

HBC   —   Hydrazinobenzoylcurcumin

DMC   —   Demethoxycurcumin

BDMC   —   BisDemethoxycurcumin

iNOS   —   inducible nitric oxide synthetase

GST   —   Glutathione S – transferase

AD   —   Alzheimer's disease

DMSO   —   Dimethyl Sulphoxide

EAC   —   Ehrilich Ascites Carcinoma

COSY   —   Correlation Spectroscopy

DLA   —   Daltons Lymphoma Ascites

ILS   —   Increase in life span

Ar   —   Aryl group

FAB   —   Fast atom bombardment

ppm   —   parts per million

TLC   —   Thin layer chromatography

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PART –I
GENERAL INTRODUCTION

GENERAL INTRODUCTION

Medicinal properties of myriads of plants, in addition to their odour and flavor, are known from ancient times, besides their odour and flavor. Traditional medicines mainly contain medicinal plants as their key ingredients. The positive effect of natural life style is adopted by Indians and they believe that the medicinal plants to a certain extent can improve one's health conditions. Work on powerful anticancer drugs progressed in last few decades and rapidly involved investigations on herbal products. There are so many factors like availability of medicinal plants, weather conditions and nature of body that influence a man in the selection of remedial medicines from his environment. The use of plants and their active principles in the prevention and treatment of chronic diseases[1-4](M J Balunas & A D Kinghorn, 2005; M Suffness & J Douros 1982; J. Bruhn & B Holinsted 1980; N.R Fransworth & R.W Morris 1976) is based on experience of traditional system of medicine. Numerous diseases are derooted by common medicinal plants which has no side effects. The products derived from plants have significant importance in the preparation of drugs [5-7](M F Balandrin *et al.,* 1985; L Hoareau & EJ DaSilv ,1999; R P Rastogi &B N Bhawan, 1982;). We can produce pharamaceuticals from medicinal plants economically. The chemicals derived from biologically active plants play a pivotal role in the medicinal world. Most of the people in India believe that green medicine is non-toxic and reliable in comparison with man-made drugs.

Turmeric *(Curcuma longa)* is a perennial plant species of zingiberaceae[8], (Chattopadhyay *et al.,* 2004) ginger family. Their Rhizomes are collected, warmed with water for about half an hour, dried and crushed into fine powder. It has many uses such as food preservative, food colouring agent[9-11](V P Menon & A R Sudheer, 2007; F DiMario *et al.,* 2007; Aggarwal *et al.,* 2007) and spice. Moreover it has extensive therapeutic usages. Curcumin, the main ingredient of turmeric exhibits wide range of biological and medicinal properties. But these properties have a restricted value in future because of its low solubility in aquous medium. Their water solubility increases 12 fold by mere heating. Curcumin display biological actions like anti-inflammatory[12](Krishnakumar Lohidashan *et al.,*2018), antioxidant,

anticancer[13](LoTempio et al., 2005) , antidiabetic, antiallergic[14](Suzuki*et al.*, 2005), antiviral[15](Si*et al.*, 2007),antiprotozoal [16](Reddy*et al.,* 2005) and antifungal properties. Curcuminoids, a very powerful phytonutrients present in the curcumin inhibit the cancer development at the early stages. The anti-bacterial function of curcumin has also been proved[17] (Mathew *et al.,*2002). The current clinical experiments on humans[22](Bush *et al.,* 2001) is based on the analysis which had already done in cells and animals[18-21](Swarnakar *et al.*, 2005; Gopinath *et al.*, 2004; B O Wahlstrom &G. Blennow, 1978; V.Ravindranath &N Chandrasekhara., 1980). Pharmacological effects[23](K.Krishnankutty *et al.,*2009) of curcumin which were ranging from anti-oxidant to anti tumour properties is due to its inhibitory effects on metabolic enzymes .The curative properties of curcumin is attributed mainly to its chemical structure[24-26](Weber *et al.,*2005; Priyadarsini *et al.*, 2003, Daniel *et al.*, 2004) and characteristic physiochemical and biological things. It is a diferuloyl methane molecule with two ferulic acid residues joined by a methylene bridge. It has three chiefly functionalities: an aromatic o-methoxy phenolic group, α, β unsaturated β- diketo moiety and seven carbon linker[27-28](V Lampe &J Milobedzka, 1913; Milobedzka *et al.*,1910).Extensive research in the last two decades has provided evidence for the role of different functional groups[29-30](A.T Dinkova-Kostova &P Talalay, 1999, Woo *et al.*, 2005) in its crucial biological activities.The bioavailability[31-36](Sharvil Patil *et al.*, 2015, Zhao *et al.*, 2012, Xie *et al.*, 2011, Antony *et al.*, 2008, Kuo-Yi Yang *et al.*, 2007, Preetha Anand*et al.*, 2007) of the curcumin is significantly dominated by its low water solubility as well as degradation at higher pH value.

In order to overcome these difficulties serious studies are taking place to derive a super curcumin which will retain the curing properties. In this process numerous methods are adopted to bypass the limitations. These studies focus on finding a natural alternative for curcumin from turmeric itself ; the manufacturing of a synthetic curcumin analogue reformulating curcumin with preventors of metabolic activities; creatingliposomal curcumin[37](Bansal *et al.*, 2009) and nanoparticle formulations [38-39](Wehrung*et* al., 2012,Dumortier*et* al., 2006)of curcumin;conjuction of curcumin prodrugs;creation of micellar structure[40](Gaucher *et al.*, 2005) and relating curcumin with polyethylene glycol[41](Butt*et al.*, 2012).The higher compounds of curcumin which   have enhanced activity[42-46](Arietta *et al.*, 1988;K.Krishnankutty&P.Venugopalan,1998;R.C.Srimal&B.N.Dhawan,1973;

A.Banerjee &S.S Nigam, 1978; K.V.D.Babu & K.N.Rajasekharan, 1994) are produced by changing the functional moiety of natural curcumin. Skeletal homologues including alteration of all groups existing in curcumin have been synthesised. Attempt in this regard is to synthesis curcumin equivalents and its metal complexes by understanding its vital role as antibacterial, antifungal and antioxidant agents. The phenyl ring in natural curcumin was replaced with various groups. Parbon in 1964 established a common scheme[47](H.J.J.Pabon, 1964) for producing curcuminiods. Here the raw materials are aromatic aldehyde,acetyl acetone,boric oxide with right amount of n-butyl amine and tri(sec-butyl) borate. In the current situation fourteen variety of aromatic aldehydes are reacted with 1,3 di-ketone (1,3 cyclohexanone) in dry ethyl acetate medium.

All the prepared equivalents are characterized by different spectroscopic methods[48-49](P.J. Roughly & D.A.Whiting, 1973; V.S.Govindarajan, 1980) such as UV,IR ^{1}H NMR,^{13}C NMR,Mass spectral techniques and TG, DTA techniques also etc. Cytotoxic, antitumor, antibacterial and antifungal studies were also conducted to establish its biochemical properties.

Role of curcuminoids in cancer therapy

Chemoprevention [50](P.U.Mathew,2002) means the treatment by tablets, vitamins or further agents for inhibition, suspension and delay the growth of cancer. It is a truthful medical methodology to diminish the threat of cancer. Chemo preventive anti-inflammatory[51](P.U.Mathew & K.Krishnankutty,2002) activity possessed by curcuminoid is considered as the best anticancer agent. The anti-carcinogenic properties of curcumin have been studied in a number of animal tumour systems[52](John*et al.*, 2002) and anti-cancer studies are also conducted in animals. They offer an actual means of recognizing complexes which can be used carefully and also deliver evidence for emerging intervention trials in individuals. Several studies of curcumin on various animals have shown that it has an anti-chemo effect on the organs like colon[53](Deshpande *et al*., 1997), oesophageal[54] (Chuang*et al.,* 2000) intestinal[55](Rao *et* al., 1995)stomach[56](Lee *et al*., 2005)andoral[57](Kuttan*et al*.,1985) carcinogenesis. It has been shown to reduce tumours induced by benzpyrene and 7,12 dimethyl benzanthracene[58-59] (Azuine and Bhide,1992;Singh *et al*., 1998;), tumour promotion induced by phorbol esters[60](Huang et al., 1988) on mouse skin, on carcinogen-induced tumorigenesis in the fore stomach and N-ethyl-

N'-Nitro-n-nitrosoguanidine-induced duodenal tumours[61](Huang et al.,1994). It has been identified that the decreased rate of bowel cancer among Indians is due to the use of turmeric in various dishes[62] (Mohandas and Desai, 1999). Executive of man-made curcumin in the food throughout the development stage significantly prohibited the multiplicity of persisting adenocarcinomas of the colon[63](Kawamori et al., 1999). Curcumin has been verified to bring programmed cell death in a range of cells as well as prostate cancer cells[64](Dorai et al., 2001).The earlier studies have proven that curcumin has a very significant effect on the enzymes like protein tyrosine kinases[65](Zhang *et al.*, 1999), cyclooxygenase[66](Liu *et al.*, 1993), protein kinase C [67] (H.W Chen & H.C Huang, 1998). The studies which have been conducted in recent years have found that curcumin prevents the phosphorylation of cytosolic phospholipase (Cpla(2) and brings great change in metabolism and it also results in the reduced cyclooxygenase- 2-(COX) expression. In addition to this, 5-lipooxygenase's (LOX)[68](Hong *et* al., 2004) catalytic characteristics are also decreased. The property of curcumin against inflammation and cancer is because of these two prominent characteristics. Curcuminoids is established as a worthy anti-angiogenesis agent[69](Arbiser *et al*., 1998) clarifying its chemopreventive function at the level of tumour growth. Anticancer properties of curcumin appear to be potentialized in the presence of estrogen in breast cancer and it inhibits genes which are under the influence of oestrogen receptor[70](Shao *et al*., 2002).It permits preparing ovarian cancer cells to cisplatin, and thereby improve chemotherapeutic treatment[71](Chan *et al*., 2003).Investigations prove that curcumin has powerful ability towards numerous cancers such as leukemia,lymphoma, melanoma and sarcoma as well as breast,ovarian,lung and neurological cancers[72](Anand *et al*., 2008). It displays its activity at different stages of tumour. It prevents development, advancement, invasion, angiogenesis and metastasis. From the animal behaviour and in vitro experiment it was concluded that curcumin destroys carcinogenesis[73] and it blocks rapid growth of varied range of tumour cells (Aggarwal *et al*).

Research works also support the cytotoxic character of curcumin related compounds. Carbonyl ligated curcumin[74]prevents the rapid cell growth and it is more effective than the normal curcumin (Zheng et al., 2013). One more derivative, hydrazinobenzoylcurcumin (HBC), reveal effective suppress activities[75] towards the proliferation of numerous tumour cell lines (Shima *et al*.,2004). In addition, a relative compound of curcumin namely, bis-Dehydroxycurcumin(bDHC) generate

autophagy on human colon cancer cells[76](Basile *et al.,* 2013). In the current study in vivo and in vitro experimental techniques are used to study the antitumour properties of complexes.

Anti-microbial properties of curcuminoids and its analogues

The growth of microbes[77] can be prevented by the curcumin and its synthetic equivalents. (Chai *et al* 2005).various studies have identified the prohibition of microbial properties[78] (Negi *et al* 1999). In recent years the researchers are giving more importance to find compounds with anti-bacterial properties from plants mainly due to increasing acceptance of conventional drugs as well as adverse effect of antibiotics in human body. Artificial alteration of formerly pronounced antibacterial compounds has been eminent in the development of innovative agents which may have an enriched antibacterial property or novel pharmacological characteristics. Like the curcumin, its oil extract, also destroy many species of bacterias namely, Streptococcus, Staphylococcus, Lactobacillus[79](Bhavani Shankar, 1979)etc. The antibacterial effects of aqueous layer[80](Kumar *et al*., 2001) of curcumin is so much noted now a days. Curcumin stops the development of *Helicobacter pylori* CagA+ strains *in vitro*[81](Mahady *et al.,*2002).Its powder form has positiveresponse on stomach. Report says that extracts of curcumin in ether,ethanol and $CHCl_3$[82-84](S.K Misra & K.C Sahu, 1977; A.Banerjee & S.S Nigam, 1978;Apisariyakul *et al*., 1995) have sufficient antifungal activities[85](Krishnakumar K.L *et al.,2017*) Turmeric oil is also active against *Aspergillus flavus,Aspergillusparasiticus,Fusariummoniliforme* and *Penicillium digitatum*[86](Jayaprakasha *et al*., 2001).

The present study focus on the synthesis of new curcuminiods analogues,(4E,6E)bis (aryl)cyclohexane-1,3-dione that possess modified aryl ring system with improved biological activity.

Revising the capability of curcumin and equivalents as origin of antimicrobial medicine with respect to antifungal and antibacterial agents, **a systematic investigation was undertaken to screen the antibacterial and antifungal activity of the synthesized curcuminoid analogues.**

Metal binding chemistry of Curcuminoids and its Analogues

Curcumin exhibits a very interesting structure, ie. diketo moiety is exactly associated to olefinic group eventhough they possess a 1,3 diketone structure. The keto-enol tautomerism is one of the key feature of 1, 3 diketones. Elimination of methine proton from the keto form and hydroxyl proton from enol form which is already acidic produces an anion which facilitates the complex formation with transition metal[87-91](D.W.Thompson, 1970; K.C.Joshi & V.N.Pathak, 1977; R.E.Sievers & J. J .Fortman, 1971; J.Emsley, 1984; Mehrotra*et al*., 1978). Process of complexation includes the formation of cyclic intermediate by combining a metal with a ligand through a coordinate bond. The binding capability of β-diketone moiety was first explained by Werner[92](A.Werner, 1901) and Morgan[93](G.T.Morgan & H.W.Moss, 1914).Application of 1,3-diketone-metal complexes are significant so their synthesis gets much attention now a days.

Curcuminiods have 1,3 diketone structure so that their metal complexation is highly deliberated in the branch of coordination chemistry.The identification of Boron (B) in the trace amount has been carried out by preparing the complex of boron and curcumin was started in the middile of 20th century itself. Preparation of Boron-Curcumin complex[94](M.R.Hayes & J.Metcalfe, 1962) in the determination of trace amounts of Boron dates back to the 1960ʾ s.One noticeable thing is that in acidic medium curcumin doesn't change its colour but when it combines with slaked lime it turns to red. Slaked lime is chemically calcium hydroxide and in the basic media curcumin turns to red. Here, Ca^{2+}reacts with curcumin and this interaction cause the colour change.Very few alkali, alkaline earth metal-curcumin complexes are reported. But there are large number of metal curcumin complexes of transition metals reported. Until now the majority of the research and studies regarding copper curcumin chelates[95] (Bachar Zebib et al., 2010)succeeded by ruthenium, zinc,manganese,iron and nickel[96](Larry Baum & Alex N.G, 2004). Generally curcumin forms 2:1 stoichiometric complexes with metals and non-metals.Rarely does it form 3:1 stoichiometric complexes with some metal salts. Physical and chemical characteristics of curcumin can be altered by the process of complexation with metal.

Through complexation the biological activities of metal are also changed. Complex formation with curcumin diminishes the toxic effect of metals. Zinc(II) and Ru(II) complexes of curcumin exhibited powerful cytotoxicity towards prostate cancer and neuroblastoma cells[97](Valentini *et al*., 2009) as reported by Samya Banerjee *et al*.Curcumin is the best medicine for Alzheimers disease when it chelated to Al(III) ions,because it is accepted as an embryonic natural drug to cure the Alzheimer's diseases. The studies which were carried out by Zhou *et al* have identified that the antibacterial characteristic of rare earth chelates[98](Yu Min Song *et al*., 2009) of curcumin is significantly greater compared to curcumin. The 1:1 stoichiometric complexes of curcumin containing copper metal atom is considered for studying the superoxide dismutase[99](Atann Barik *et al*., 2005) activity.The cytotoxic characteristics of Ruthenium-arene chelates of curcumin[100](Francesco Caruso *et al*., 2012)was studied. Curcumin associates with transition metals to form complexes with stoichiometries such as ML,ML_2, ML_3 and so on, among which ML_2 is most stable chelate. The synthesis of metal ligand chelate is carried out by dissolving the ligand and metal salt in appropriate solvent and it is heated for 3 hrs for its precipitation. The solid product isolated and checked its purity by thin layer chromatography. The metal complex of the ligand exhibits significantly improved biological activities than that of the unsaturated ligand.**In the current study transition metal ions such as Cu^{2+},$Zn^{2+}$and Ni^{2+}were chelated with synthetic curcuminoid equivalents. Their structural elucidation was also done by spectral techniques. Then they were assessed for their biological properties.**Several metal complexes of curcumin are shown to be cytotoxic with cancer causing cells. The extensively used chelate in cancer chemotherapy is cis-dichlorodiamine platinum (II), usually known as cisplatin.

Cytotoxic copper complexes

Composite ligands of copper (II) complexes display considerable cytotoxic result for example Cu(SalNEt$_2$)salicylate(Coats *et al*., 1976) complex. Generally the existence of electron-releasing moiety enhances cytotoxic character[101](Coats *et al*., 1976). Anticarcinogenic character of di-Schiff base copper (II) complex (two novel di-Schiff bases coordinated with active center analogs of Cu_2 Zn_2 superoxide dismutase) efficiently accelerates the yield of hydroxyl radicals in the presence of polymorphonuclear leukocytes, which causes reduction in tumor size, delay of

metastasis and a significant increase in life span of the hosts[102](R Miesel &U Weser, 2006)Copper (II) chelates of phenanthroline similarly showes substantial antitumor task[103](J Kuncheria &K K Aravindakshan., 1993).

In addition to natural curcumin its synthetic equivalents of copper also deliveres improved antitumor character[55].Many artificial curcuminoids3-ethoxy-4 propanoyloxy benzaldehyde,1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (curcumin-1), 4-butanoyloxy-3 ethoxy benzaldehyde,1,7-bis(4-triphenyl siloxy-3 methoxy phenyl)-1,6-heptadiene-3,5-dione(piperonylcurcumin);1,7-bis(2-hydroxynaphthyl)-1,6-heptadiene-2,5-dione(2-hydroxy naphthyl curcumin); cinnamyl curcumin and their copper chelates were examined for their antitumour character and were detected to be extremely effective.Enrlich Ascites cancer cells were checked with fourteen different para substituted phenyl glyoxal –bis-(4-methyl-3-thiosemicarbazone)[104](Singh*et al*., 2003) zinc chelates and they displayed supreme activity. Assessable comparative study implies that this particular nature towards the cancer cell organ can be enhanced by substitution with moiety having electron attracting and lipophilic character. The introduction of cancer cells to the mixtures of ascorbate and copper chelates, particularly Cu^{2+}-o-phenanthroline and Cu^{2+}-2,9-dimethyl-o-phenanthroline complexes caused in the decay of enormous portion of cell groups[105](S.H Chiou &N Ohtsu, 1985) Copper (II) complex of N´-[(2-hydroxy phenyl) carbonothioyl] pyridine-2-carbohydrazide inhibits the expression of c-Src, a non-receptor tyrosine kinase, which performs a substantial role in the growth-mediated signalling pathway, thus displaying cytotoxicity upon the colon cancer cell line[106](Shrivastav*et al*., 2006) Tris-(hydroxymethyl)phosphine copper(I) complexes with the new bis(1, 2, 4-triazol-1-yl)acetate ligand had showed in-vitro antitumor character similar with that of cisplatin, the widely employed metal-based antitumor medicine[107](Marzano*et al*., 2006). Copper(II) complex of trans-bis(salicylaldoximato) revealed cytotoxicity equivalent to that of adriamycin by making cell cycle arrest and apoptosis[108](H Elo, 2004). Thus the significance of Cu (II) ion in cytotoxic nature is properly confirmed.Generally copper complexes of curcumin are well-known for its cytotoxic character whereas aluminium complexes are for antimicrobial character[109](Katherine.H.Thompson *et al*., 2004).It has been earlier stated that vanadyl curcumin is more efficient for inhibiting synoviocyte proliferation, smooth muscle cell growth and mouse lymphoma cell growth than curcumin alone[110](Khosro Mohammadi *et al*., 2005) Mohammadi et al. synthesized

vanadyl curcumin [VO(cur)$_2$] and vanadyl diacetylcurcumin [VO(DAC)$_2$], and examined them. The medicinalapplications of Vanadyl curcumin complex as cytotoxic agents were studied by Khosro Mohammadi *et al* (2005). Studies by Moamen.S.Refat revealed that complexes of Ni(II),Cu(II) and Zn(II) with curcumin ligand has antitumour activity[111](Moamen.S.Refat *et al*., 2013). The metal chelates displayed antifungal character upon Aspergillus flavus, Candida albicans, Aspergillus avus, Microsporum canis, Fusarium solani and Candida glabrata fungal strains based on literature protocol.

Hence the present study has undertaken with the following objectives:

1. Synthesis and structural characterization of a series of curcuminoid analogues.

2. Synthesis and structural characterization of transition metal complexesof synthetic curcuminoid analogues.

3. To evaluate the antitumour activity of a series of synthetic analogues of natural curcuminoids and their synthesized transition metal chelates.

4. To evaluate the antibacterial activity of a series of synthetic analogues of natural curcuminoids

PART – II

LITERATURE REVIEW

CHEMICAL AND ANTIMICROBIAL STUDIES ON CURCUMINOIDS AND THEIR ANALOGUES-A REVIEW

Introduction

Recently most of the research works focused on the therapeutic plants with understanding of its significance on the health care. Certain plants[112] (K.L.Krishnakumar & Mathew Paul, 2013) have some functional properties besides fragrance and flavor. Even in the current extent, environment is still the ultimate source of medicines. There are numerous plant products endowed with curative properties. WHO evaluates that the emerging world requires conventional, curing plants for primary treatment [113](Bannerman, 1982). Plant parts have medicinal values for treating common diseases. Therapeutic[114](Abdul Kawy & A.S.Waly, 1978) plants have a noticeable place in the plant kingdom. Recently people use lot of drugs made from medicinal plants and these medicines played a key role[115,116](M.S.Butler, 2004;D.J Newman &G.M Cragg, 2007) in health issues.There is herbal based medicine,- phytomedicine which associate classical medicine with modern medicine. Irrespective of all the growth in man-made chemistry and biotechnology, plants play a conistent role in medicinal preparations. Right from the start, the records of information particularly on the therapeutic usage of plants have provided several significant drugs of modern day[117](Aggarwal *et al*., 2006). By the arrival of latest methods it has come to be possible to separate and distinguish the bioactive chemical compounds existing in the therapeutic plants. India is blessed with rich and diverse native health customs which in agreement with similarly rich varied plant genetic resources. In this condition one of the finest known sample is turmeric[118,119](M.M Khanna, 1999; C.CAraujo &L.L Leon, 2001) (Curcuma longa Linn). Phytochemicals [120-122](CManach *et al*., 2004;B Halliwell, 2007;Hung *et al*., 2004) are the bioactive chemical components which are derived from plants. Plants produce these chemicals to protect themselves and they also protect humans against diseases. They can be classified into different organic compounds such as carotenoids, flavonoids, isothiocyanates, phenols, sulphides/thiols, whole grains, vitamins etc. Some plants exhibit medicinal properties due to the presence of these compounds.The phytochemicals which we consume through our food include carotenoids phenols piperine,curcuminiods Cassumuin A, Nomillin, Ellagic acid,

Quercetin etc[123](Dhar*et al*., 1968) In the midst of vast number of phytochemicals, curcuminiods display a very distinct structure and reasonable applications.

TURMERIC

In India the use of plants for treating disease dates back approximately 4000 years to the Vedic age. In South East Asia, turmeric was used not only as a principal culinary spice but also as a component in religious ceremonies. Since then turmeric has been using as a cure for many diseases[124-126](Funk *et al*., 2006;Deodhar*et al* 1980; Sidhu *et al*., 1998) including digestive disorder,rheumatism, cough, liver disease, pulmonary diseases and as antiseptic for wounds. Turmeric is also used as a paste for shinier skin and it also prevents some harmful bacteria entering into the body. Curcuma longa linn comes under the plants family zingiberaceae and it is a perennial plant and it is generally farmed in Asia. The rhizome is employed widely for colour[127-128](N.B.Sankaracharya, 1974; F.Mayer, 1943) and essence to foods. Turmeric obtained from rhizomes is very important in medicinal field.(Srimal 1997). Over the past few years, several studies have been showed to recognize the curative properties[129-133](Gujral *et al*., 1953; Arora *et al*., 1971; Kiso *et al*., 1983; Ammon *et al*., 1992; D Eigner & D Scholz, 1999) of turmeric. India harvests approximately the world's entire turmeric crop and uses up 80% of it. Turmeric contains[134-137] (Balakrishnan*et* al., 2007; Selvam *etal*., 1995; Leela et *al*., 2002; Albert .E.Leach, 1904)*condensed* liquid, curcumin, oils, protein, K, Ca, carbohydrates etc (Balakrishnan 2007). It also contains various fatty acids and some α-linoleic acid[138] (Goud *et al*., 1993). Curcuminiods actually contains three curcumins such as curcumin I, curcumin II, and curcumin III and it gives yellow colour to turmeric[139,140].(Chainani –Wu, 2003).

The relation of turmeric and curcumin

The fundamental biological character of turmeric depends on a yellow bioactive [141-146](K.I. Priyadarsini, 2013; Gupta *et al.,* 2011;Esatbeyoglu *et al*., 2012;G.Grykiewicz & P. Silfirski, 2012;Ruby *et al*., 1995;Subash.C.Gupta *et al.,* 2012) pigment called curcumin. Curcumin is the chief colouring material in curcuma longa and two connected compounds demethoxy curcumin (DMC) and bisdemethoxy curcumin (BDMC), are in total identified as curcuminoids. Curcumin is the lively constituent of the nutritional supplement in Tumeric. The curcuminoids ⁽Kulkarni *et* al., 2012) can be taken out from Tumeric[147] by Soxhlet extractorand separation and refinement [148-152] (Kim *et al.,* 2013; Patel *et al*., 2000; Lee *et al* .,

2012; Andrew.M.Anderson *et al*., 2000; Revathy *et al*., 2011)was done by column chromatography [153](Srinivasan et al 1953). Vogel and pelletier [154,155](Vogel A, 1842; Vogel Pelletier,1815) stated that the ''yellow colouring matter'' of turmeric is due to the high levels of curcumin it contains. 17-bis (4- hydroxyl- 3methoxy phenyl) 1, 6 – heptadiene -3,5 –dione is now known as curcumin and its structure is recognized by Milobedzka and Lampe [27,28]
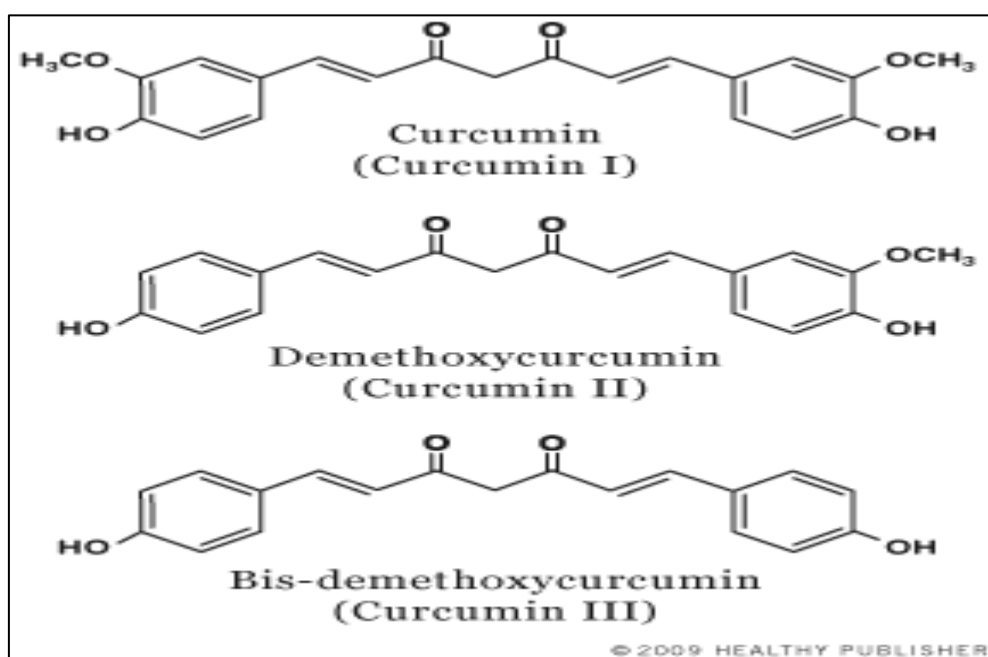
Parbon described a humble method for the synthesis of curcumin and in this the –CH$_2$ group of 1,3 diketone was blocked with boron oxideand reacted with an aldehyde (vaniline). Knoevenagel condensation is taking place here. The nucleophilicity of C3-carbon of 1,3 diketone (Acetyl acetone) is reduced by applying theboric oxide .The two terminal methyl groups of 1,3 diketone undergo aldol condensation.In between an intermediate is formed which upon hydrolysis gives the product curcumin.

CURCUMIN-PROPERTIES & BIOAVAILABILITY

Investigation has recognized that curcumin is the basic chemical component which is responsible for most of the biological properties of turmeric like anti-inflammatory[156-160](Menon *et al*., 2007;H.K.Biesalski,2007;Nurfina *et al* ., 1997; Siwak *et al*., 2005; Holt *et al*., 2005) antioxidant [161-166](P.Scartezzni & E. Speroni, 2000;Kim *et al*., 2003;Sun *et al.,* 2002;Dutta *et al*., 2005;O.P.Sharma, 1976;Khan *et al*., 2008) antiprotozoal [167-169](Rasmussen*et al*., 2000;Iwu *et al*.,1994;Araujo *et al*., 1999) nematocidal[170](Kiuchi *et al*., 1993)antibacterial[171-174](Shagufta Naz *et al*.,

2010;Paramasivam *et al*., 2007;A.Pal &A.K.Pal,2005;Mahony *et* al., 2005) antiviral[175] (Kim *et* al., 2009)antitumour[176-180] (Wilken *et* al.,2011; Aggarwal *et* al., 2003;Simoni *et al*., 2008;Fang *et al*., 2005;A.K. Chakravarty &H. Yasmin, 2005) activity etc. Fundamentally curcuminoids are 1,7-diaryl heptanoids [181](Gorbitz *et al*., 1986) in which diketo moiety is directly associated to olefinic groups . The characteristic structural features of curcumin include two methoxy phenol units, two enone moieties, and a 1,3-diketone system. Curcumin shows [182](Daijiro Yanagisawa *et al*., 2010) characteristic keto-enol tautomersim. It lacks its solubility in polar water medium but to very extent it is soluble in certain solvents like DMSO,chloroform, Ethanol,ethyl acetate, methanol, ethyl acetate, etc.Curcumin has been verified to be harmless in searching studies in human being even at great regular doses of up to 8 gm,[183-184](Bharath *et al*., LD Kapoor, 1990)with minor side effects .The major problem faced in experimental trials with curcumin is its reduced bioavailability in body.The restricted water solubility of curcumin ,low concentration, quick uptake and systemic elimination have been the leading causes for dropping its bioavailability [185](Antony *et al.,*2008)can be improved by enclosement of curcumin in the cavities of cyclodextrins[186](Chowdhury *et al* .,2008) *or* the advance of [187,188](Tiyaboonchai *et al.,* 2007; Karikar *et* al .,2007)nanoparticles and ceramic particles, change new formulations based on biocompatible organic materials like liposomes[189](Li *et al* .,2005),micells [190] (D.Suresh & K. Srinivasan, 2007) glycols,cellulose biopolymers etc. Some methods to increase the bioavailability are depicted under.

Nanoparticles: By the application of nanoparticle science we can bring out targeted and activated drug sending systems with nano paricles. There is particularly noticeable change in the bioavailability of therapeutic agents. By applying this nano delivey system we can overcome the reduced bioavailability of curcumin. Currently a nanocurcumin is reported by Bisht et al. based on its production, description and its tumour applications. Fundamentally it is a polymeric particle. This polymer based "nanocurcumin" has comparable property as that of natural curcumin.

Liposomes are outstanding methods used in providing drugs since it can transfer both hydrophilic and hydrophobic molecules. Li *et al* considered the antitumor activity of liposomal curcumin compared to human pancreatic carcinoma cells and certified that liposomal curcumin can avoid pancreatic carcinoma growth and in

addition exhibits antiangiogenic properties.Ruby *et al* also stated the anti-cancerous and antioxidant activities of neutral unilamellar liposomal curcuminoids in mice.

Micells and phospholipid complexes can improve the gastrointestinal absorption of natural drugs thereby giving higher plasma levels and lower kinetic elimination resulting in improved bioavailability. The intestinal absorption of curcumin and micellar curcumin formulation with phospholipid was evaluated using an in vitro model consisting of everted rat intestinal sacs. Scientists hope to attain significant antimicrobial actions by structural alteration on curcumin. Several works dealing with the improved biological properties of curcumin derivatives and analogues[191,192](Mosley *et al*., 2007;Shen *et al* .,2007) can be seen in literature . A systematic effort made by   Mosley *et al*. s describes some studies dealing with the biological activity connection of curcumin and its derived forms.

Complex formation by coordinate bond[109,110](Katherine.H.Thompson *et al*.,2004; Khosro Mohammadi *et* al., 2005) is the another way to enhance the biological properties of curcumin. Curcumin possesses two phenolic groups and one active methylene group and this makes it as an outstanding ligand for binding metals. A number of metal chelates of curcumin are found to have   biological activity over free curcumin.

Curcuminoids:Biological actions and   medicinal applications

In recent years, great extent of work has been completed to make the biological behaviour and Pharmacological actions of curcuminoids. The biological properties[84] of turmeric to a great extent depend on curcumin, the yellow bio active constituent of turmeric. These include its anti-inflammatory, hypocholesteremic, antidiabetic, antibacterial, antiviral, antifungal, antioxidant, antiprotozoal, antiviral, antiulcer, and hypotensive activities.Curcumin is considered as a dietetic phytochemical with low harmfulness. Many factors such as medicinal activities, pharmacological effects, and its colour made this as Indian solid gold.

Drug effect of curcumin as an anti-inflammatory agent [44] has been inspected by Srimal and Dhawan (1973). They informed that curcumin was operative in acute and chronic representations of inflammation. The power of curcumin is identical to a reference drug, phenylbutazone in carrageenan-stimulated edema test in mice. Mukophadhyay et al (1982) has proven the anti-inflammatory property of curcumin and sodium curcuminate by doing tests in cotton pellet granuloma models of inflammation in rats[193](Mukhopadhyay *et al*., 1982) also disclosed the performance

of sodium curcuminate[194](Ghatak & Basu (1972) as an anti-inflammatory agent. As reported by Huang et al (1992), "curcumin showed inhibitory effects on the proliferation of blood mononuclear cells and vascular smooth muscle cells"[195](Huang *et al*., 1992). Ammon et al (1992) demonstrated curcumin as an "inhibitor of leukotriene formation in rat peritoneal polymorphonuclear nuetrophills"[196](Ammon *et al*., 1992) Curcumin gives anti- inflammatory property by means of blocking Nuclear Factor Kappa B[197](S.Singh &B.B.Aggarwal, 1995)(NF-kB), a particle that moves into the nuclei of cells and stimulate genes associated to inflammation. The anti-inflammatory character of curcumin can be settled by down regulation of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthetase (INOS) through suppression of NFκB activation[198](Surh *et al*., 2001)

In 1975, a researcher named Sharma O.P studied about the antioxidant character of curcumin. The functioning antioxidant source in *curcuma longa* has been pinpointed as curcumin [199,200] (Moken *et al*., 1984; Choiu*et al.,* 1983). Because of its chemical configuration it can neutralize free radicals. So it can be considered as useful antioxidant. As reported by Pulla Readdy and Lokesh(12992) curcumin can diminish lipid peroxidation [201](Pulla ReddyAch &B.R.Lokesh, 1992) by retaining the vitality of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxide. They further realized that curcumin is apt for scavenging oxygen free radicals such as superoxide anions and hydroxyl radicals and that initiate lipid peroxidation.Confirming to Joe and Lokesh 1994, Curcumin retards the formation of reactive oxygen species (ROS), $H_2 O_2$ and nitrite radical generation [202](B.Joe & B.R.Lokesh, 1994) that has serious role in inflammation. As ROS have been interested in the advance of varied pathological conditions curcumin has the power to dominate these diseases[203,204](B.Halliwell, & J.M.C Gutteridge, 1990; Bandhyopadhyay *et al*.,1999)with its powerful antioxidant activity. Joe B *et al* announced that the phenolic and methoxy group on the phenyl ring and the 1, 3-diketone system in curcumin appear to be a relevant structural feature[205](Joe *et al*., 2004) and that assists to its antioxidant activity.Sharma *et al* noticed that the phenolic hydroxyl groups are needed for antioxidant activity and double the number of this functional group provide advanced activity than the curcumin. The function of β-diketone component for the antioxidant property was proposed by Sugiyama *et al*[206](Sugiyama *et al.,* 1976).

The regular build up of bacterial resistance to currently obtainable antibiotics has demanded the look for innovative and adequate antimicrobial composition, particularly of plant source. The development of several bacterial strains such as Staphylococcus albus and Staphylococcus aureus[207] (Chopra et al., 1941)are removed by the application of curcuma oil. Bhavani Shankar and Murthi [82](1979) reviewed the activity of curcumin opposite to intestinal bacteria in vitro and checked the hinderence of growth of Lactobacilli. Mahady et al disclosed [84] that curcumin, in addition avert the buildup of Helicobacter pylori cag A^+ strains in vitro. The research led by Dipit Rai *et al* (2008) has stated [208](Dipti Rai *et al*., 2008) that curcumin, a digestable polyphenolic compound, has been exhibited to have a powerful antibacterial activity compared to lot of pathogenic bacteria along with Staphyylococcus aureus, Staphyylococcus epidermidis and Enterococcus. Curcumin hinders FtsZ assembly in E.Coli.[209](Rai *et al*., 2008) a striking mechanism for its antibacterial property. Curcumin repressed the assembly of FtsZ protofilaments and too improved the GTPase activity of FtsZ . Curcumin lowered the aggregation of FtsZ protofilaments in vitro. The result indicates that the perturbation of the GTPase activity of FtsZ assembly is lethal to bacterial and suggested that curcumin inhibits bacterial cell proliferation by inhibiting the assembly dynamics of FtsZ in the Z-ring.Curcumin shows antiprotozoan activity. Curcumin and man-made derivatives have anti-Leishamania and anti L-amazonensis phenomenon[210,211](C.Gomes Dde & L.V.Alegrio, 2002; Koide*et* al., 2002). Curcumin displays activity when it reacts with plasmodium falciparum, and that is a protozoan.Curcumin possesses antiviral property.Mazumber *et al* (1995) demonstrated [212] (A.Mazumber &K.Raghavan, 1995) that " Curcumin being a HIV-I integrase inhibitor may be developed as anti-aids drug". Eigner and Scholz (1999) described [133] that curcumin exhibit anti HIV-1 and HIV-2 activities.

When they react with fungal [213] (Upendra *et al*.,2011) *infection* they have noticeable inhibitory property. Showed activity[214] (Jim *et al* .,2003*)* against fungi cretures namely R.Solani, Pu.recondita and P. infestans. They also showed antifungal property when they react with few phytophagous fungi such as Fusarium solani abd Helmintho sporium oryzae[215] (Chowdhury *et al*., 2008) S hadomy *et al* developed a new method, diffusion disc method and this is extremely efficient to analyze the antifungal property of curcumin. Turmeric oil has medicinal property in guinea pigs[84], diseased by T. rubrum. This kind of infection is removed by the dermal

operation of turmeric oil. The consideration of curcumin among 14 dissimilar strains of Candida group revealed that curcumin displays significant fungicidal activity when it reacts with them even at low concentration.The desirable mechanism showing the antifungal application was established by the down regulation of ERG3 gene guiding to major minimization in ergosterol of fungal cell. This results in the generation of biosynthetic precursors of ergosterol and that creates cell death by formation of reactive oxygene species[216] (Sharma *et al*., 2010). Decrease in proteinase secretion and modification of membrane related characteristics of ATP ase activity are another probable serious factors for antifungal activity of curcumin[217] (Neelofar *et al*., 2011)

In addition to the above properties curcumin has anti- carcinogenic [70] properties. There are numerous mechanisms for its activity where induction of apoptosis has an important part. A researcher named R.S.Ramsewak *et al* studied the effect of curcumin ina number of cancer cells such as melanoma[218] (Ramsewak *et al*.,2000) leukemia,colon and breast. He concluded that when polarity hikes cytotoxic effect is lowered. So the electron releasing groups such as methoxy has important role in exhibiting cytotoxicity in curcumin. As reported by Chen *et al* 1998. Curcumin brings about programmed cell death and hinders cell – cycle progression[67], both these avert cancerous cell development in rat aortic smooth muscle cells. The programmed cell death can be regulated by the suppression of protein tyrosine Kinase, protein Kinase C and bcl-2 m RNA expression. Curcumin creates programmed cell death by triggering damage to DNA in human cancer cell lines[219] (MartinCordero *et al*.,2003) Conforming to Duvoix et al[220] (Duvoix *et al*., 2003)"expression of glutathione-S-transferase P1-1(GSTPI-I) is associated to carcinogenesis and curcumin has been shown to induce apoptosis in K526 leukaemia cells by inhibiting the expression ofGSTP-1 at transcription level".Colon carcinoma is prevented by curcumin over the capture of cell-cycle progression independent of suppression of prostaglandin synthesis[221] (Hanif *et al*., 1997) .The curcumin also stamps out tumour development. Nitric oxide (NO) and its derived forms execute a leading role in tumour production. Curcumin constrains iNOS and COX-2 production[222] (Brouet*et al* ., 1995)by abolishment of NFκB activation.

Synthetic analogues of curcumin as chelating agents

Naturally occurring 1,3 diketones are curcuminoids and which are very interested in complex formation with many metals[223-225](Vajragupta *et al.*,2004; Khalil *et al.*,2013;Ferrari *et al.*,2013)So there is pronounced significance in studying their properties functions, characterization and its medicinal applications. Most of the metallic compounds[226,227](Borsari et al., 2002;Subhan et al., 2014) forms complexes with curcumin or their synthetic equivalents .

The ligating capacity of curcumin depends on its β- diketo component and it is the best example for didendate ligand. Thus they form ML_2 and ML_3 stoichiometry[228,229](Wanninger *et al.*,2015; Bagchi *et al.*,2015). The enolic in the curcumin plays a major role in complex formation and an enolic proton is substituted by the metal.

Chemical research on metal chelates of commonly occuring ligand structures and their implication in biology and medicine constitute one of the vibrant areas in inorganic chemistry. Biochemical activity of numerous plant chemicals are recognized to be connected with their capacity to form chelates with metal ion.

Medicinal applications of complexes

There are number of activities exhibited by curcumin metal complex. Some of them are antimicrobial, anti HIV, anticancer, antioxidant, and anti-Alzheimer's disease [230-232] (Chauhan *et al.*, 2013; Hatamie *et al.*, 2012; Sharma et al., 1987) etc. Since curcuminoids are good chelating agents they can form complex with various block metals such as representative group[233] (Radhika Pallikkavil *et al.*, 2013) d block elements and f block elements[98,111].Various Indium, Boron. Gallium[234,235](Asti *et al.*,2014;Ferrari *et al.*, 2014) Aluminium[236,237](Ahmadi *et al.*,2011;Jiang *et al.*,2014) complexes by curcuminoid equivalent ligands have been available. Most of the transition elements such as Fe, Mn, Ni, Zn, V, Ru, Zn, Co, Cu, etc make a range of curcumin equivalent complexes[238-240](Sumanont *et al.*, 2007; Khalil *et al.*, 2014; Goswami *et al.*, 2013). In very rare case these form complex with lanthanides and actinides[98,227,241] (Zhou *et al.*,2012) The best stimulating feature of metal curcuminiod equivalent complexes is that several of them display very different and extremely potential health properties. Tentative studies on anticancer features of metal-curcumin complexes predates to the year 1997. Abundant transition metal complexes of curcuminoid ligands have been efficiently tested in vitro and in vivo

for anticancerous property[242,243](Leung *et al*., 2013; Ali *et al*.,2013) Amongst the various transition metal complexes Cu(II) complexes showed maximum cytotoxicity and also display extensive contraction in solid tumour volume in ascites tumour burden mice[52]. Vanadium curcumin[109,110] complex showed antioxidant property, zinc curcumin complex showed anticancer effect, group 13 elements and transition elements with curcumin showed antitumour activity.copper complexes exposed better antitumour activity when they react with three human cancer celllines specifically ASPC-I,MCF-T and HeLa(Cervical Cancer)[245](Mei *et al*., 2011).

Certain metal complexes like aluminium, gallium, copper and zinc curcumin complexes behave as effective agent for certain diseses like Alzheimer's. Metal ions which can serve as powerful risk cofactors in AD[246](Jiang *et al*., 2012) comprise , $Al^{3+,}$ Mn^{2+},Fe^{3+}, Cu^{2+} and $Zn^{2+.}$nuerotoxicity ix controlled by metal curcumin complexes. Certain curcumin complexes of Ga and Tc verified with success for careful staining of β-amyloid plaques of AD[247](Triantis *et al*.,2013). It has been proven that Al-curcumin complexes are authentic in restricting proteins from the development of amyloid fibrils and even pull out the preformed amyloid deposits.

Antioxidant actions have being conferred by curcumin complexes of vanadyl,copper, gallium and indium. Copper and manganese complexes considered for their super oxide dismutase property, free radical scavenging and antioxidant potential.as reported by Barik A *et al* the medicinal properties of metal curcumin complexes depends on the type of ligand, for example Cu(Cur)$_2$has limited antioxidant activity [248](Barik *et al*., 2007) than Cu (Cur) (OAc) (OH).These two complexes much differ in their structure. The Cu (Cur)(OAc)(OH) complex suffers a greater distortion from the square planar structure, showed more activity than Cu(Cur)$_2$. From the current investigation it was found out that the 1:1 complex would be capable to go through and continue the distortion from square planar geometry to the distorted tetrahedral one during its action with superoxide radical. This permits the compound to experience several redox cycles and therefore works as a precise expert antioxidant. The complex Cu(Curc)$_2$ experience less distortion and therefore it behaves as cheaper antioxidant.the gold curcumin combination display the antioxidant property as reported by Singh *et al*.

Several vanadyl and gold complexes of curcumin exhibited antiarthritic property. When the gold curcumin complex was applied on the rat polyarthrit there was a contraction in paw swelling[232]. K.H. Thompson *et al* reported that "VO(curc)$_2$

exhibited significant antiarthritic activity compared with curcumin alone. Its activity was observed to be twice that of curcumin". Antiviral studies were so effective and it was conducted by B and Cu complexes. Vaginal gel is prepared by copper curcumin complex and that is used for curing viral infections. Z Sui *et al* have found "curcumin-boron complex[249](Sui *et al*., 1993) is a modest inhibitor of HIV-1 and HIV-2 proteases". Some homoleptic complexes have powerful anti-osteoporotic activity [250](Y. Mawani & C. Orvig, 2014) as mentioned by Y.Mawani and C.Orvig, 2014. Cisplatin like activity is found in some of the lanthanide curcumin complexes as reported by M.A.Subhan *et al* Escherichia Coli, Klebsiella and Pneumonia when treated with some of the complexes such as Pd,Y,Eu,Cr metal complexes , the synthetic analogues showed some antibacterial activity[230].some metal complexes showed DNA splitting skill . Some nanometal complexes of Co and Ag have anti-microbial[251](Sakey *et al*.,2012) feature. The synthetic silver complex was operative towards some bacterial strains namely Bacillus Cereus[252](Haroon Khalid *et al*.,2015) and E.coli.Compared to normal curcumin, $(cur)_3$ possess more antibacterial[253](Saeed Tajbaksh *et al*.,2008) property.

Curcuminiods are bioactive composites with dynamic bio-medicinal resources existing in turmeric. The current search is an effort to make synthetic equivalents of the active fundamental chemical component namely curcuminoids found in the herbaceous Indian medical plant turmeric. Coordination aspects of these medicinal plants have gained extensive thought in modern years.This is obvious from the later year's research in these areas. Here all the efforts are made to synthesis curcuminoid derivatives from the cycic 1, 3 diketones.

PART-III

MATERIALS, METHODS AND INSTRUMENTAL TECHNIQUES

MATERIALS, METHODS AND INSTRUMENTAL TECHNIQUES

3.1 Materials for synthesis of curcuminoid analogues and metal complexes:

The compounds consumed in the current study for production and analytical determinations were of analar grade and obtained from Sigma Aldrich, USA.Fourteen different aldehydes such as 2-methyl benzaldehyde,2,5-dimethyl benzaldehyde, thiophen-2-aldehyde, 3-methyl thiophene-2-aldehyde,2-chloro benzaldehyde,4-chloro benzaldehyde, 3,4-dichloro benzaldehyde, 3,4,-trimethoxy benzaldehyde, 3-ethoxy-4-hydroxy benzaldehyde,2,4-dihydroxy benzaldehyde, 1-naphthaldehyde, 2-methoxy naphthaldehyde, 2-hydroxy naphthaldehyde and anthracene-9-carboxaldehyde were used along with the 1,3-diketone,cyclohexane 1,3 dione.Metal salts used for the synthesis of metal complexes include copper (II) acetate monohydrate, nickel (II) acetate tetrahydrate and zinc(II) acetate.Marketable solvents were distilled and castoff for synthesis.

3.2 Methodsfor synthesis of curcuminoid analogues and their metal complexes Cu(II),Zn(II) &Ni(II) .

3.2.1 Synthesis of different curcuminoid analogues

1,3-cyclohexanone-boron complex is achieved by adding boric oxide (0.0035mol, 0.25g) to 1,3 cyclohexanone (0.005mol, 0.5g) by one hour agitation. . Then to the reaction blend, the aldehyde (0.01mol) which is dissolved in dry ethyl acetate (7.5ml) having tri(sec-butyl) borate (0.02mol,5.4 ml) were added and the temperature was retained above 80°C. The reaction mix was agitated further and n-butyl amine (0.1ml dissolved in 1ml dry ethyl acetate) was added slowly throughout 40min. Stirring was sustained for an additional period of ~ four hours and the solution was set aside overnight. The very next day, Hot (~60°C) HCl (0.4M, 7.5ml) was prepared and reacted to the above mixture by stirring one hour. After the stirring, organic layer was separated from aqueous layer by the solvent ethyl acetate. Then the solvent layer removed by evaporation and the remaining compound was stirred with HCl for ~1h. The mass products detached together, were washed away with water and dehydrated in vacuum. The product obtained was purified by column chromatography via silica gel (60 – 120 mesh)

3.2.2 Separation and purification of (4E, 6E) bis (aryl) cyclohexane 1,3 diones

The concrete output acquired on acidification was liquefied in least amount of ethyl acetate and fixed up the column thickly packed with silica gel. The eluting solvent used was 1:5 (v/v) acetone: chloroform mixture. After the elution, the product was examined by TLC and the combined extracts on removing the solvent in vacuum yield (4E,6E)bis(aryl) cyclohexane 1,3 diones in 60 − 70% yield. The isolated (4E,6E)bis(aryl) cyclohexane 1,3 diones were recrystallised twice from hot benzene to get chromatographically pure compound.

3.3   Synthesis of metal complexes with Cu (II),Zn(II) &Ni(II)

Metal salt used for the synthesis were Nickel (II) acetatetetra hydrate, copper (II) acetate mono hydrate and zinc(II)acetate. The complexes were available by the addition of a a methanolic solution of metal salt (25 ml, 0.001mol) to a solution of curcuminoid analogue (25 ml, 0.002 mol) in methanol and warmed mildly for 2 h. Subsequently dropping the volume to half, the solution was brought to room temperature.The precipitated complex was cleaned, lapped with 1:1, methanol:water mixture and recrystallized from hot methanol.

3.4 Characterisation of the ligands and metal complexes:

The synthetic ligands and metal complexes were identified by numerous spectral and analytical methods. The spectral systems include UV,IR,^{1}H NMR,^{13}CNMR, 2D COSY NMR and Mass spectra . Elemental analysis was to find out the C, H, S and metal percentage in them by Vario EL III analyzer.

UV-Visible Spectra

Absorbance spectroscopy was performed on a Schimadzu UV-VIS -1601 spectrophotometer using a solution of (10^{-3}M) of compounds in ethanol.

IR spectra

IR spectra gives a particular consideration of their molecular structure.IR spectroscopy is frequently used to recognize configurations because functional groups bring about representative bands both in terms of intensity and position(frequency).IR spectra (KBr pellets) of compounds were recorded on 8101 Schimadzu FTIR spectrophotometer.

¹H NMR spectra

The magnetic character of particular nuclei can be established by way of NMR spectroscopy. Different protons such as aryl,methine,alkenyl and enolic protons can be recognized in the spectrum [257-259]. The Varian 300 NMR spectrophotometer was used for recording ¹HNMR spectra. The spectra were recorded using MeOD as solvent and TMS as internal reference.

¹³C NMR spectra

Proton NMR detects H atoms while carbon NMR spectroscopy detects the presence of carbon atoms in a nuclei. It is almost same to ¹H NMR and the only difference is that nuclear magnetic resonance applied to carbon. Equivalent carbon atoms and dissimilar ones can be easily identified from¹³CNMRspectrum and its splitting pattern determined by the H-atoms linked to individual carbon atoms.Bruker, AV 400 – AVANCE III NMR spectrophotometer is used for recording ¹³C NMR spectra.

2D COSY NMR

The most common two dimensional NMR spectroscopic techniques is the cosy NMR. Generally it is applied for those compounds in which 1D NMR analysis is too much difficult and it usually delivers significant information of test compounds than the usual NMR specta reveals. Cosy NMR displays frequencies in vertical as well as in horizontal axes. Diagonal and cross peaks are the two important peaks observed in cosy NMR. Diagonal peaks give information about the 1 D NMR analysis and the cross peaks revealcoupling among the pairs of nuclei [260] .COSY NMR spectra were recorded using Bruker, AV 400-AVANCE III FT-NMR spectrophotometer.

Mass Spectroscopy

The technique is based on the fact that it ionizes the entire compound and converts it into chemical fragments that possess charge. The power of mass spectral technique to explore the structure of transition metal co-ordination complexes[261] has been revealed by Kentaro Yamaguchi. There are much noticeable differences in the mass spectra of beryllium β-diketonates [262] with that of equivalent β-diketone. Joel SX–102 mass spectrophotometer from CDRI, Lucknow, India is used for mass spectral analysis.

C, H, N analysis

        C, H, N analysis was carried out by Vario EL III analyzer. Metal percentage was also calculated by standard methods.

3.5 Thermogravimetric studies

        The phase change and thermal decomposition of analyzing compound can be very well explained by Thermogravimetry (TG) technique. A TGA[263] makes a continuous weighing of a small sample in a controlled atmosphere as the temperature is increased at a programmed linear rate. TG studies were conducted using Perkin Elmer Diamond Thermal Analyser System.

3.6 Biological studies of Curcuminoid analogues and their Transition metal chelates

The Invitrocytotoxic study,Invivoantitumour study, effect of compounds on solid tumour, Antibacterial and Antifungal studies were the parameters used in order to assay the biological activities of the compound.

3.7.1 Invitrocytotoxicity study

Materials

The entire reagents and chemical compounds applied for the investigation are pure quality grade.

Cell lines: Adayar Cancer Research Institute, Chennai, India provides cancerous cell lines namely, Daltons Lymphoma Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC).

Preparation of Reagents:

        Normal Saline: It can be made by dispersing A.R.NaCl (0.85g) in 100 ml distilled water. Phosphated Buffer Saline(PBS): it is a water based solution to maintain constant pH. One of the main advantages is that it is non-toxic to many cells. One noticeable thing is that it is isotonic with human body. It is made by dissolving NaCl (8g),KCl(0.2g),$Na_2HPO_4.2H_2O$(1.44g) and KH_2PO_4(0.2g) in one litre distilled water.

Trypan Blue dye: The living cell doesn't absorb Trypan Blue Dye while dead cells display a specific blue colour when dye is passing through it i.e living cells are excluded from this method of determination.It is prepared by dissolving 1 g trypan blue in 100 ml distilled water.

Method

In vitro cytotoxicity testing methods are applied primarily to recognize possibly harmful chemicals and/or to check the lack of certain toxic characters in the early stages of the development of potentially valuable drugs.The unsaturated diketone based ligand and its metal chelates were taken in least amount of DMSO. The extracted cancer cells (DLA &EAC) were cleansed with PBS (Phosphate buffered saline) and centrifuged at 1000 rpm for 5 min. The growth conditions were checked out by trypan blue exclusion method. Diketone ligand and metal chelates are taken in various concentrations and viable cells (1×10^6 cells in 0.1 ml) are added in to it and the volume is fixed to 1 ml with the help of PBS. One tube is treated as the control tube which includes nothing but cell suspension. Incubation is carried out for the text mixtures for a time period of 3 h at 37°C. Later, trypan blue dye is added to the text mixture containing cell suspension and colour change has been noticed after few minutes. Live cells have undamaged cell membranes that exclude dyes whereas dead cells do not.The number of marked and unmarked cells were numbered and percentage cytotoxicity (cell death) was assessed by Trypan blue dye exclusion technique.

% cytotoxicity=[No:of dead cells/ No:of dead cells+No:of live cells] x 100

3.7.2 Invivo antitumour studies

Cell lines: The cancerous cells EAC and DLA were extracted from mice and washed several times with PBS solution. After that it is suspended in normal saline water in order to get cell suspension. This method provides 1 million cells/ml and one ml of this solution were introduced into the peritoneal cavity of fresh Swiss albino mice. The creatures under examination were fed normally and within two weeks there is buildup of fluid that comprises ascites cancer cells were deposited particularly in peritoneal cavity. The tumours developed in the creatures and were died within 25 days. These cells are circulated commonly by transporting it to new normal mice as stated above and thus the cell lines were maintained.

Animals:Swiss albino mice(7-8 weeks old weighing 25-30 g,male) were acquired from the Small Animal Breeding Station (SABS), Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala. The animals were preserved in well ventilated cages under standard conditions of temperature and humidity in animal house of Amala Cancer Research Centre. The creatures were fed with

standard mouse chow (Sai Durga Feeds,Bangalore,India) and water *at libitum*. All animal experiments in the investigation were done with the prior sanction of the Institutional Animal Ethics Committee (IAEC) and were carried out strictly agreeing to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (No.ACRC/IAEC/16-01/(1) constituted by the Animal Welfare Division, Govt. of India.

Preparation of drug suspension

Drug suspension preparation follows the following procedure.

1. Carboxymethyl cellulose (CMC,0.05g) dissolved in distilled water(10ml)
2. 0.01 gm (4E,6E)-4,6-bis(aryl) cyclohexane -1,3 dione and their metal(Cu,Zn,Ni) chelates were dissolved suitable solvent.
3. Combine these two solutions and preserve the mixture in a water bath for evaporation of solvent in order to get the drug in the slurry form.
4. The reference drug cyclophosphamide was prepared by dissolving 25mg/kg body weight of mice in phosphate buffered saline (PBS) solution.
5. About 0.01 ml of this std. drug was given to each mouse for ten days.

Methods

Determination of the effect of compounds in reducing ascites tumour development

Six animals form a study group and there were many groups. Ascites tumour was made in these animals by introducing viable EAC cells in 0.1 ml of PBS into the peritoneal cavity. One group was kept as control group which wasn't treated with any drug. Another group was treated with the standard drug,cyclophosphamide and the remaining ones are treated with drugs after the tumour induction. The creatures were observed for survival for 1 month from the 15th day onwards and their increase in life span(ILS) was calculated using the formulae,

[% ILS= {(T − C)/ C} X 100, where T and C are mean survival of treated and control mice respectively.]

Determination of the effect of compounds on solid tumour development

The Internal introduction of DLA cells on the right hind limb of mice was carried out through injection and this lead to the development of solid tumour. For comparison of the end result one group was retained as standard, and it was treated with reference drug. Another group was retained as control group and no drug was

given to it. The remaining groups were treated with unsaturated test compounds for about 10 days continuously and number of days of procedure were kept as same for the control group and standard group also. The tumour volume was obtained by measuring the diameter of tumour and it was applied in the formula $V = 4/3\pi \, r_1^2 r_2^2$ and this procedure was repeated in every third day for a period of one month. Where r_1 and r_2 are the minor and major radii respectively.

3.8 Antibacterial assay (Agar well diffusion method)

The influence of several test compounds on the numerous bacterial strains was assessed by agar well diffusion method. Muller-Hinton (MH) agar medium is mixed with 1000 ml of water to prepare the agar plates. The three Bacterial strains namely EscherichiaColi, Klebsiella Pneumoniae and Bacillus Subtilis were freshly seeded and regularly spread into the exterior of the agar plates by using a stick. Wells were cut into agar plates with sterile gel puncture. The unsaturated diketone based curcuminoid equivalent and their metal chelates were dissolved in DMSO and were supplied into the wells. Here the reference drug is streptomycin and it is treated as positive control and DMSO act as negative control. The antibacterial activity was measured in terms of mean diameter of the zone of inhibition in mm.

3.9 Antifungal activity by Kirby Bauer or Disc Diffusion Method

Disc diffusion method is adopted to test the antifungal properties of unsaturated diketone based ligand and its metal complexes. Sabouraud's Dextrose broth is used for preserving the fungal creatures. Homogeneous distributions of fungi on the SDA plates were done with the help of sterile swabs. Sterile filter paper discs of 3mm diameter were placed on the surface of SD agar plates at a distance of 2cm using sterile forceps. The medicinal drug which is used for the antifungal investigation was dissolved in DMSO having a concentration of 2%. One noticeable thing is that the solvent DMSO has no pronounced effect on the entire fungal species.
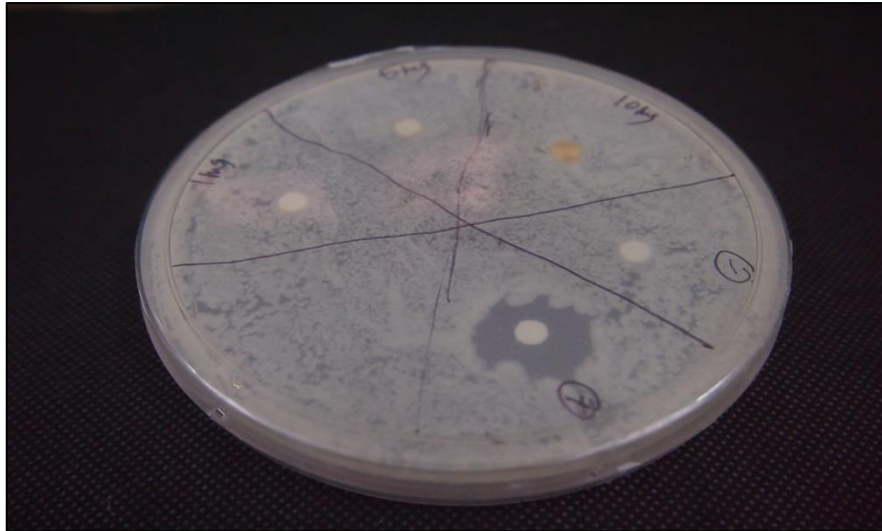
Media used and their composition

Muller Hinton Agar Medium(1 L)Muller Hinton Agar Medium (HiMedia) is mixed with distilled water.The dissolved medium was autoclaved at 15 lbs pressure at 121°Cfor 15 minutes. The autoclaved medium was mixed well and poured into100mm petriplates (25-30ml/plate) while still molten.

Nutrient agar media was used for maintaining pure fungal / bacterial culture and to lawn the fungus / bacteria for detecting the anti -microbial activity. It was prepared by dissolving peptone (1g), meat extract (0.5g), NaCl (0.5g) and agar (2.5g) in distilled water (100ml) and adjusting the pH of the medium to 7.2 – 7.4 using 10% NaOH.Sabaraud's Dextrose broth was used for maintaining pure culture for fungi samples. It was prepared by dissolving peptone (1g), D-glucose (4g) and agar (2.5g) in distilled water (100ml) and adjusting the pH of the medium about 5.6 - 6.0 using 10% HCl. A suspension of fungal / bacterial spores in normal saline (by dissolving 0.95g of NaCl in 100mldistilled water) was used for lawning / spreading. Solutions of the test compounds were prepared in DMSO and for sterilizing, all the media used were autoclaved at 121°C for 20min.

PART-IV
EXPERIMENTAL

CHAPTER-I

SYNTHESIS,CHARACTERIZATION AND BIOCHEMICAL ACTIVITIES OF MONO AND DISUBSTITUTED (4E,6E)-4,6-BIS(ARYL)CYCLOHEXANE-1,3-DIONESAND THEIR TRANSITION METAL COMPLEXES WITH CU(II), Zn (II) &Ni(II)

INTRODUCTION

This chapter is divided into five sections. Section I is on the synthesis and characterization of (4E,6E)-4,6-bis(aryl)cyclohexane-13-diones. In section II the synthesis and characterization of metal complexes of (4E,6E)-4,6-bis(aryl)cyclohexane-1,3-dionesare considered. Antibacterial studies of (4E,6E)-4,6-bis(aryl)cyclohexane-1, 3-diones and its metal complexes are included in section III and antifungal studies of (4E,6E)-4,6-bis(aryl) cyclohexane-1, 3-diones and its metal complexes are discussed in section IV. Section V deals cytotoxic studies of (4E, 6E)-4, 6-bis(aryl) cyclohexane-1, 3-diones and its metal complexes.

SECTION I
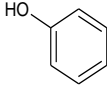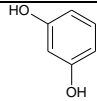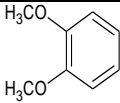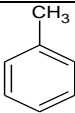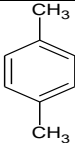
THE SYNTHESIS AND CHARACTERIZATION OF (4E, 6E)-4,6-BIS (ARYL) CYCLOHEXANE-1,3-DIONES WITH MONO & DISUBSTITUTED PHENYL RINGS

1.1.1. Synthesis of mono & disubstituted (4E,6E)-4,6-bis(aryl)cyclohexane -1, 3-diones

The compounds were prepared by the reaction of the aldehydes (2-hydroxybenzaldehyde, 2,4-dihydroxybenzaldehyde,2,3-dimethoxybenzaldehyde,2-methylbenzaldehyde,2,5 dimethyl benzaldehyde)with cyclohexane-1,3- dione as in **scheme 1.1.1**. The solvent used was ethyl acetate and the condensation was carried out in the presence of tri-butyl borate and n-butyl amine. The synthesized compounds were purified by column chromatography using 4:1 chloroform: acetone mixture as eluent. For better purification the products were recrystallized from hot benzene.
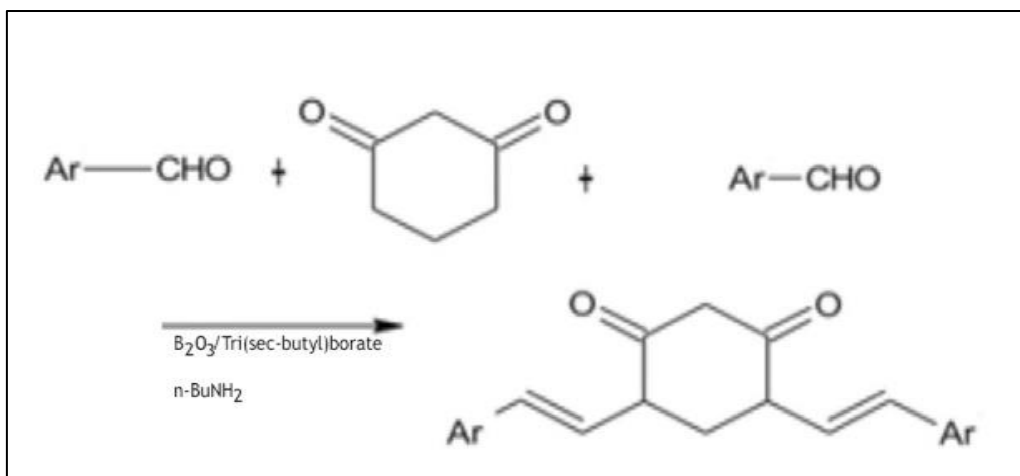
Scheme 1.1.1.
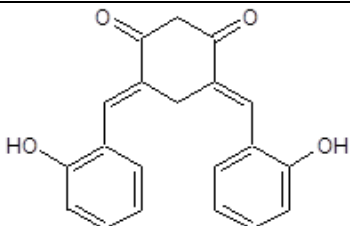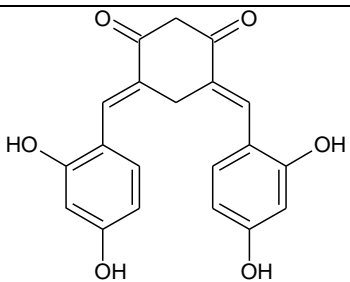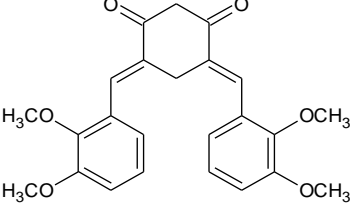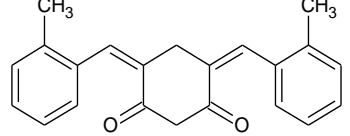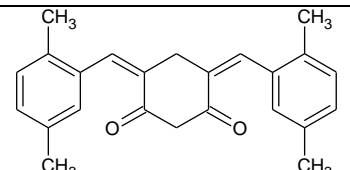
Compound	Ar
1a	
1b	
1c	
1d	
1e	

The starting aldehyde andyield are given in **Table 1.1.1.**

All the curcuminoid analogues are crystalline in character with sharp melting point.

The analytical and physical data of the compounds are shown in **Table 1.1.2.**

Table 1.1.1 Synthetic details of (4E,6E)-4, 6-bis(aryl)cyclohexane -1, 3- diones.

	Aldehydes	Structure of synthetic analogue	systematic name	Yield (%)
1a	2-hydroxy benzaldehyde		(4E,6E)-4,6-bis(2-hydroxybenzylidene) cyclohexane-1,3-dione	72
1b	2,4-dihydroxy benzaldehyde		(4E,6E)-4,6-bis(2,4 dihydroxy benzylidene)cyclohex ane -1,3-dione	68
1c	2,3-dimethoxy benzaldehyde		(4E,6E)-4,6-bis(2,3-dimethoxy benzylidene)cyclohex ane -1,3-dione	77
1d	2-Methyl benzaldehyde		(4E,6E)-4,6-bis(2-methyl benzylidene)cyclohex ane-1,3 dione	64
1e	2,5-dimethyl benzaldehyde		(4E,6E)-4,6- bis(2,5-dimethylbenzylidene) cyclohexane-1,3-dione	67

1.1.2 Characterization of the phenyl substituted ligands

The synthesized compounds were characterized on the basis of UV, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral techniques.

Table 1.1.2 Analytical and electronic spectral data of (4E,6E)-4,6-bis(aryl)cyclohexane -1, 3 –diones.

| Compounds | M.P. (°C) | Elemental analysis (%) | | Molecular weight | Electronic spectra λmax (nm) |
| | | C | H | | |
		Found/(Calculated)			
1a, $C_{20}H_{16}O_4$	185	74.01(75.33)	4.99(5.05)	318(320)	278 466
1b,$C_{20}H_{16}O_6$	189	67.87(68.18)	4.92(5.00)	351(352)	276 465
1c,$C_{24}H_{24}O_6$	197	69.148(70.58)	5.96(6.14)	406(408)	271 454
1d,$C_{22}H_{20}O_2$	147	83.4 (83.54)	5.34(5.95)	315(316)	265 358
1e, $C_{24}H_{24}O_2$	163	82.17 (83.73)	5.52(5.87)	343(344)	292 417

Electronic spectra

The electronic spectra were taken by dissolving the test compounds in the solvent; methanol and the result were shown in **Table 1.1.2.**All the compounds in the region showed two broad brands due to n$\rightarrow \pi *$ and $\pi \rightarrow \pi *$transitions.

Infrared spectra

The IR spectra of the compounds displayed two prominent bands at about 1630cm^{-1}and 1650cm $^{-1}$, and assignable to the v(C=O) cinnamonyl and cyclohexyl chelated carbonyl vibrations. The Presence of a broad band in the region of 3200-3600 cm^{-1} supports the existence of compounds in the intramolecularly H-bonded enolic form.The alkyl and aryl vibrations of the compound were detected in the area of 2400-3000cm $^{-}$1.The most important peak lies at 1625 cm^{-1} which corresponds to v(C=O cyclohexyl) in the compounds.This clearly indicates that C=O group in diketone –curcuminoid analogue are in conjugation with alkenyl bond. Furthermore it strongly supports the presence of intra molecular hydrogen bonding in the compound. The IR peaks as a result of phenyl and alkenyl vibrations are specified in **Table 1.1.3.**The bands in the range 1237 cm^{-1} belong to the in plane C-H vibrations of phenyl ring and at 880 cm^{-1} is due to out of plane C-H vibrations of

aromatic ring. The IR spectra of the compounds **1a**, **1c** and **1e** are reproduced in **Fig.1.1.1, Fig.1.1.2** & **Fig.1.1.3** respectively.
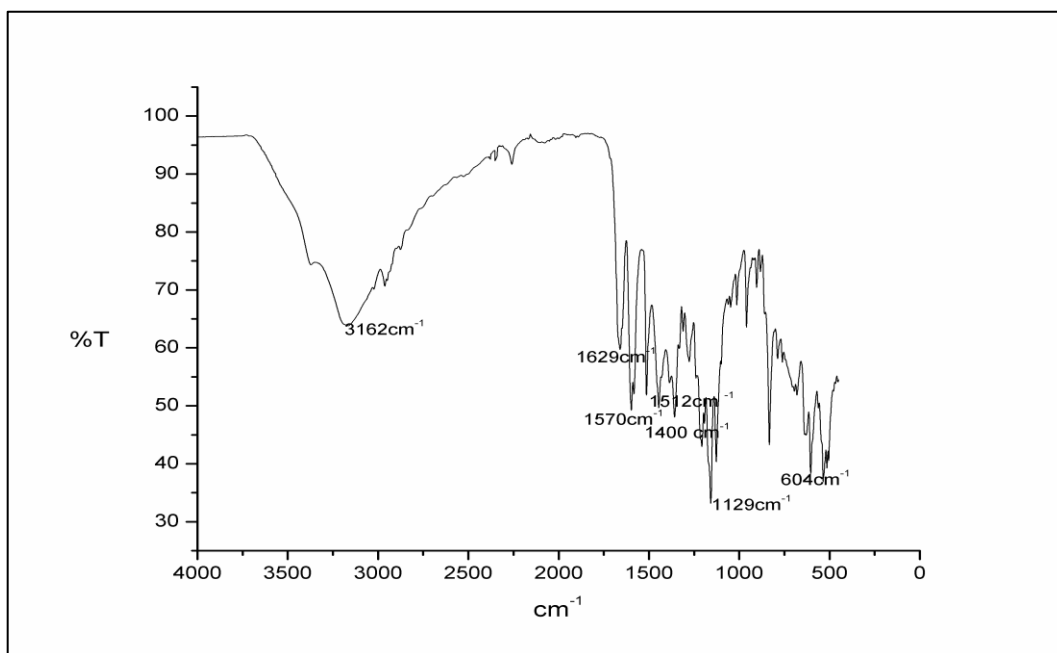
Fig.1.1.1   IR spectrum of 1a.

Table 1.1.3 IR spectral details of (4E, 6E)-4, 6-bis (aryl)cyclohexane -1, 3-diones.

Compounds					Feasible IR assignments
1a	**1b**	**1c**	**1d**	**1e**	
1651	1666	1655	1689	1692	ν(C=O) cyclohexyl
1629	1625	1641	1634	1617	ν(C=O) cinnamonyl
1570	1561	1590	1597	1553	ν(C=C) phenyl
1512	1514	1513	1532	1515	ν(C-C) alkenyl
1400	1474	1503	1506	1480	ν_{as}(C-C-C) chelate ring
1421	1428	1411	1313	1450	ν_{s}(C-C-C) chelate ring
1129	1160	1128	1136	1175	$\nu\beta$(C-H) chelate ring
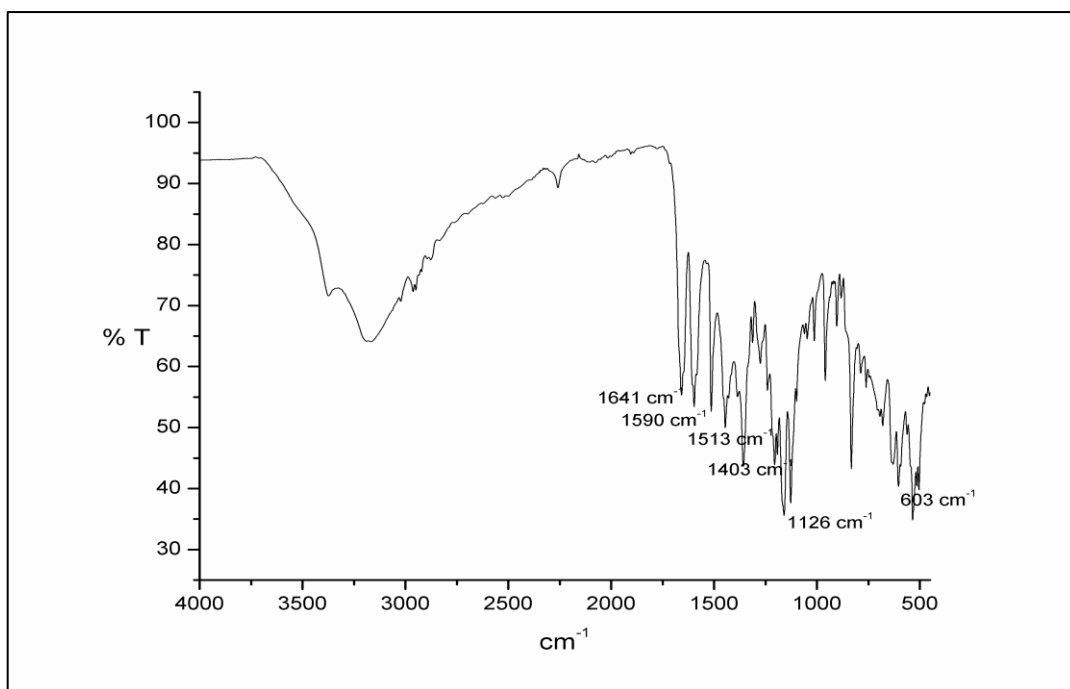
Fig.1.1.2   IR spectrum of 1c.
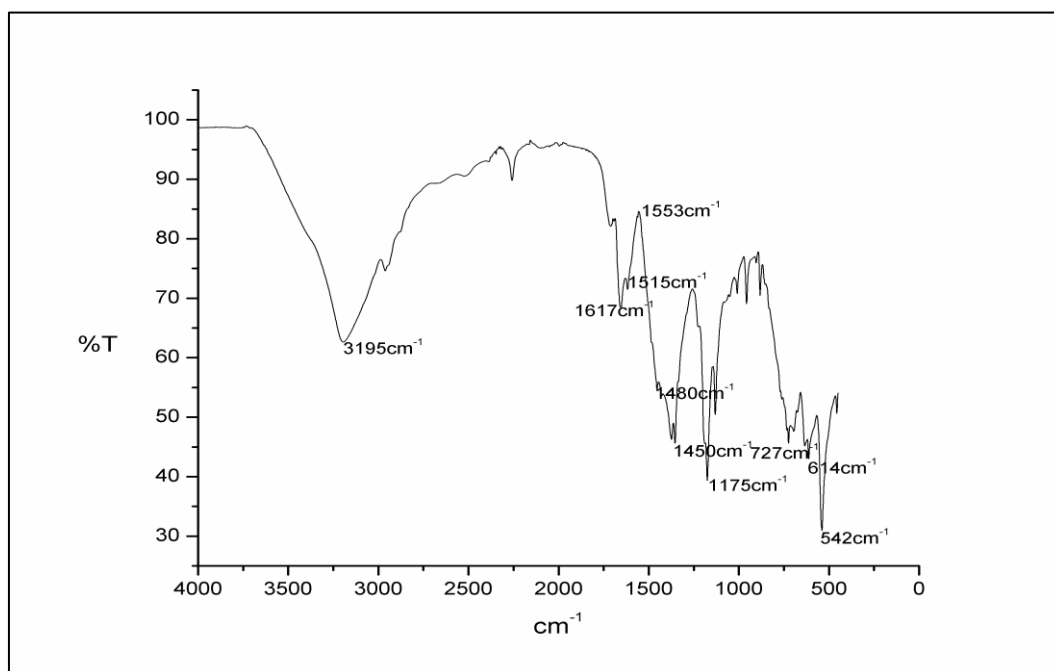
Fig. 1.1.3   IR spectra of 1e.

^{1}H NMR spectra

The ^{1}H NMR spectra of(4E,6E)-4,6-bis(aryl)cyclohexane-1,3-diones displayed peaks conforming to enolic, methine, alkenyl, methyl, phenyl and phenolic groups.

Table 1.1.4 ^{1}H NMR spectral data of(4E,6E)-4,6-bis(aryl) cyclohexane -1,3-diones

Compound	Chemical shifts (δ ppm)				Substituent			
	Enolic	Methine	Alkenyl	Phenyl		OCH$_3$	Phenolic	CH$_3$
1a	16.07	5.71	6.11-7.42	7.27-7.41		-	9.73	1.44-1.51
1b	16.11	5.33	6.8 – 7.81	7.12 – 7.63			10.11	
1c	16.4	5.67	6.44-7.39	7.13-7.39		3.68–3.97	-	-
1d	16.48	5.44	6.925-7.681	7.013-7.512				2.51
1e	16.30	5.52	6.933-7.812	7.47-7.49				2.43

The presence of the peak above 16 ppm of the compounds (4E,6E)-4,6-bis(aryl) cyclohexane -1,3-diones suggests a the existence of the compounds predominantly in the enolic structure. The presence of proton at 7 ppm indicates a methine proton which is also intra-molecularly H-bonded. The identification of these two protons can be carried out using the structure which is depicted below.In the region of 7 to 8 ppm the presence of phenyl protons can be detected while the region of 6.11-7.85 ppm indicates the distribution of alkenyl protons. The ^{1}H NMR spectra of **1a**, **1c&1e** are depicted in **Fig.1.1.5- 1.1.7**. The observed peaks and their assignments are given in **Table 1.1.4.**
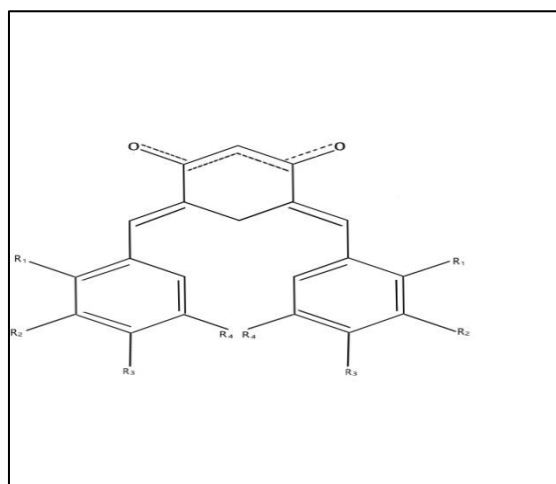
Fig 1.1.4 Enolic structure of ligands.

^{13}C NMR spectra

The ^{13}C NMR spectral data of the compounds are given in **Table 1.1.5, 1.1.6, 1.1.7, 1.1.7 & 1.1.8**. ^{13}**C NMR spectra of 1a & 1c** are given in **Fig.1.1.8 & 1.1.10**. at 114 ppm the peak which is generated by methine (C1) is seen.The deviation of C1 carbon at 100 ppm confirms the presence of keto- enol tautoumerism. The chelated carbonyl carbon generates a peak at 186 ppm and it is labeled as C2 carbon. In the neighbourhood of aromatic ring the presence of alkenyl carbon can be detected and in the region of 116-166 ppm the occurrence of aromatic carbon atoms are observed. The aromatic carbon atoms are present between 117 – 154 ppm.The presence of hydroxy group which is in association with for a C9 and C5 carbons, can be detected in the region of 117 ppm. In **1d** & **1e** the carbon which is attached to the alkenyl carbon atom (C5) is down shielded and present at a position ~ at 134ppm. The methyl carbon on the aryl ring of 1a is present at a position ~ at 30ppm whereas two methyl carbons of 1b are present at positions 20.9 & 19.3 respectively. The carbon atom (C6) which is attached to methyl group is downshielded and is ~ at 140ppm.
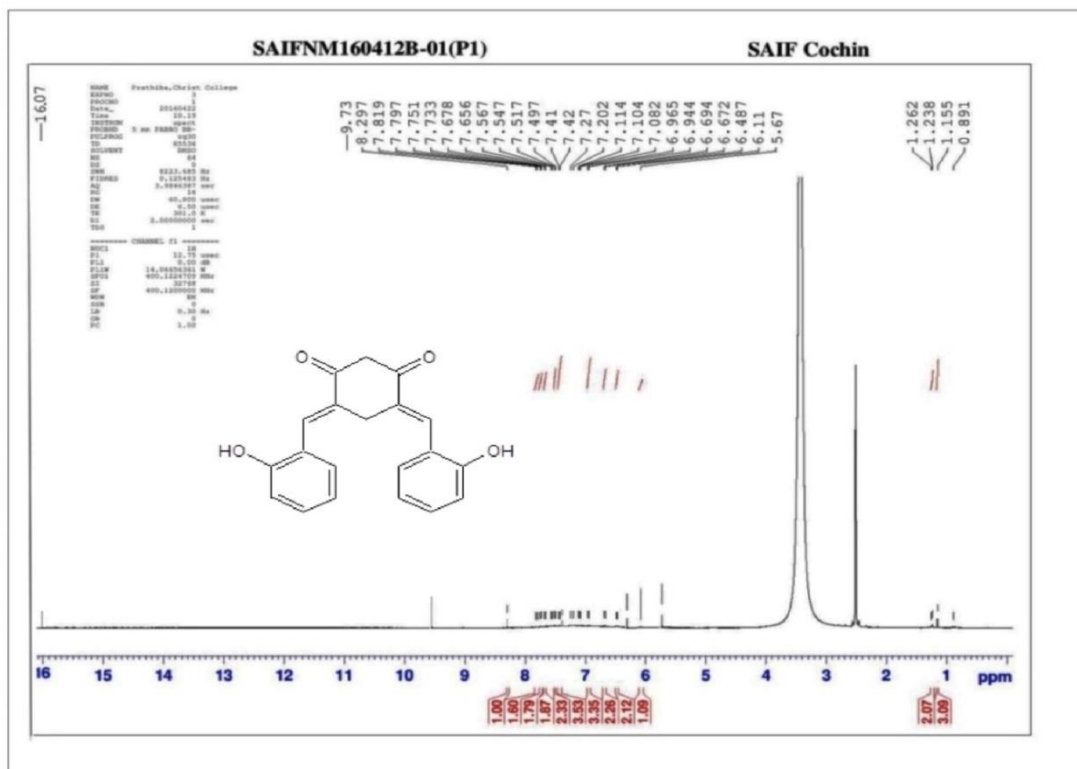
Fig.1.1.5 ^{1}H NMR spectrum of 1a.
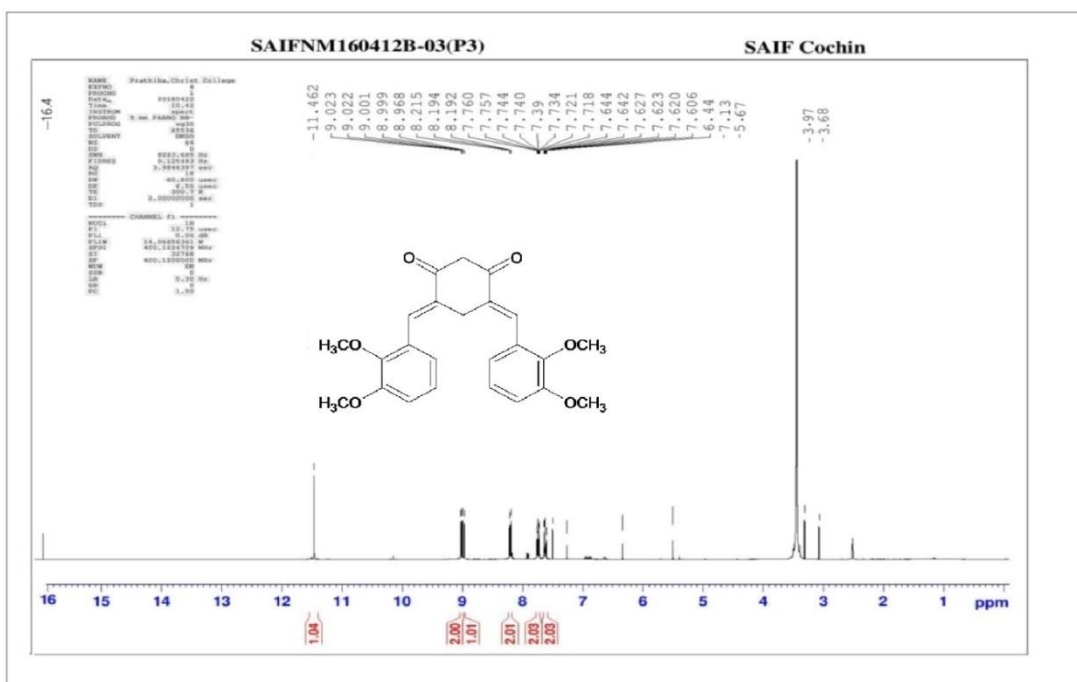
Fig.1.1.6[1]H NMR spectrum of 1c.
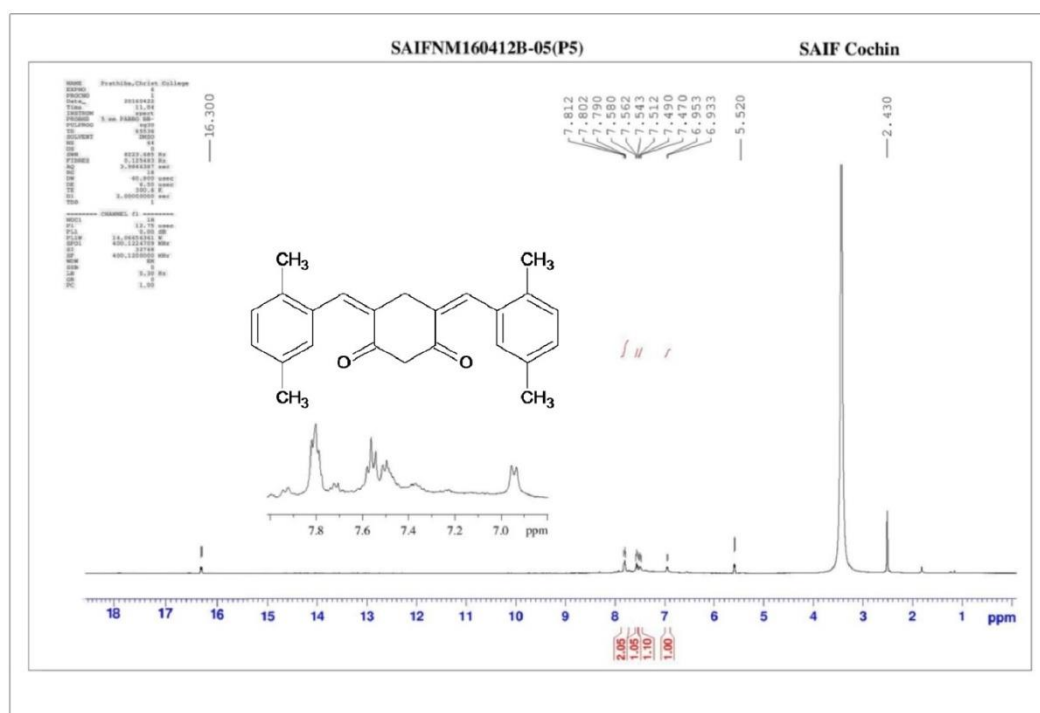
Fig.1.1.7^{1}H NMR spectrum of 1e.

Table 1.1.5 ^{13}C NMR spectral data of 1a (chemical shift in ppm)

C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'
114.01	186.45	147.28	117	151.74
C6,C6'	C7,C7'	C8,C8'	C9,C9'	C10,C10'
137.27	166	154.42	119	117

The identification of the carbon atoms which doesn't have any association with the hydrogen atoms can be carried out by comparing the DEPT-**135** spectrum with ^{13}C NMR Spectrum. The occurrence of negative peak is a clear indication of the presence of methylene group in the compound.The absence of peaks in the DEPT spectrum on comparison with the ^{13}C NMR spectrum reveals that there is no association between C2,C3, C5,C10 with hydrogen atom.
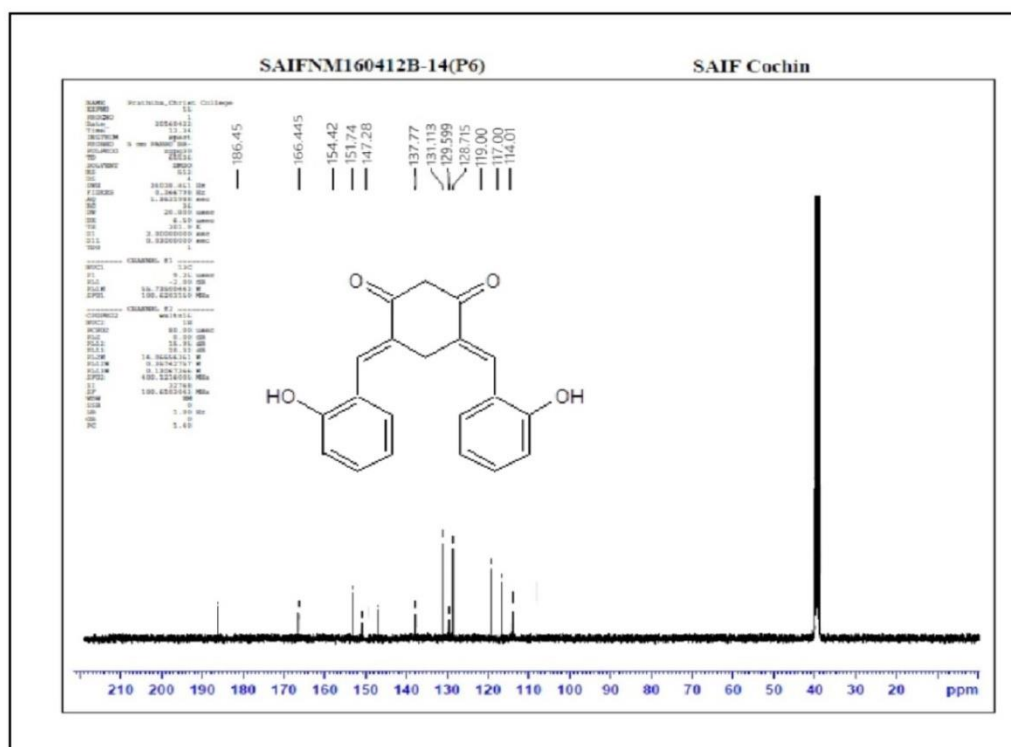
Fig.1.1.8 ^{13}**C NMR spectrum of 1a.**
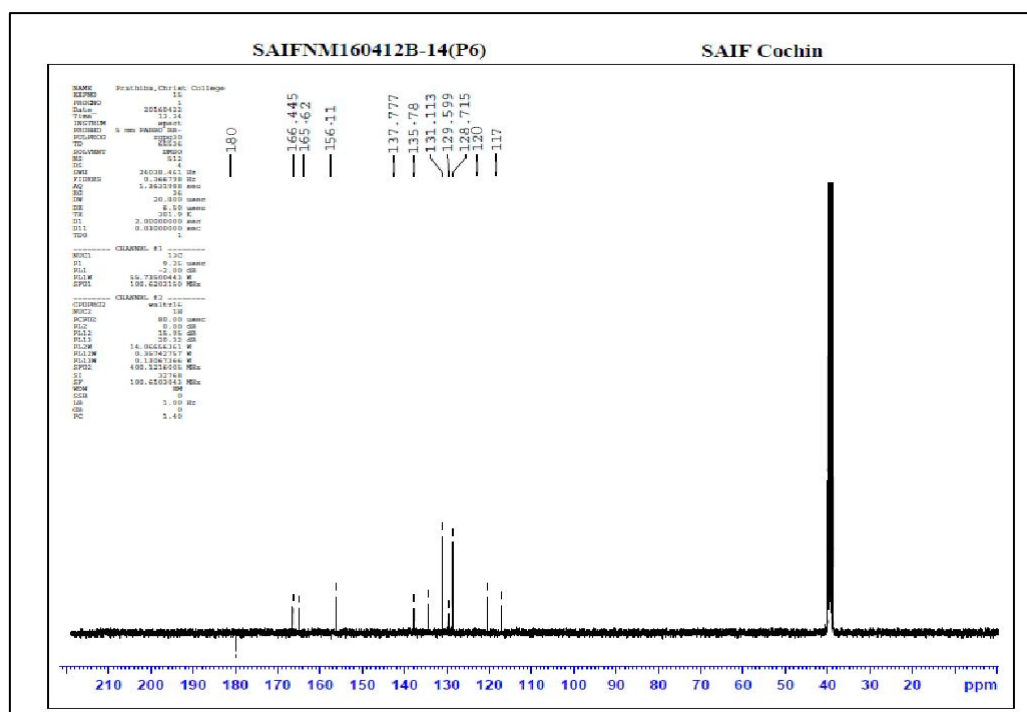
Fig.1.1.9 DEPT-135 spectrum of 1a.

Table 1.1.6 ^{13}C NMR spectral data of 1b (chemical shift in ppm)

C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'
110.43	187.62	142.79	123.74	149.25
C6,C6'	C7,C7'	C8,C8'	C9,C9'	C10,C10'
138.03	128.59	137.92	120.82	131.22

In (4E,6E)-4,6-bis(2,4 dihydroxy benzylidene) cyclohexane -1,3- dione, the C1 carbon flanked by C=O moiety produced a peak at 110 ppm whereas carbonyl carbon C2 generates a peak at 187 ppm.at regions of 138 and 137 two peaks are observed corresponding to C6 and C8 respectively, they are associated with the hydroxyl group. The hydroxyl groups are attached to carbons C6 & C8 and the peaks are observed at positions 134.35 & 135.65 respectively. In **1c** OCH$_3$ groups are associated to carbon atoms C6, & C7. The peaks are down shielded and are detected at range 153.23 ppm for C6 and 151.49 ppm for C7

Table 1.1.7 ^{13}C NMR spectral data of 1c

C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'
111.08	189.24	141.74	121.29	148.23
C6,C6'	C7,C7'	C8,C8'	C9,C9'	C10,C10'
152.23	151.49	142.58	119.43	133.71

Table 1.1.8^{13}C NMR spectral data of 1d (chemical shift in ppm)

C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'	C6,C6'
107.44	195.1	135.76	123.11	130.92	139.42
C7,C7'	C8,C8'	C9,C9'	C10,C10'	C11,C11'	
125.67	120.81	118.38	133.17	24.22	
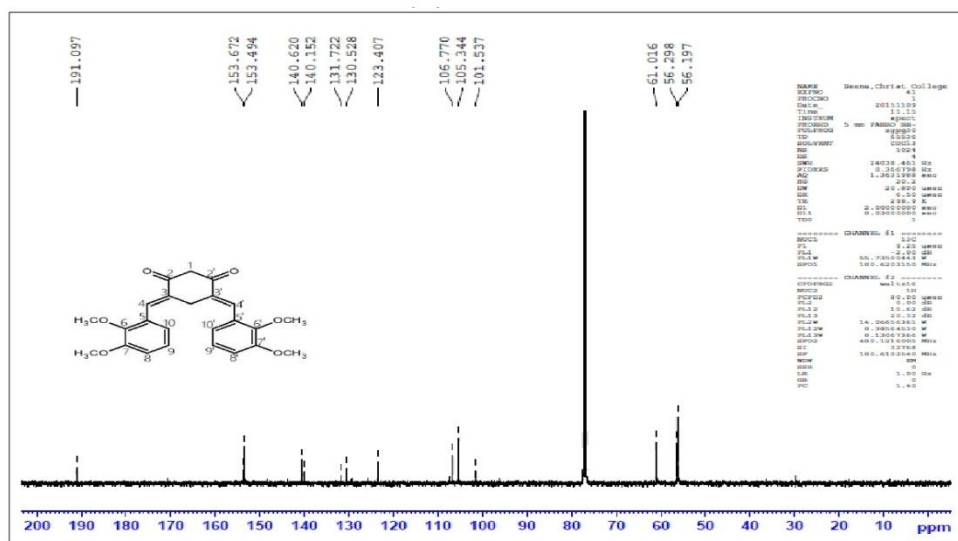
Fig.1.1.10 ^{13}C NMR spectrum of 1c.
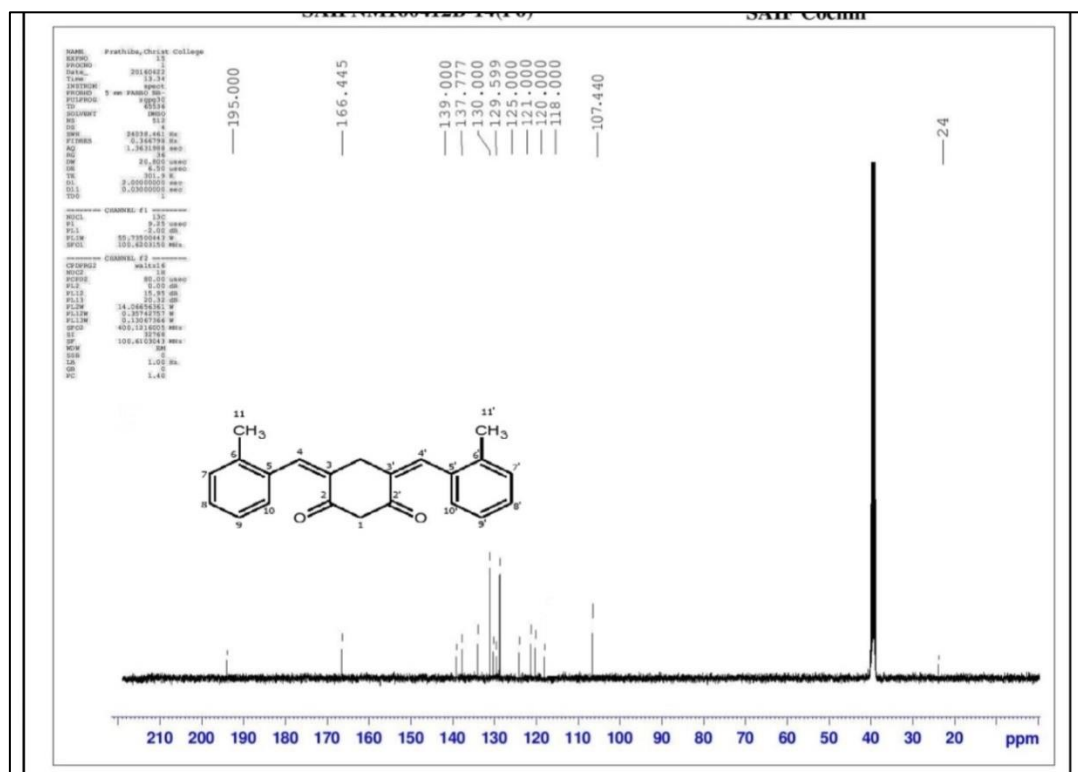
Fig.1.1.11 ^{13}C NMR spectrum of 1d.

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Table 1.1.9 ^{13}C NMR spectral data of 1e (chemical shift in ppm)

C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'	C6,C6'
102.82	181.48	134	125.68	131.37	135.42
C7,C7'	C8,C8'	C9,C9'	C10,C10'	C11,C11'	C12,C12'
1130.585	122.51	120.739	123.495	18.4	17.72

Mass spectra

The analysis of mass spectrum of **1a** reveals the presence of a prominent [P+1] ion peak at the region of 321.Formation of important fragments from the molecular ion are given in **Scheme 1.1.2** and its data are mentioned in the **Table 1.1.10**.The base peak is observed at 160 which is due to structure B [Ar-CH=C(CH$_2$)-CO]+ and 174 is due to structure A. The peak at 133 is due to structure C [Ar-CH=C(CH$_2$)]+ and at 104 is due to the structure D [Ar-CH]+. In the mass spectrum of **1b**, the base peak is observed at 176 and can be assigned to the peak of B [Ar-CH=C(CH$_2$)-CO] $^+$ and 122 is due to to structure D [Ar-CH]+. The mass spectrum of **1d** is given in **Fig.1.1.12** and **1e** is given in **Fig 1.1.13**. The peaks due to the removal of minor components such as O, OH, CH$_2$ etc. from the molecular ion are also appeared in the spectrum.The important peaks appeared in the mass spectra of **1d** & **1e** can be explained from their fragmentation patterns.

Scheme labels: A, B, C, D, E, F, G, H; M⊕; Ar; CH₂; CO; G - C₂H₂

$$G - C_2H_2 \quad H$$
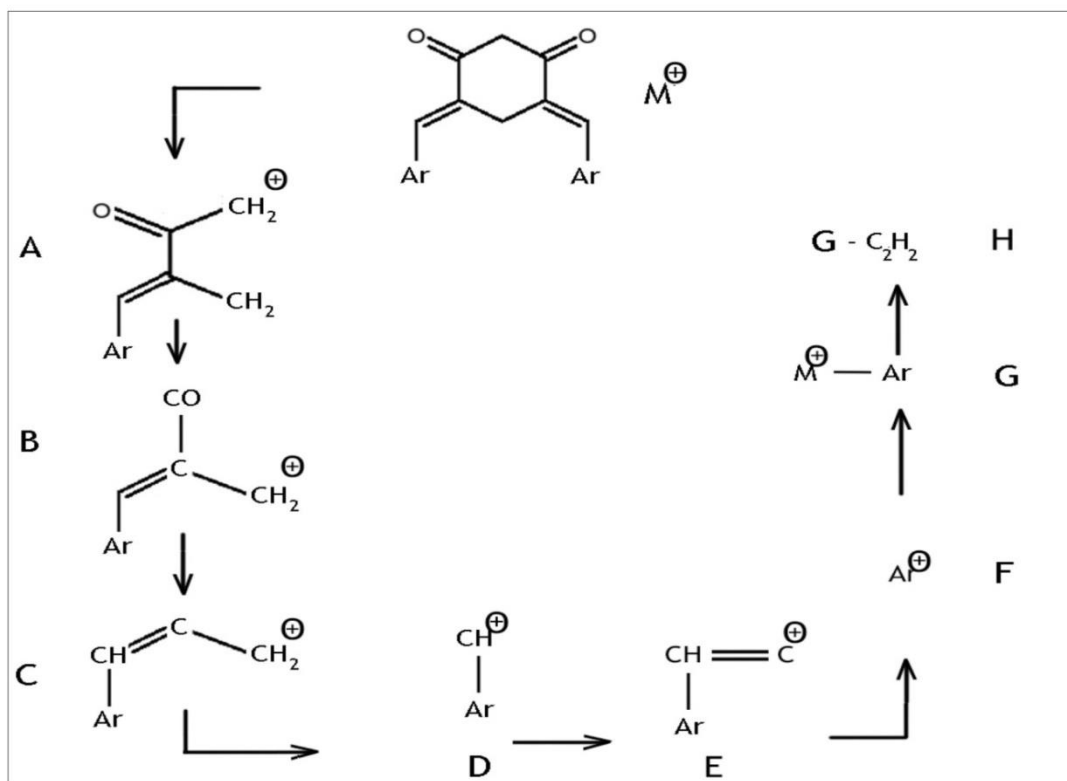
Scheme 1.1.2.

Table 1.1.10   Mass spectral data of 1a, 1b,1c,1d & 1e

Fragments	diketones	M+/ M+1 Ion	I	II	III	IV	V	VI	VII	VIII
Mass pattern	$C_{20}H_{16}O_2$	321	174	160	105	117	93	110	227	201
	$C_{20}H_{16}O_4$	352	190	176	122	134	109	126	243	217
	$C_{24}H_{24}O_6$	408	218	204	150	161	137	154	271	245
	$C_{22}H_{20}O_2$	317	160	146	104	116	91	108	225	199
	$C_{24}H_{224}O_2$	344	186	172	118	130	105	122	239	213
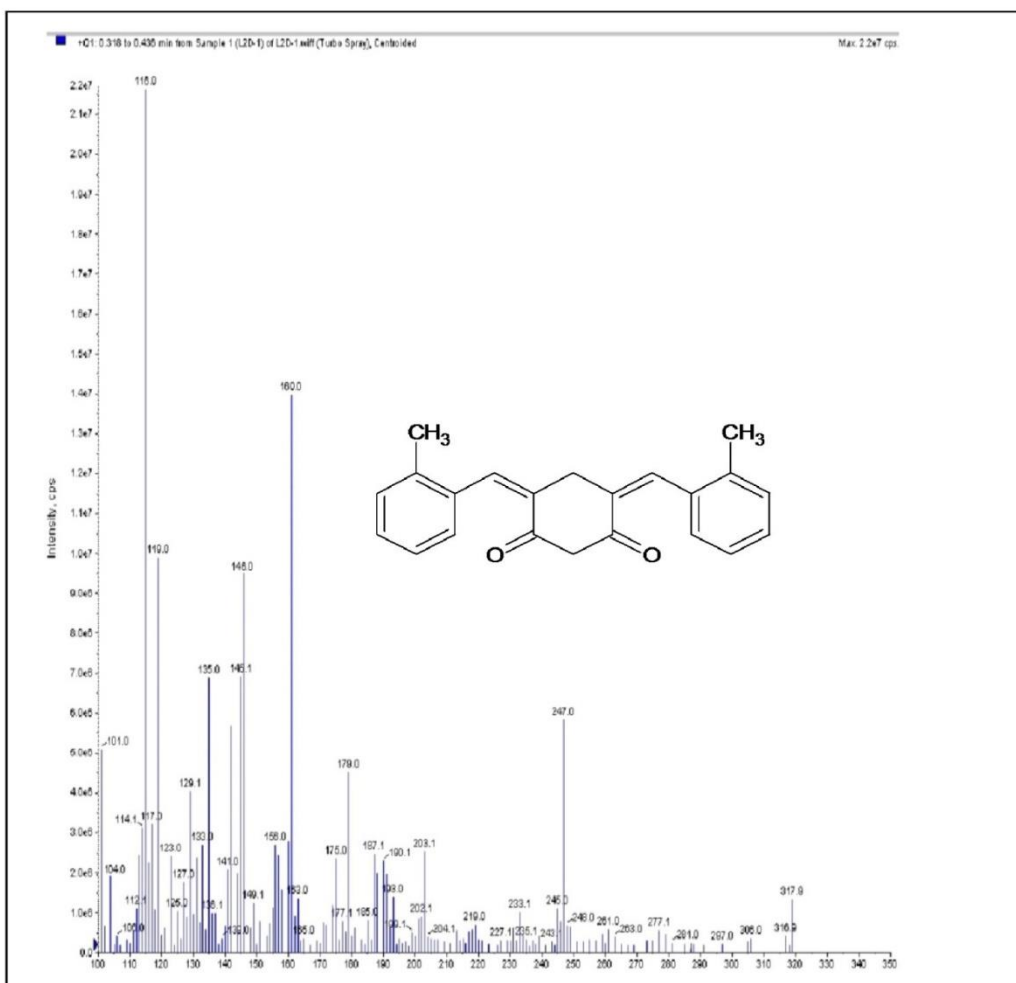
Fig.1.1.12 Mass Spectrum of 1d.
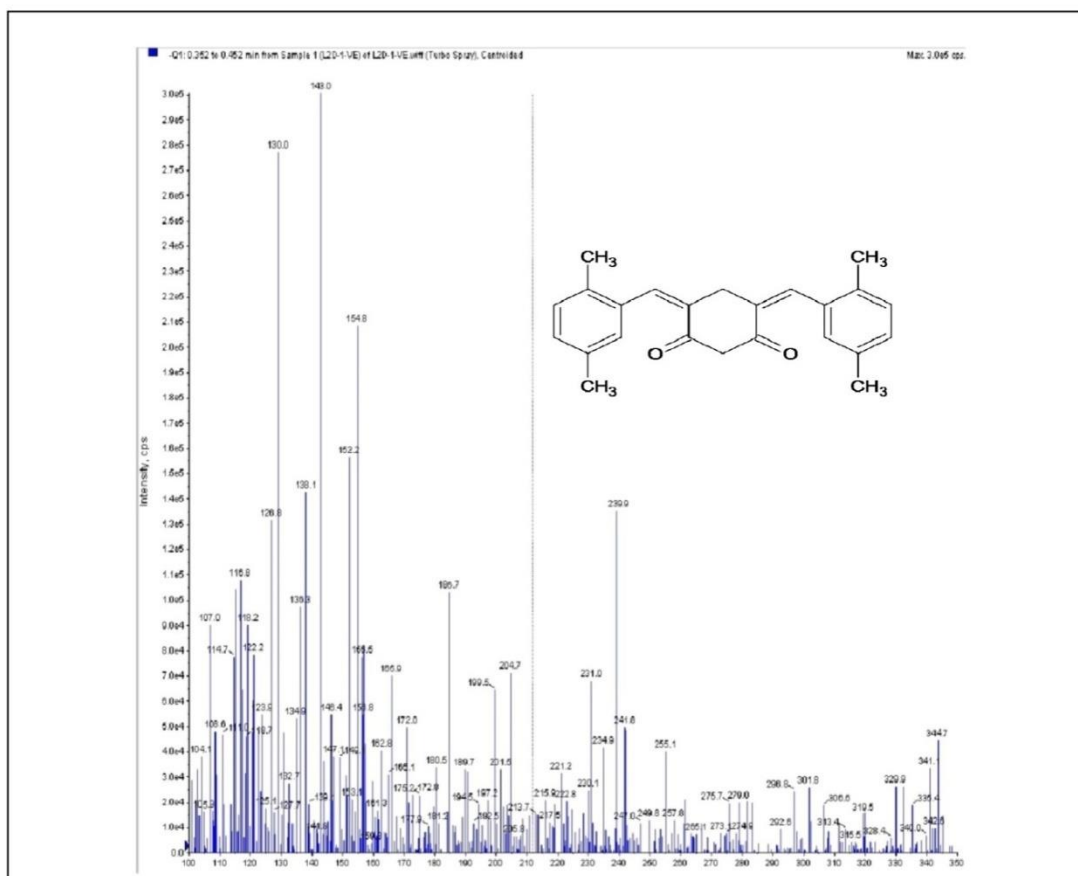
Fig.1.1.13 Mass Spectrum of 1e.

Thus all the spectral evidences such as UV,IR,NMR,mass spectra and TG analysis support the structure of the compounds as in **Figure 1.1.14.**
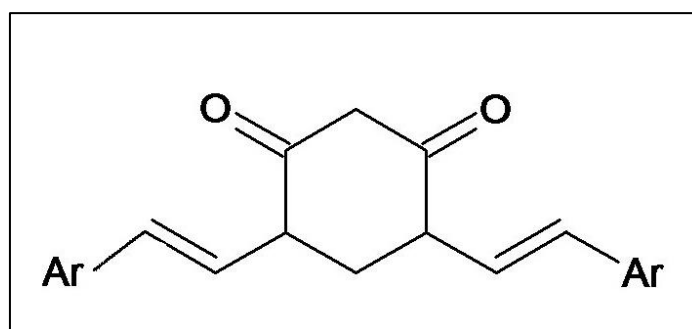
Fig 1.1.14 structure of ligand.

SECTION-II

SYNTHESIS AND CHARACTERISATION OF TRANSITION METAL CHELATES OF MONO AND DISUBSTITUTED (4E,6E)-4,6-BIS(ARYL) CYCLOHEXANE -1,3 DIONES

1.2.1 Synthesis of metal complexes of (4E,6E)-4,6-bis (aryl)cyclohexane -1,3 diones

   Copper (II), zinc (II) and nickel (II) complexes of the curcuminoid analogues considered in section 1, were synthesized by adopting parbons method.The generally adopted scheme is outlined here.To a heated solution of the diketone (**1a**,**1b**,**1c**,**1d**&**1e**) (0.002mol) in methanol (25ml), an aqueous solution of metal salt (0.001mol) was added and the reaction mixture was refluxed for almost 4 hours and cooled to room temperature. The precipitated complex was filtererd, washed with 1:1 methanol: water mixture and recrystallized from hot methanol. The metal salts used were copper (II) acetate, zinc (II) acetate &nickel (II)acetate.

1.2.2 Characterization of metal complexes

Metal chelates were characterized on the basis of the spectral technique such as UV,IR, NMR and mass data. Mass spectra support the [ML$_2$]configuration of curcuminiod –diketone metal complexes. And their datas were depicted in **Table 1.2.1- 1.2.5**.

Table 1.2.1 Analytical and spectral data of metal complexes of 1a

Metal used	M.P. (°C)	Elemental analysis (%) Found/(calculated)			Electronic spectra λmax (nm)	Feasible IR stretching bands (cm⁻¹)		
		C	**H**	**M**		**(C=O)**	**(C-C-C)**	**(M-O)**
Cu(II)	243	67.35 (68.27)	4.001 (4.551)	8.04 (8.96)	283 472	1588	1506	460 411
Zn(II)	217	67.98 (68.04)	4.26 (4.53)	8.92 (9.26)	284 477	1577	1501	447 423
Ni(II)	203	67.72 (68.69)	3.94 (4.57)	7.83 (8.40)	281 475	1582	1518	441 417

Table 1.2.2   Analytical and spectral data of metal complexes of 1b

Metal used	M.P. (°C)	Elemental analysis (%) Found/(calculated)			Electronic λmax nm	Characteristic IR stretching bands (cm⁻¹)		
		C	H	M		(C=O)	(C-C-C)	(M-O)
Cu(II)	193	61.92 (62.58)	4.02 (4.17)	7.86 (8.21)	273 468	1613	1510	463 418
Zn(II)	178	61.94 (62.38)	3.86 (4.159)	7.902 (8.49)	277 471	1617	1515	455 414
Ni(II)	171	61.75 (62.93)	3.94 (4.19)	7.04 (7.69)	279 474	1610	1519	462 417

Table 1.2.3   Analytical and spectral data of metal complexes of 1c

Metal used	M.P (°C)	Elemental analysis (%) Found/(calculated)			Electronic λ max nm	Characteristic IR stretching bands (cm⁻¹)		
		C	H	M		(C=O)	(C-C-C)	(M-O)
Cu(II)	202	64.47 (65.52)	5.14 (5.46)	6.79 (7.16)	286 478	1581	1502	464 412
Zn(II)	219	64.59 (65.35)	5.39 (5.44)	6.63 (7.41)	288 464	1588	1517	471 417
Ni(II)	223	62.16 (65.85)	5.22 (5.48)	6.16 (6.70)	261 461	1585	1521	465 419

Table 1.2.4 Analytical and spectral data of metal complexes of 1d

Metal used	M.P (°C)	Elemental analysis (%) Found/(calculated)			Electronic λmax nm	Characteristic IR stretching bands (cm^{-1})		
		C	H	M		(C=O)	(C-C-C)	(M-O)
Cu(II)	183	76.24 (75.91)	5.01 (5.75)	9.01 (9.13)	268 362	1594	1517	463 418
Zn(II)	187	75.01 (76.44)	5.39 (5.79)	8.77 (8.49)	271 364	1591	1522	467 429
Ni(II)	182	74.13 (75.71)	5.44 (5.73)	9.11 (9.37)	283 367	1588	1521	465 431

Table1.2.5 Analytical and spectral data of metal complexes of 1e

Metal used	M.P (°C)	Elemental analysis (%) Found/(calculated)			Electronic λ max nm	Characteristic IR stretching bands (cm^{-1})		
		C	H	M		(C=O)	(C-C-C)	(M-O)
Cu(II)	185	75.01 (76.64)	6.94 (6.38)	8.99 (8.45)	273 369	1608	1521	467 458
Zn(II)	186	78.03 (77.14)	6.98 (6.42)	7.97 (7.86)	277 371	1604	1527	463 431
Ni(II)	183	75.61 (76.45)	6.89 (6.37)	8.91 (8.67)	288 373	1610	1532	478 433

Electronic spectra

The electronic spectra of thetransition metal complexes are almost similar and the bathochromic shift indicates the involvement of of carbonyl group in metal complexes.

IR spectra

The Infrared specta of all (4E,6E)-4,6-bis(aryl)cyclohexane-1,3-diones are similar but somewhat dissimilar from transition metal chelates.A typical IR spectrum of the transition metal complex has been illustrated in **Fig.1.2.1**. In the complexes, theC=O stretching of the free ligand is moved considerably to lower frequencies. This kind

of observation strongly supports the participation of carbonyl moiety in complex formation. The transition metal ions replaced the enolic proton so that the broad band which was originally present in(4E, 6E)-4,6-bis(aryl)cyclohexane -1,3 diones is controlled . IR spectrums of complexes also possess some additional peaks in the range of 410-460cm $^{-1}$ due to M-O bond which further supports the complex formation.The IR spectrum of complexes are depicted in **Fig.1.2.1- 1.2.3**.
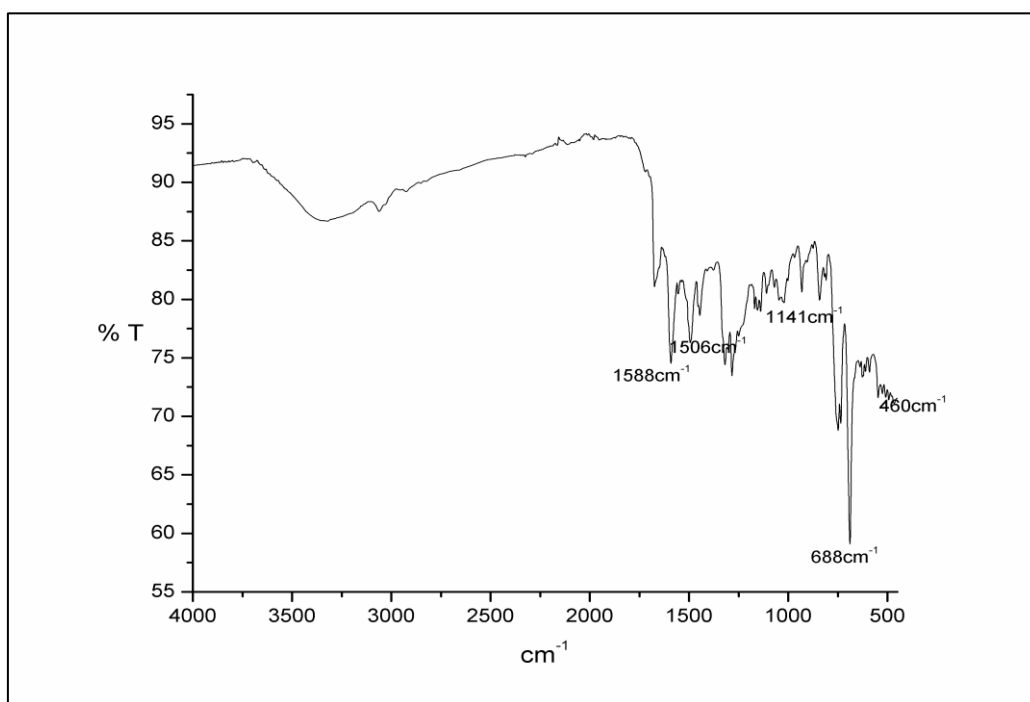
Fig.1.2.1.IR spectrum of Cu(II) complex of 1a.
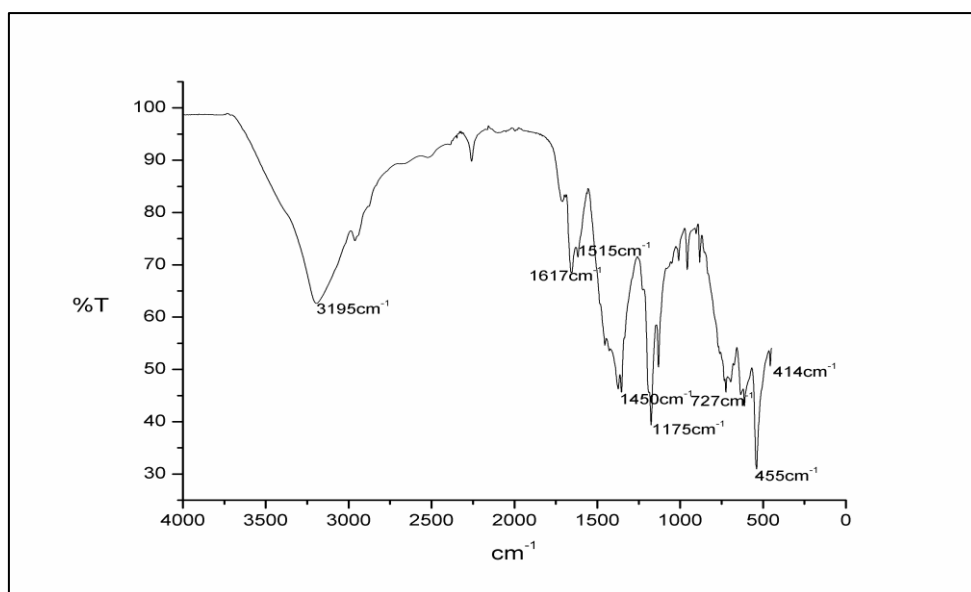
Fig.1.2.2   IR spectrum of Zn(II) complex of 1b.

IR spectrum of copper complex of 1e displays the ν_{M-O} vibrations are in the region of 400-490cm^{-1} as medium intensity bands.
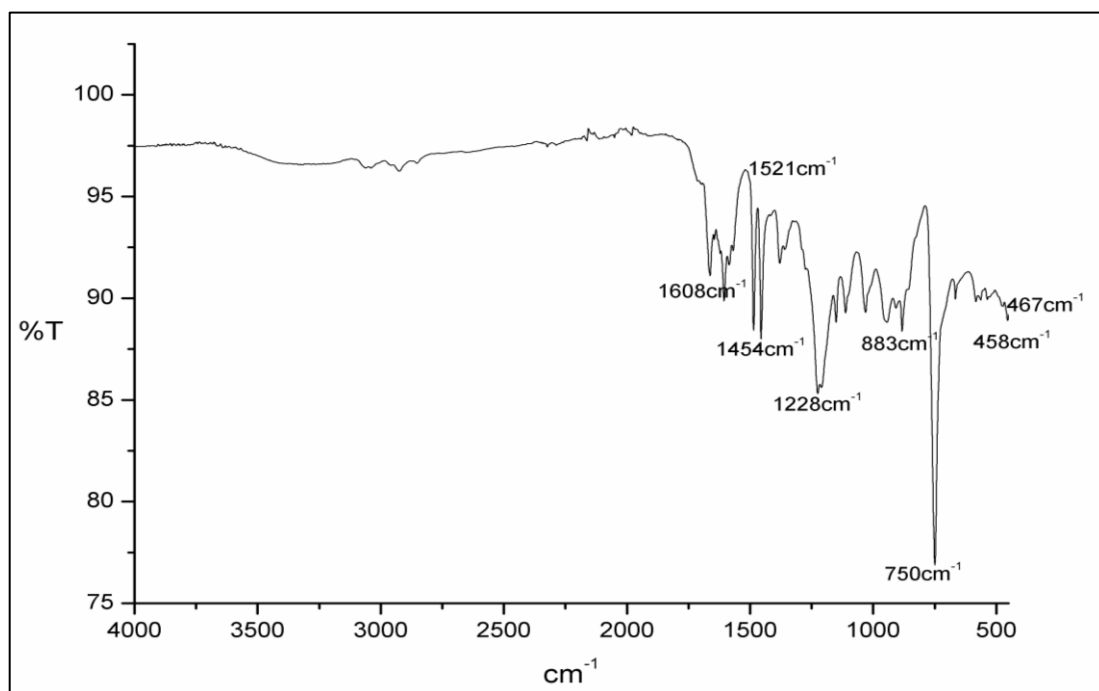
Fig.1.2.3 IR spectrum of Cu(II) complex of 1e.

^{1}H NMR spectra

The absence of enolic peak around 15 ppm in the^{1}H NMR specta of the complexesgave significant evidence that in complex formation process enolic proton is replaced by transition metal ions. The peak due to aryl and olefinic protons is the same both in the ligand and its metal chelates. There is minor displacement of methine peaks to the downfield of the spectra. In total both these spectrums resembles with each other and the only difference is in the case of enolic peak.The ^{1}H NMR spectrum of Ni(II) complex of ligand **1d** is depicted in **Fig 1.2.4**
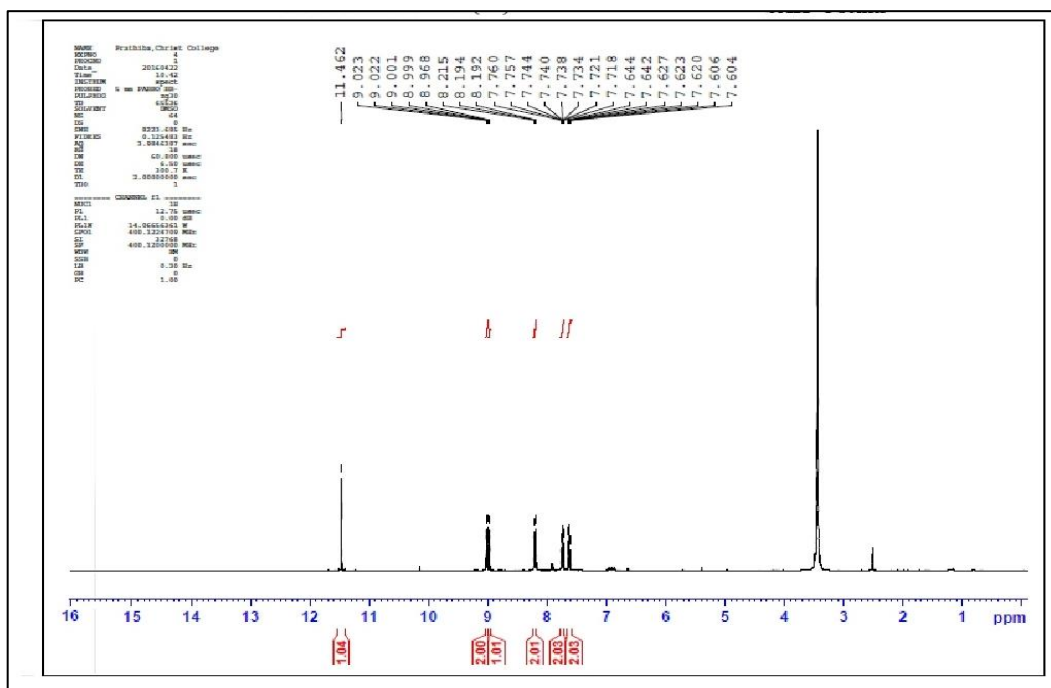
Fig 1.2.4 ^{1}H NMR spectrum of Ni(II) complex of ligand 1a.

Mass spectra

The mass spectra clearly indicate the[ML_2] stoichiometry of the metal complexes. Successive removal of organic moiety from (4E, 6E)-4, 6 bis (aryl) cyclohexane -1, 3 doines has been observed in most cases. Peaks due to [ML]$^+$, [L$^+$]and fragments of [L$^+$]are also detected in the spectra. General fragmentation patterns are depicted in **scheme1.2.1**.
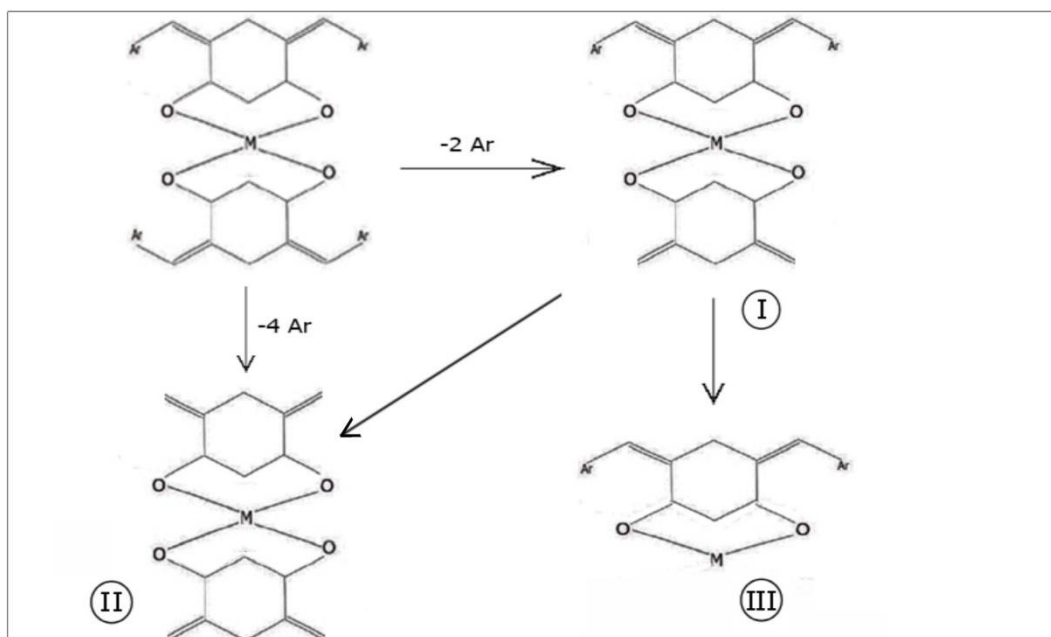
Scheme 1.2.1.
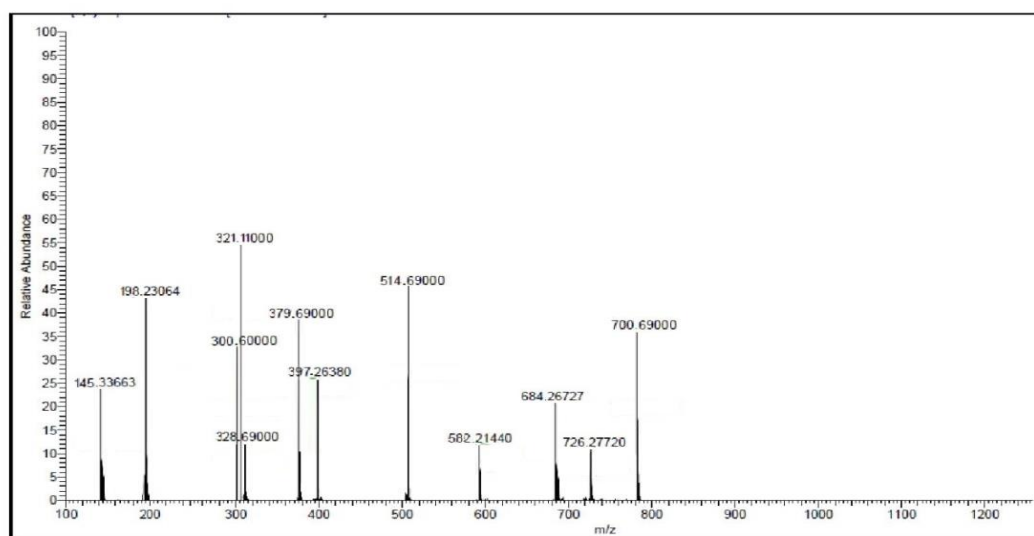
Fig.1.2.5 Mass Spectrum of Ni(II) complex of 1a.
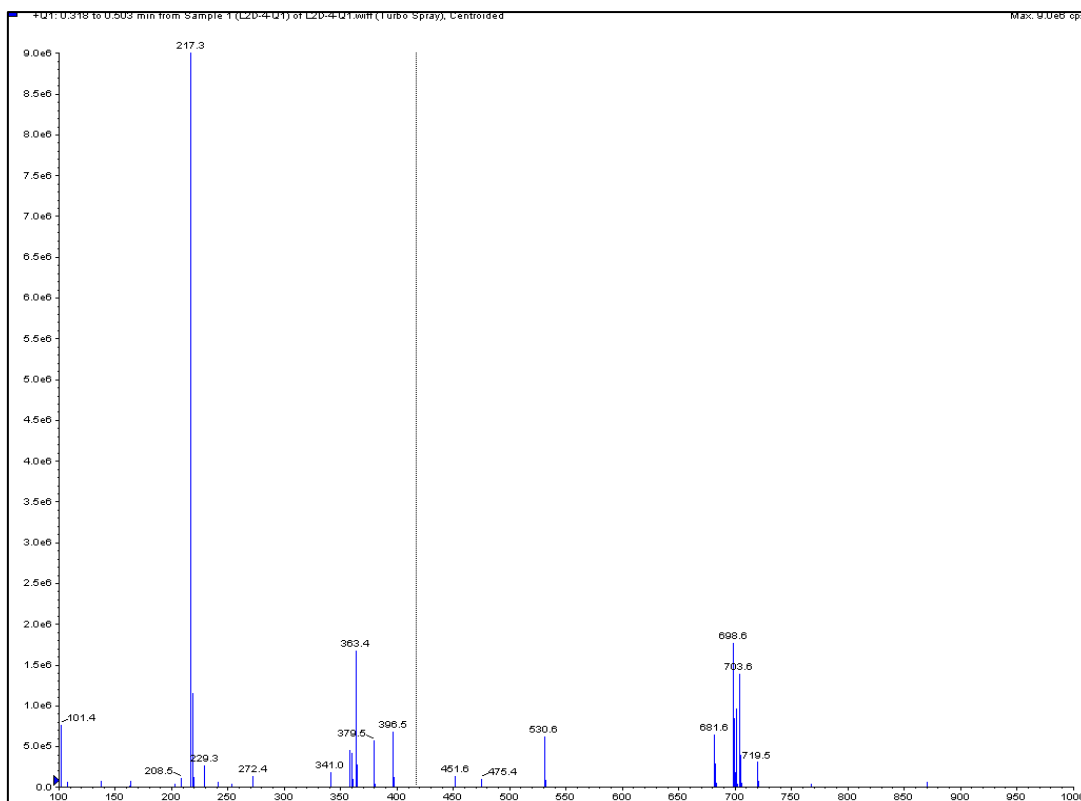
Fig.1.2.6 Mass Spectrum of Cu(II) complex of 1d.
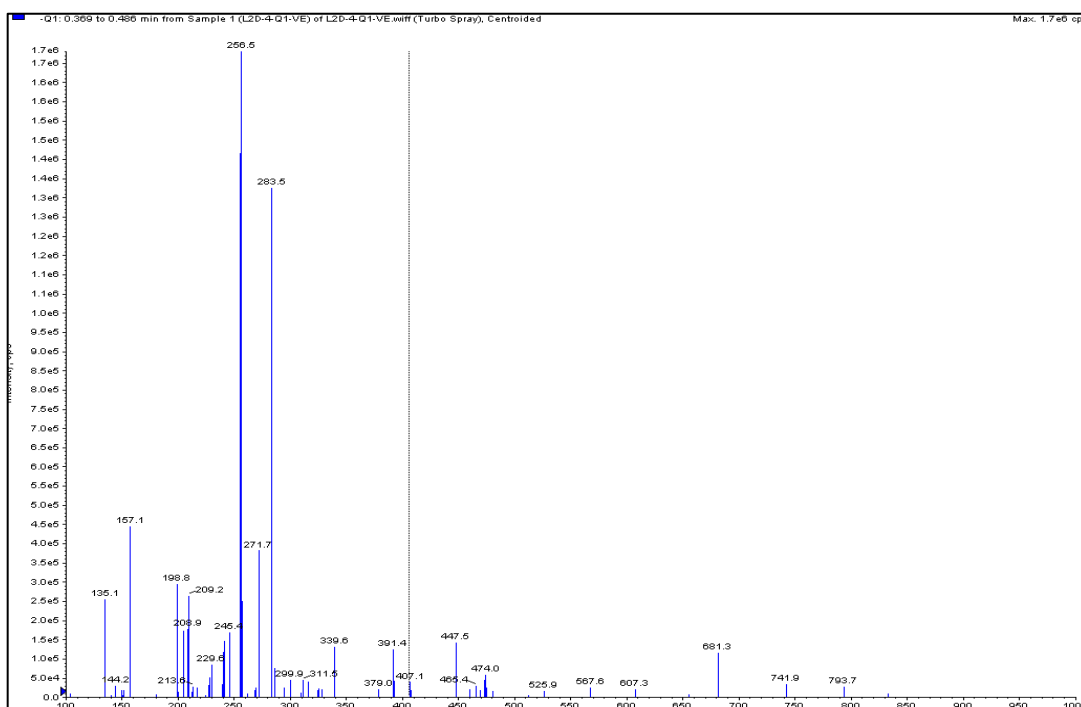
Fig.1.2.7 Mass Spectrum of Ni(II) complex of 1e.

Typical fragmentation pattern of the complexes are given based on the **scheme 1.2.1**.The peaks in the mass spectrum of Cu(II) complex of compound **1d** at 698 is

due to M+2 ion peak.The peaks at 530and 217 are due to the removal of two and four aryl groups respectively from the molecular ion peak.

The observed UV, IR, ^{1}H NMR and Mass spectral data of the metal complexes strongly supports the structure of the compounds as
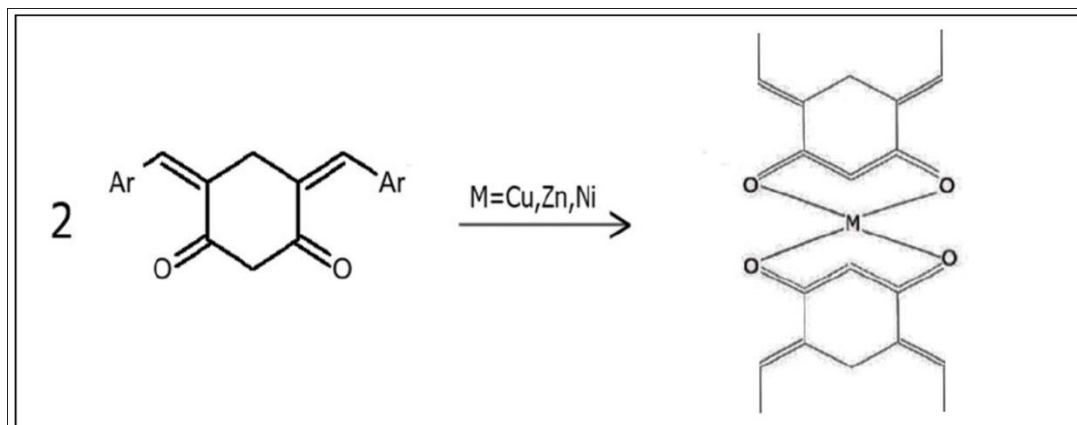
THERMOGRAVIMETRIC ANALYSIS

Thermogram of (4E, 6E)-4,6-bis (2,5-methylbenzylidene)cyclohexane -1, 3 doine (**1e**) and its Cu (II) complex are given in **Fig 1.2.8 & 1.2.9** respectively. The thermogravimetric analyses of synthetic analogues were studied in the temperature range,39.2^0 to 718.9^0. The present study indicates that the thermogram is a two stage decomposition pattern for the ligand. It could be inferred from the curve that up to120^0C the compound was almost steady and after that decomposition initiates slowly with a sharp fall in the mass (30.5%) upto about 283^0 C. This may be due to the removal of aryl part from the ligand. The detachment of aryl group is responsible for the second stage decomposition and the decomposition continues to a temperature of 435^0C with a mass reduction of 61%. The peak temperature inDTG is at 310^0.
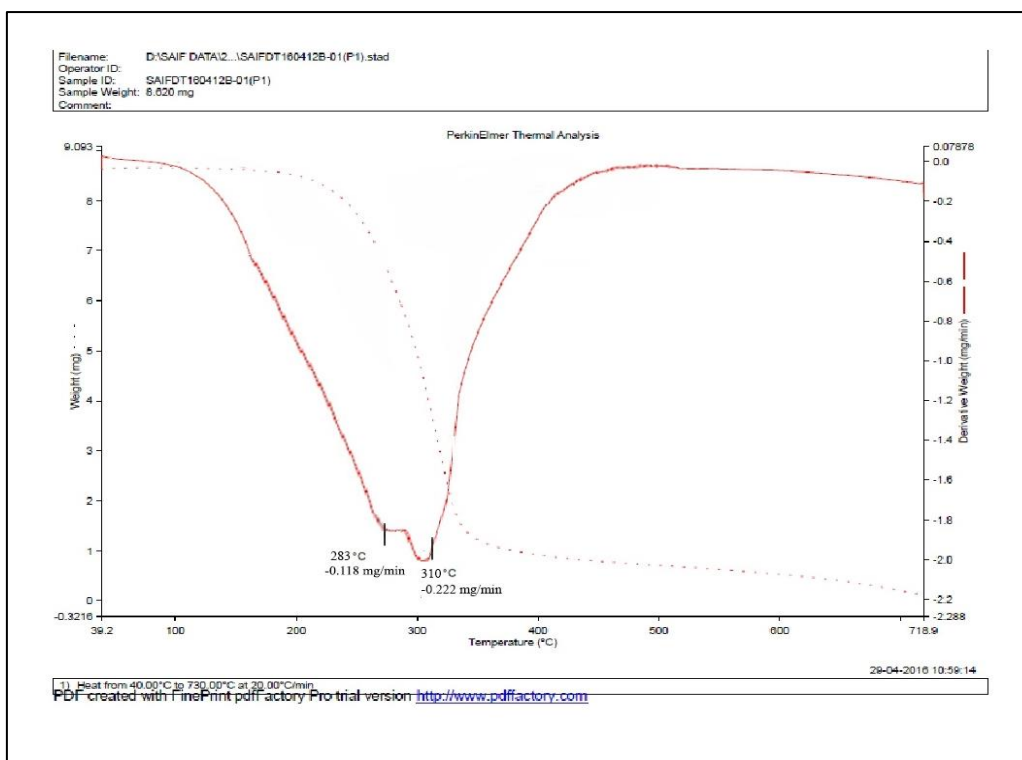
Fig.1.2.8 Thermogram of 1e.
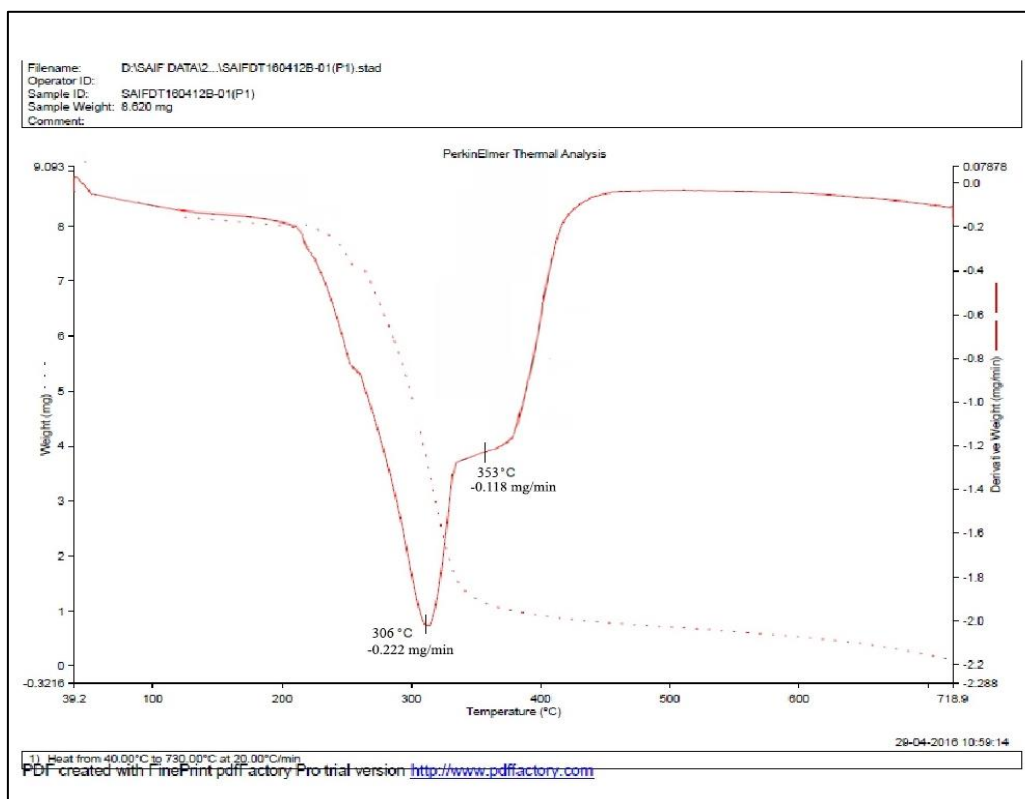
Fig.1.2.9 Thermogram of Cu (II) complex of 1e.

The thermogram of copper complex of (4E,6E)-4,6-bis(2,5-dimethylbenzylidene) cyclohexane-1,3-doine showed a two stage decomposition pattern.In the begininig the decomposition occured within a temperature range of 210^0Cand 330^0C. In the course of decomposition 45.80% of mass was lost from the complex and it was due to the removal of one ligand and it was coincides the theoretical value. The second weight loss corresponds to 91.6% of reduction of mass within a temperature range of 330^0Cto 420^0C, from the complex, suggests that the second ligand also decomposed from it and it shows a peak temperature of 353^0 C. The ultimate product was the metal oxide ie. CuO.

IN VITRO ANTITUMOUR STUDIES

In the third section we deal with the cytotoxic character of metal complexes of (4E,6E)-4,6-bis (aryl) cyclohexane-1,3diones and its metal complexes. Cytotoxic analysis done by Trypan Blue Exclusion method and the cells used were DLA and EAC.

1.3.1. In vitro analysis of 1a

The test compounds were studied for short term in vitro cytoxicity using Daltons's lymphoma ascites (DLA) cells. The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by Trypan blue exclusion method. Viable suspension (1×10^6 cells in 0.1 ml) was added to tubes containing various concentrations of the test compounds and the volume was made up to 1 ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. These assay mixture were incubated for 3 hour at 37^0c.Further cell suspension was mixed with 0.1ml of 1%Trypan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of Trypan blue while live cells do not take up the dye. The numbers of stained and unstained cells were counted separately.

% cytotoxicity =   No.of dead cells/No. of live cell+No.of dead cell×100.

 The data of EAC cells and DLA are depicted in **Table 1.3.1** and **Fig.1.3.1** -**1.3.2**.

Table 1.3.1. *In vitro* analysis of 1a (L$_1$)and its metal complexes to EAC& DLA

Med Con. µg/ml	% Cell death to EAC				% Cell death to DLA			
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$
200	28	87	68	37	29	89	77	41
100	17	79	55	31	21	77	64	37
50	13	66	39	23	15	69	49	28
20	9	44	29	11	9	53	36	17
10	3	27	17	7	4	31	27	13
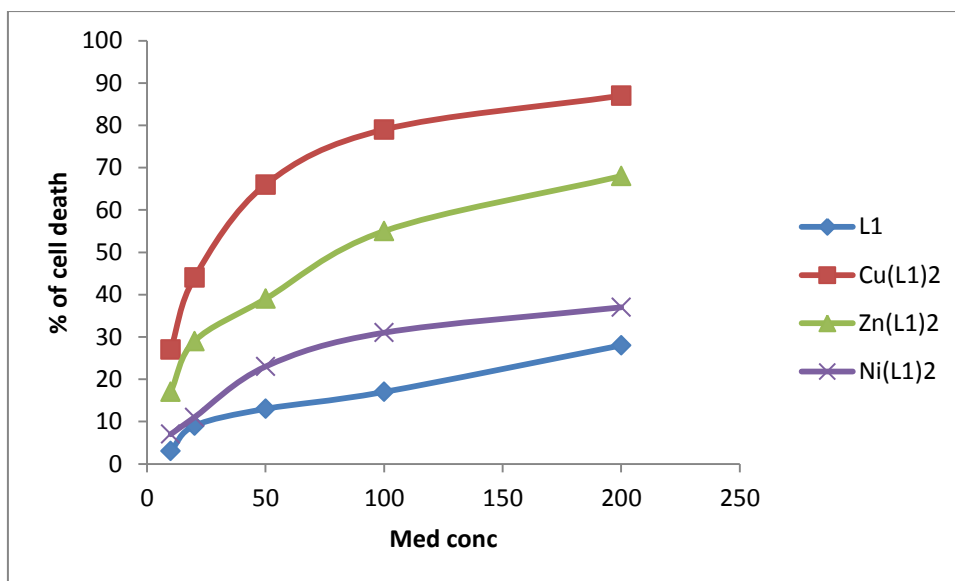
Fig.1.3.1. *In vitro* **analysis of 1a and their metal complexes towards EAC**
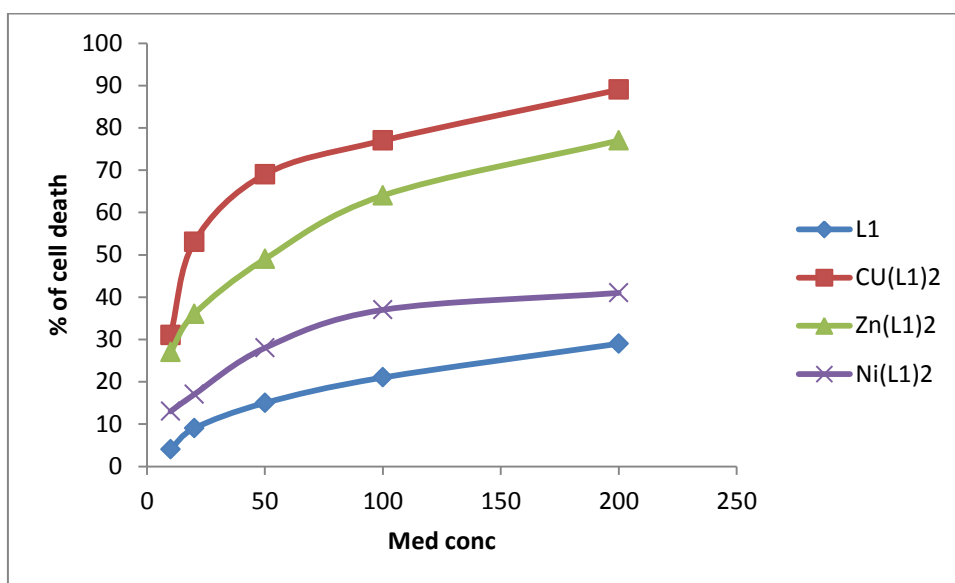
Fig.1.3.2. *In vitro* **analysis of 1a and their metal complexes towards DLA**

When (4E,6E)-4,6-bis (2-hydroxybenzylidene) cyclohexane -1,3 dione and its metal chelates were applied as drugboth EAC and DLA cells gave almost similar results in the cytotoxic analysis. The ligand gives 29 % of cell death against DLA whereas 28% towards EAC.Compared to the ligand its transition metal chelates have major cytotoxic activity. From the careful analysis of the study it can be concluded that the copper chelates produces more activity than the other ones. The outlined data have shown that DLA cells made more % of cell death. Because of chelation effect the

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complexes possess greater % of cell death than (4E,6E)-4,6-bis (2-hydroxybenzylidene) cyclohexane -1,3 dione.At 200 μg/ml copper complex of (4E, 6E)-4,6-bis (2-hydroxybenzylidene) cyclohexane -1,3 dione produces a cell death of 89% which is about three times greater than the unsaturated diketone based ligand. Out of the three complexes, Nickel complex possess minimum % of cell death but it is far better than the ligand. Hence it can be concluded that chelation increases the antitumour activity and it also depends on the nature of metal ion.

1.3.2. *In vitro* **analysis of 1b (L$_2$) and their metal complexes**

In the same manner we conducted the cytotoxic analysis of 1b and their metal complexes such as copper, zinc and nickel.All the results are projected in **Table 1.3.2.**

Table 1.3.2. *In vitro* **analysis of 1b (L$_2$) and their metal complexes against**

EAC& DLA

Drug Con. μg/ml	% Cell death to EAC				% Cell death to DLA			
	L$_2$	Cu(L$_2$)$_2$	Zn(L$_2$)$_2$	Ni(L$_2$)$_2$	L$_2$	Cu(L$_2$)$_2$	Zn(L$_2$)$_2$	Ni(L$_2$)$_2$
200	33	57	51	44	37	58	46	33
100	18	39	26	21	21	42	32	24
50	11	28	19	13	15	26	21	19
20	8	14	10	9	7	17	14	11
10	3	11	7	5	2	9	5	3
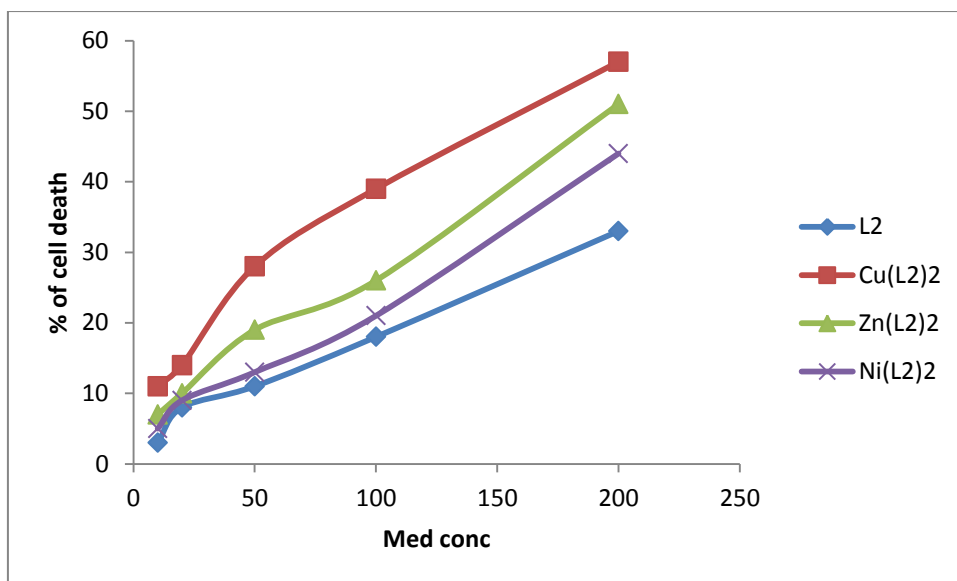
Fig.1.3.3. In vitro studies of 1b (L$_2$) and their metal complexes against EAC.
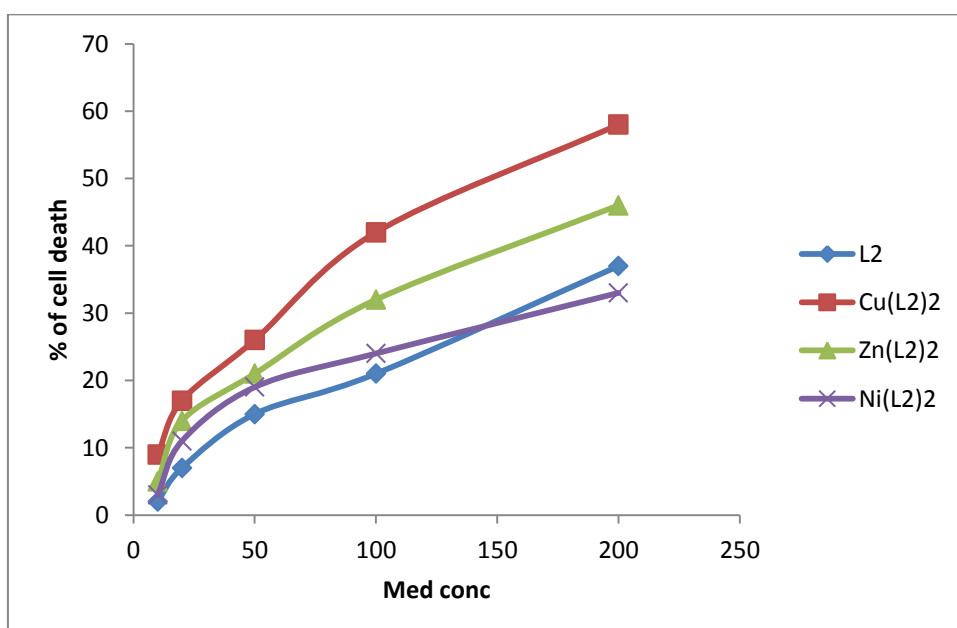
Fig.1.3.4. In vitro analysis of 1b (L$_2$) towards DLA.

Compared to **1a**, the ligand (4E,6E)-4,6-bis(2,4dihydroxy benzylidene)cyclohexane-1,3dione(1b) have less cytotoxic activities. At maximum concentration say 200 μg/ml the ligand itself shown 37% of cell death.compared to other complexes, copper complex reported much result with 58% cell death. The two complexes namely copper and zinc made somewhat noticeable cytotoxic character. At very dilute concentration both the unsaturated diketone ligand and its transition chelates

show minimal activity against the cancer cells i.e their effect is observed to be insignificant up to a concentration of 50µg/ml. The cytotoxic nature has improved merely marginally even after metal introduction.

1.3.3. *In vitro* analysis of 1c (L₃)and their metal complexes

In vitro analysis of (4E,6E)-4,6-bis(2,3dimethoxbenzylidene)cyclohexane -1,3 dione (**1c**) and their metal complexes using cancer cells, EAC and DLA are described in **Table 1.3.3**&**Fig.1.3.5**. and **Fig.1.3.6**.

Table 1.3.3.*In vitro*analysis of 1c (L₃)and its metal complexes to EAC& DLA

Med. Con. µg/ml	% Cell death to EAC				% Cell death to DLA			
	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$Ni(L_3)_2$	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$Ni(L_3)_2$
200	43	92	77	53	45	93	81	57
100	27	84	61	31	29	85	67	39
50	13	67	52	21	21	71	56	28
20	11	41	29	14	17	53	35	19
10	7	32	18	9	8	36	25	12
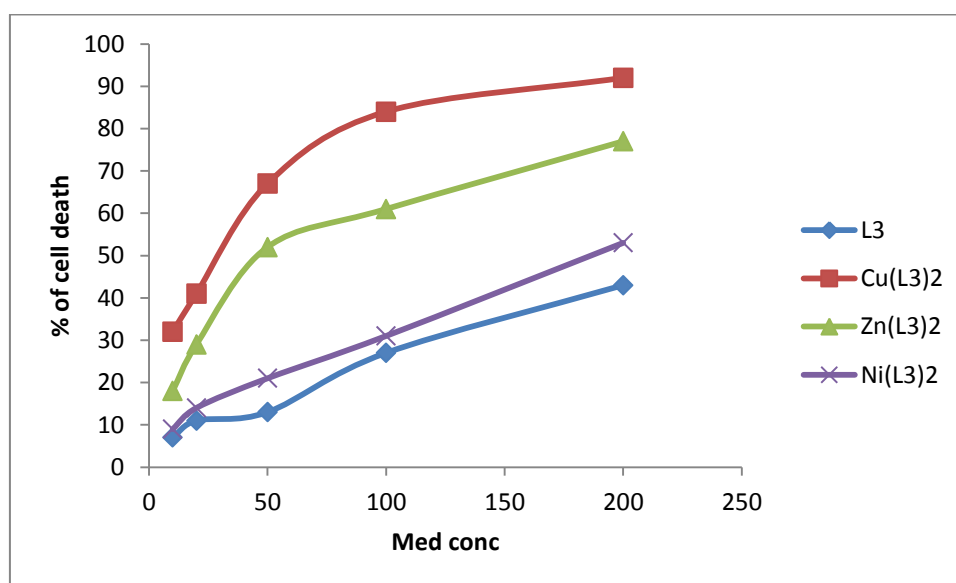
Fig.1.3.5. In vitro analysis of 4c(L₃) and **their metal complexes towards EAC.**
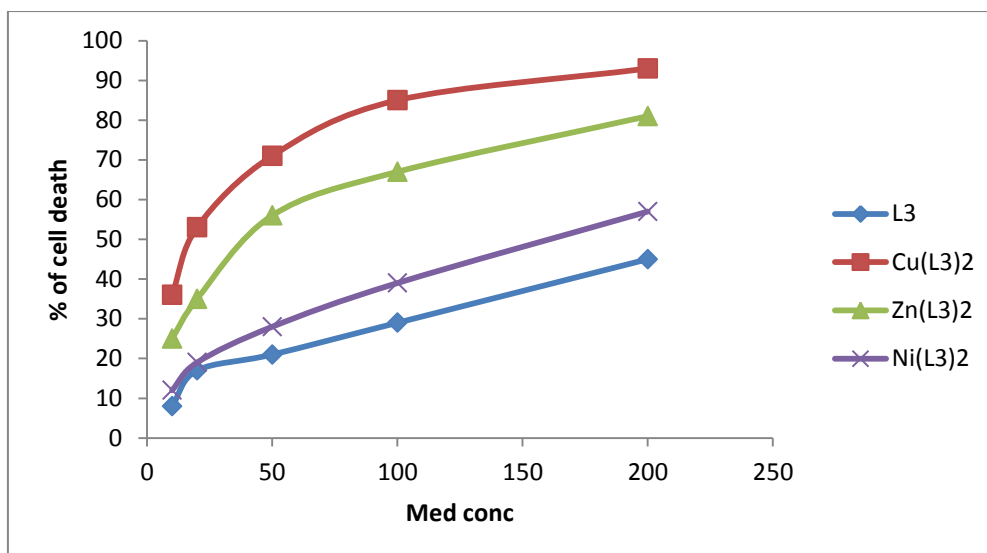
Fig.1.3.6 In vitro analysis of 1c(L₃) and **their metal complexes towardsDLA.**

At the great level concentration i.e 200µg/ml, (4E,6E)-4,6-bis (2,3-dimethoxybenzylidene) cyclohexane -1,3 dione (**4c**) and their metal complexes projected significant cytotoxic character to the cancer cells. Among the metal chelates, Ni (II) complex show minimal activity. This may be due to their carcinogenic character and can be highly toxic to living organisms.The ligand exhibited about 40% of cell death to both cells whereas its copper complex possesses about 90% cell death i.e complexation greatly increases the activity and here it is about 5 times.The Cu(II) and Zn(II) complex gave almost 93 and 81% cell death at a concentration of 200µg/ml. At lower concentration zinc might be acting as supplement which enhanced cell viability.

Table 1.3.4 In vitro analysis of 1d (L₄) and its metal complexes to EAC & DLA

Drug con. µg/ml	%cell death				%cell death			
	L_4	$Cu(L_4)_2$	$Zn(L_4)_2$	$Ni(L_4)_2$	L_4	$Cu(L_4)_2$	$Zn(L_4)_2$	$Ni(L_4)_2$
200	21	61	44	41	28	67	46	40
100	13	49	35	31	17	52	40	33
50	11	33	24	19	13	38	26	21
20	7	13	10	9	10	15	11	8
10	2	9	7	4	3	11	9	6
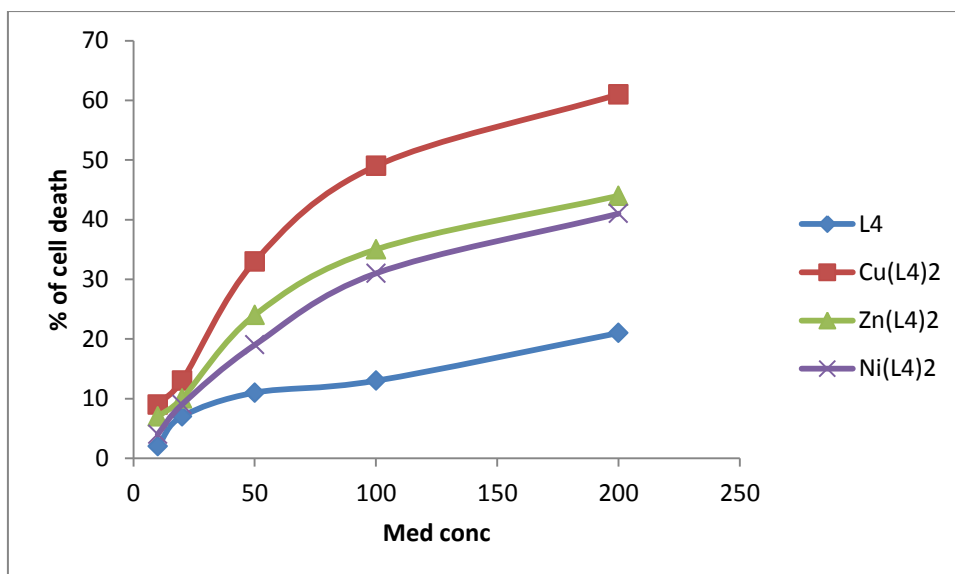
Fig 1.3.7. In vitro cytotoxic studies of 1d (L$_4$) and its metal complexes to EAC.
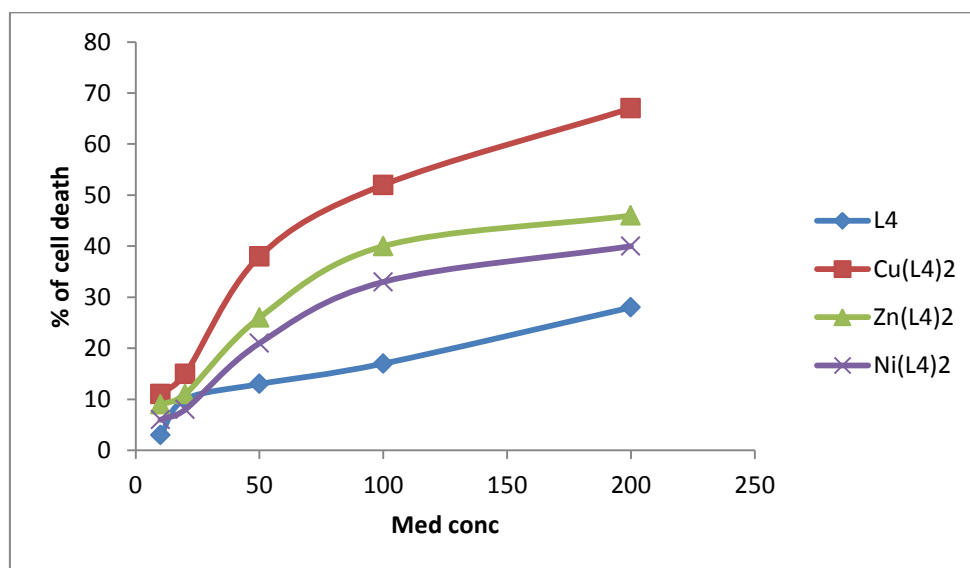
Fig.1.3.8. In vitro Cytotoxic studies of 1d and its metal complexes to DLA.

Comparing the DLA & EAC results we can see that (4E,6E)-4,6bis(2-methylbenzylidene) cyclohexane -1,3 doine is little more cytotoxic to DLA cells. Towards DLA cells the ligand showed 28% cytotoxicity where as in EAC studies it showed 21% cytotoxicity at a particular concentration namely 200µg/ml. Here also the metal chelates showed enhanced cytotoxicity towards EAC cells. All the concentrations showed activities but enhanced activity is at higher concentrations.

Table 1.3.5. In vitro studies of 1e (L₅) and its metal complexes to EAC & DLA

Drug con. µg/ml	%cell death to EAC				%cell death to DLA			
	L_5	$Cu(L_5)_2$	$Zn(L_5)_2$	$Ni(L_5)_2$	L_5	$Cu(L_5)_2$	$Zn(L_5)_2$	$Ni(L_5)_2$
200	21	84	37	33	19	79	37	31
100	9	69	21	17	11	67	21	17
50	7	43	11	10	6	45	11	9
20	5	31	7	6	4	31	9	7
10	2	13	3	3	2	17	4	5
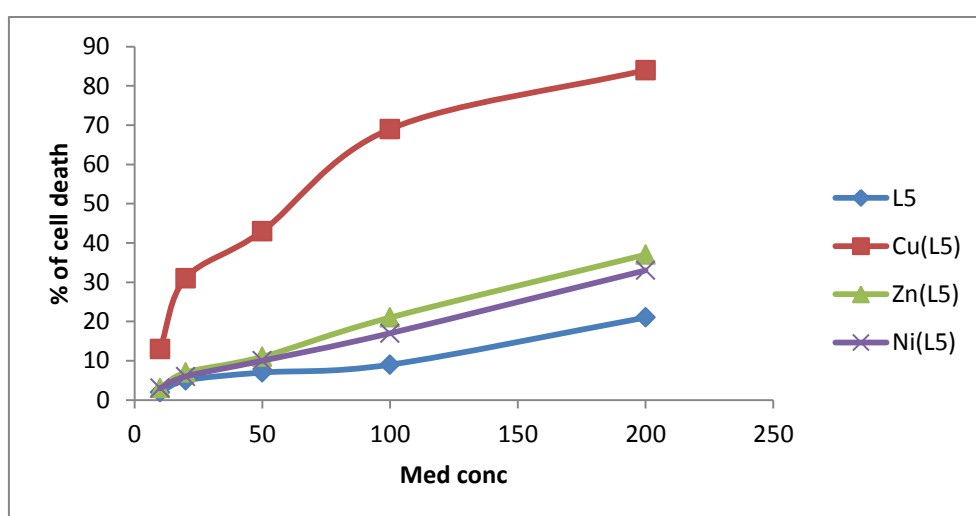
Fig 1.3.9 In vitro cytotoxic studies of 1e (L₅) and its metal complexes to EAC.
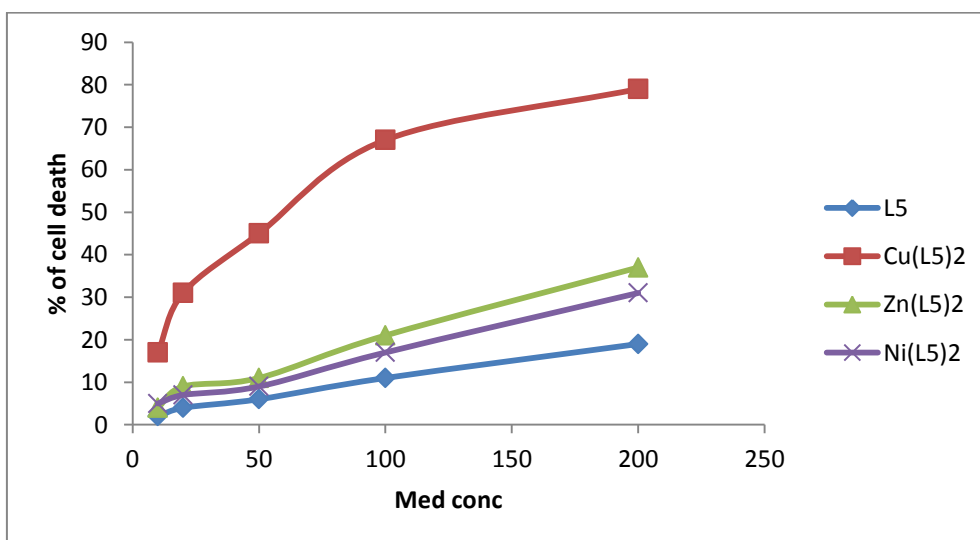
Fig 1.3.10. In vitro cytotoxic studies of 1e (L₅) and its metal complexes to DLA.

Conclusion

The ligand **1e** was not very active against DLA cells. It showed only 19% of cell death whereas metal chelation increases the % of cell death. From the result it can be shown that copper complex possess greater activity than the ligand and the entire metal complex possess inevitable role in cytotoxicity. The ligand **1e** showed less cytotoxicity than **1d.** But the entire complexes showed similar activities and they possess greater cytotoxicities.In the current section we discussed the cytotoxic nature of five unsaturated diketone based ligands and their transition metal chelates.Among this dimethoxy substituted diketone and its copper complexes projected maximum activity towards cancer cells, it is because of electron releasing character of substituent present in the unsaturated diketone.The evaluation of the cytotoxic character of transition metal complexes indicates that extreme activity was detected with the metal Cu.
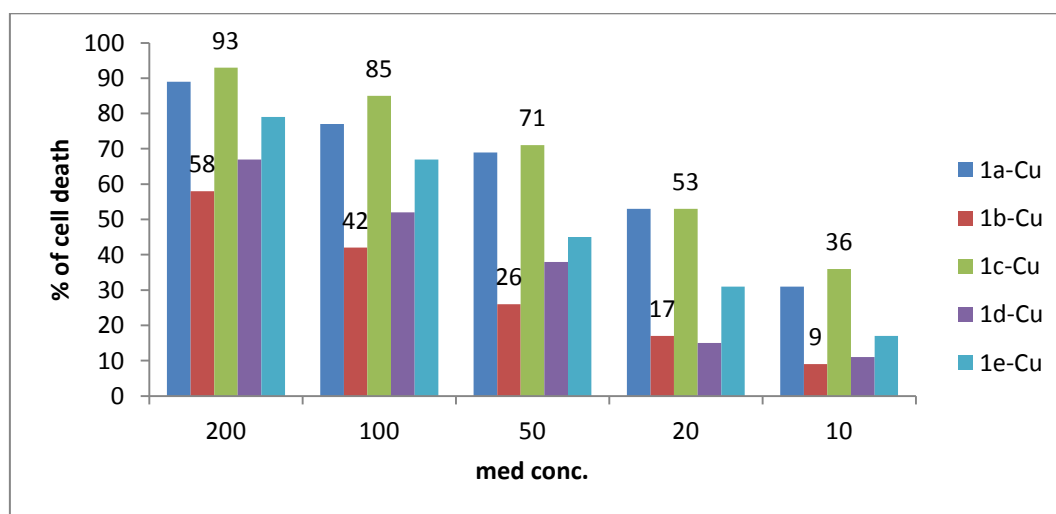
Fig.1.3.11 Comparison of *in vitro* analysisofCu(II) complexes of (4E,6E)-4,6-bis(aryl)cyclohexane -1,3 diones against DLA

THE *IN VIVO* ANTITUMOUR STUDIES

The intraperitoneal induction of (4E,6E)-4,6-bis(2,3-dimethoxy benzylidene) cyclohexane -1, 3-dione (**1c**) and its copper complex results in the extension of life period of mice group. The death rate of eight groups were noted carefully and % of ILS calculated.The results are projected in **Table 1.3.6**

Table 1.3.6. Effect of 1c and the Cu (II) complex on ascites tumour reduction

Animal groups	Concentration (µg/ml)	No.of animals with tumour	No.of days survived	%ILS
1.Control			17.6±1.7	
2.Std.drug			33.21±3.1	88.69
3. L1	20	5/5	25.4±5.1	44.31
4. L1	10	5/5	22.54.1±2.6	28.06
5. L1	5	5/5	19.1±4.50	8.52
6. Cu(L1)2	20	5/5	30.5±5.1	73.29
7. Cu(L1)2	10	5/5	28.12±6.2	59.77
8. Cu(L1)2	5	5/5	20.6±2.65	17.04

Generally the compounds which were active in cytotoxic studies were selected for in vivo antitumour studies. Animals were divided into 8 groups and ascites tumour was induced in them.The current investigation was carrying out to estimate the in vivo antitumour character of the compounds toEAC cancer cell lines by means of ascites tumour model.Ascites tumour was made in these animals by introducing viable EAC cells in 0.1 ml of PBS into the peritoneal cavity Viable EAC cells were injected into the peritoneal cavity of mice so that they develop tumours in their body.The intraperitoneally application of **1c** and its Cu(II) complex has increased the life period of tumour bearing mice significantly. The life period of mice of control group is observed to be 17.6±1.7 while it is 33.21±3.1for those which were medicated with the reference drug cyclophosphamide. The study reveals that in each concentration metal chelates have shown significantly better response when it is compared with the ligands and ultimately resulting in the extension of life period of mice. This is a clear indication that metal introduction in the diketone moiety can substantially increase the antitumour activity when it is compared to the simple ligand. Cu(II) complex of**1c** display an extension of life period of tumour burden mice and it made a % ILS 73.20% at 20µg/ml. The percentage increase in life span(%ILS) of tumour bearing mice were 44.31, 28.06 and 8.52% for diketone based ligand at various   concentrations namely 20,10,5µg/ml respectively.   There was an intensification in the average life span of creatures for both the curcumin based ligand and the transition metal complex.Among the two compounds, the analysis

reveals that in effect Cu(II) complex has a pivotal role in the antitumour studies because it acst as efficient proteasome inhibitors in cancer cells.

INVIVO CYTOTOXIC STUDY ON SOLID TUMOUR DEVELOPMENT

1.3.3 Effect of 1d and 1e and their Cu(II) complexes on solid tumour development

The synthetic analogues and their copper complexes were applied as drug to check its ability on solid tumour development in mice.DLA cells are induced in a group of mice and thus solid tumours are developed on the right hand limb.

Table 1.3.7. Effect of Compounds 1d and 1e on solid tumour

Compounds	Tumour volume on 31st day
Control group	6.031cm^3
1d	5.01 cm^3
1e	5.78 cm^3
Cu(1d)$_2$	4.55 cm^3
Cu(1e)$_2$	4.06 cm^3
Std.drug	1.982 cm^3

All the(4E,6E)-4,6 bis (aryl) cyclohexane -1,3 doines made a substantial decrease of solid tumour volume in mice.From the analysis it is observed that metal chelates of ligand gives good result compared to unsaturated diketones. For the control group,ie. the group that won't get any drug the measured reading was 6.031 cm^3 . On the 31 ^{rst} day and it were 5.01 cm^3 and 5.78 cm^3 for 1d and 1e respectively.Comparing with that of the control group,the ligands produced a decrease in volume of 1.021 cm^3 and 251 cm^3 respectively. Among the ligands ,1d was more effective than 1e in reducing the tumour volume.The tumour volumes on day 31 for copper complexes of 1d and 1e were 3.65 cm^3 and 3.05 cm^3 respectively.The decrease in tumour volume was 1.481 cm^3 and 1.971 cm^3 respectively with respect to control group.The Cu(II) complex of 1e had shown a pronounced effect in reducing tumour volume.Plasma copper concentration increases in neoplastic and autoimmune diseases as an immune mediated physiological response to the disease state. Treatment with copper complexes is a therapeutic support of this increase in plasma copper and the attendant distribution of copper to affected tissues to enable de-novo synthesis of copper dependent enzymes required to bring about remission by reestablishing normal tissue function.

SECTION-IV

ANTIBACTERIAL STUDY OF MONO &DISUBSTITUTED (4E,6E)-4,6-BIS(ARYL)CYCLOHEXANE-1,3-DIONESAND THEIR TRANSITION METAL CHELATES WITH CU(II), Zn(II) &Ni(II)

1.4.1. Antibacterialstudiesofligands 1a, 1b & 1c and theirCu(II),Zn(II)&Ni(II) complexes

The forth section describes antibacterial activity of (4E, 6E)-4, 6-bis (aryl) cyclohexane -1, 3- diones and their transition metal chelates.Agar well diffusion technique was used for the study and evaluated the antibacterial activity of diketone based compounds against bacterial strains namely Escherichia Coli, Klebsiella Pneumoniae & Bacillus Subtilis. All compounds and their complexes displayed significant activity towards the considered three bacterial strains and it was measured in terms of diameter zone of inhibition.

The results confirmed auspicious antibacterial activity of 1a and 1cand their complexes.When we compare the data of metal complex with the one obtained by reference drug we can see that the activity is almost comparable in several cases. The aryl part in the diketone moiety greatly influence the antibacterial activity.In total the nature of metal ion and the aromatic part play a pivotal role in the investigation of antibacterial study. In all the circumstances metal chelates retain improved antibacterial nature than diketone based ligands, which means that metal introduction enrich the activity. The results of antibacterial activity were projected in the Tables below.

Table1.4.1Antibacterial studies of 1a and theirCu(II),Zn(II)&Ni(II) complexes

Bacteria	Diameter of zone of inhibition in mm			
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$
E Coli	10	16.6	12	15.3
Klebsiella	12	17.3	12.8	16.8
Bacillus	11	15.9	11.2	14.7
Standard	20	20	20	20
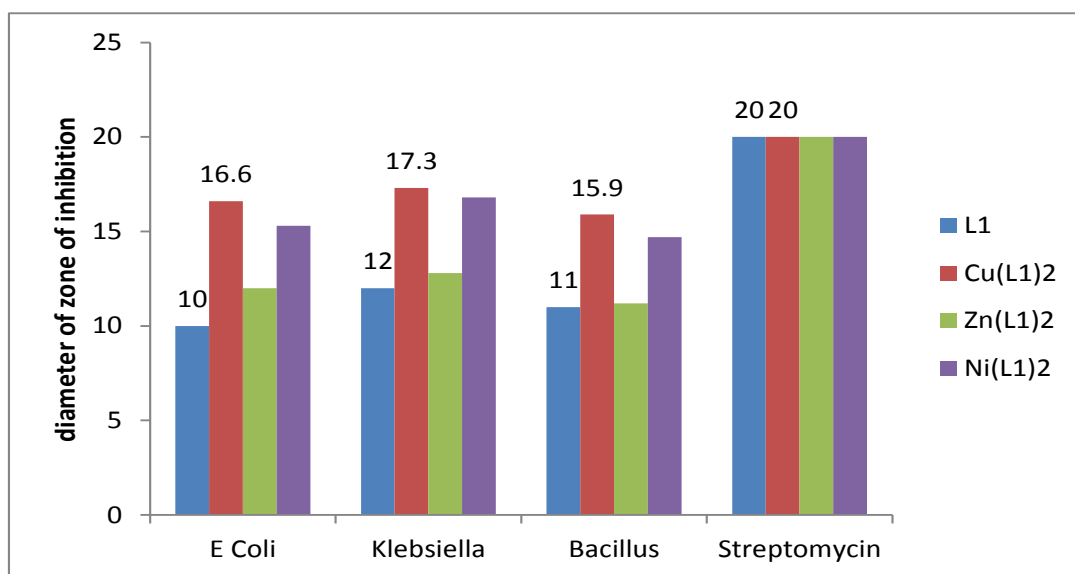
Fig.1.4.1Antibacterial studies of 1a (L1) and their Cu (II) , Zn (II) & Ni (II) complexes.

Table1.4.2 Antibacterial studies of 1b and their Cu(II),Zn(II)&Ni(II) complexes

Bacteria	Diameter of zone of inhibition in mm			
	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$
E Coli	14	17.4	16	17
Klebsiella	13	18.2	13.7	17.6
Bacillus	11	16.2	12	15.4
Standard	20	20	20	20
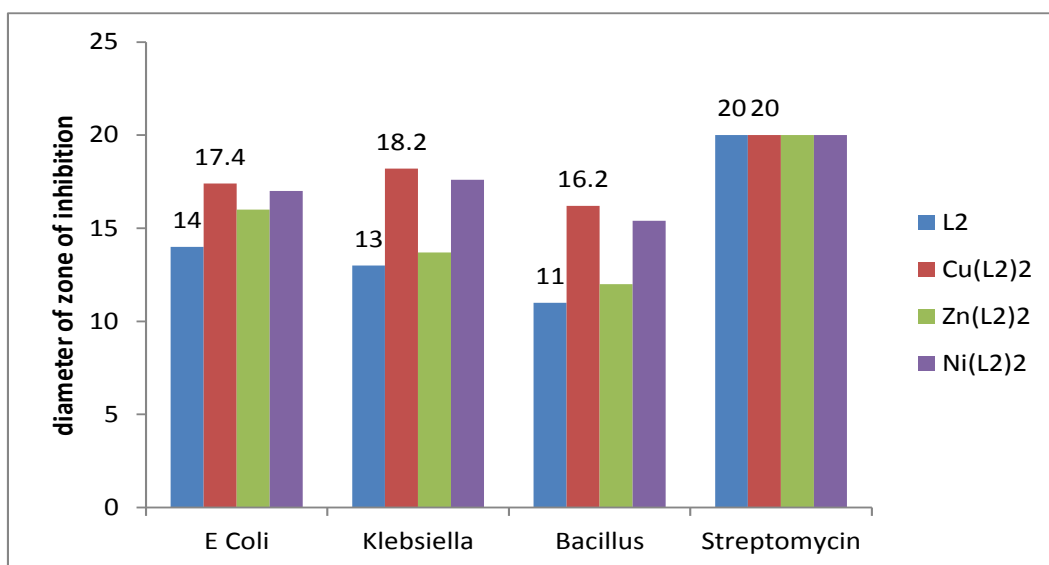
Fig. 1.4.2 Antibacterial studies of 1b (L2) and their Cu (II), Zn (II) & Ni (II) complexes.

Table 1.4.3 Antibacterial studies of 1c (L3) and their Cu (II), Zn (II) & Ni (II) complexes.

Bacteria	Diameter of zone of inhibition in mm			
	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$Ni(L_3)_2$
E Coli	12.7	17	14	16
Klebsiella	12.9	17.9	13.1	17.2
Bacillus	10	16	11.9	14.9
Standard	20	20	20	20

70
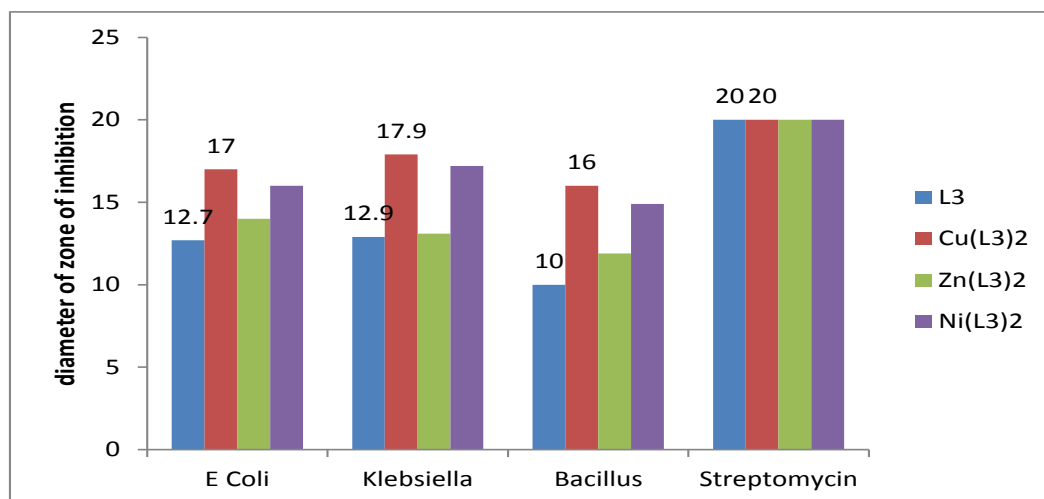
Fig.1.4.3Antibacterial studies of 1c (L3) and their complexes.

It is observed that, the one with aromatic ring contains dihydroxy group, shows significantly more activity towards every tested bacteria, than the other two compounds.The antibacterial nature is measured in terms diameter of zone of inhibition and the above mentioned compound delivered 11mm, 14mm and 13mm on Bacillus, E.coli and Klebsiella species respectively.The1c,its aromatic part contains methoxy group possess predominant antibacterial character. On comparing the (4E,6E)-4,6-bis(2,3dimethoxy benzylidene)cyclohexane -1,3- dione (1c)with the (4E,6E)- 4,6-bis(2,4dihydroxybenzylidene)cyclohexane1,3-dione(1b) it is observed that **1c** shows minimal activity. From the studies which were conducted in the past it is known that hydroxyl and methoxy moiety possess significantly great antibacterial properties. The entire metal complexes exhibited improved antibacterial activity than the equivalent ligands.Out of the nine complexes prepared from three ligands, the copper complex of **1b** projected maximum diameter of zone of inhibition of 18.2 mm towards Klebsiella.The data obtained from the investigation reveals that both the copper complex of (4E,6E) -4,6-bis (aryl)cyclohexane -1,3- dione and reference drug have displayed almost comparable activity. In most of the cases, antibacterial functioning depends on the metal ion involved in the complexation.In the examined cases the activity is more for copper complex because of the lipophilic character of the complex.

SECTION –V

ANTIFUNGAL STUDIES OF (4E,6E)-4,6 -BIS(2-METHYLBENZYLIDENE) CYCLOHEXANE-1,3-DIONE & (4E,6E)-4,6-BIS(2,5-DIMETHYLBENZYLIDENE) CYCLOHEXANE -1,3- DIONE AND IT'S METAL CHELATES.

1.5.1 Antifungal studies of 1d&1e and their Zn(II) and Ni(II) complexes.

The examination of the antifungal activities of 1d,1e and their Zn(II) and Ni(II) complexes were carried out by using the method of Kirby Baurer method againt 3 fungal culters namely Aspergilus Niger, Pencillium Chrysogenum and Alternaria Alternate .

Table 1.5.1 Antifungal studies of 1d (L_1) and its Zn(II) & Ni(II) complexes.

Fungi	Diameter of zone of inhibition in mm								
	L_1			$Zn(L_1)_2$			$Ni(L_1)_2$		
	100 µg	250 µg	500 µg	100 µg	250 µg	500 µg	100 µg	250 µg	500 µg
Aspergilus	4.7	7.2	8.6	5.2	8.4	9.6	6.6	10.3	11.3
Pencillium	6.3	8.3	10.4	6.7	8.4	11.3	7.2	11.2	12.4
Alternaria	5	6.6	7.9	5.8	9.2	10.2	7.5	12.1	12.6
Standard	21	21	21	21	21	21	21	21	21

Table 1.5.2 Antifungal studies of 1e ((L_2) and its Zn(II) & Ni(II) complexes.

Fungi	Diameter of zone of inhibition in mm								
	L_2			$Zn(L_2)_2$			$Ni(L_2)_2$		
	100 µg	250 µg	500 µg	100 µg	250 µg	500 µg	100 µg	250 µg	500 µg
Aspergilus	8.2	10.6	12.1	10.4	11.2	14.2	12.3	14.1	16.4
Pencillium	11.3	13.2	14.2	12	15.3	17.3	14	17.1	19
Alternaria	10.7	12.7	15.2	11.4	13.1	16	13.2	16.3	17.2
standard	21	21	21	21	21	21	21	21	21
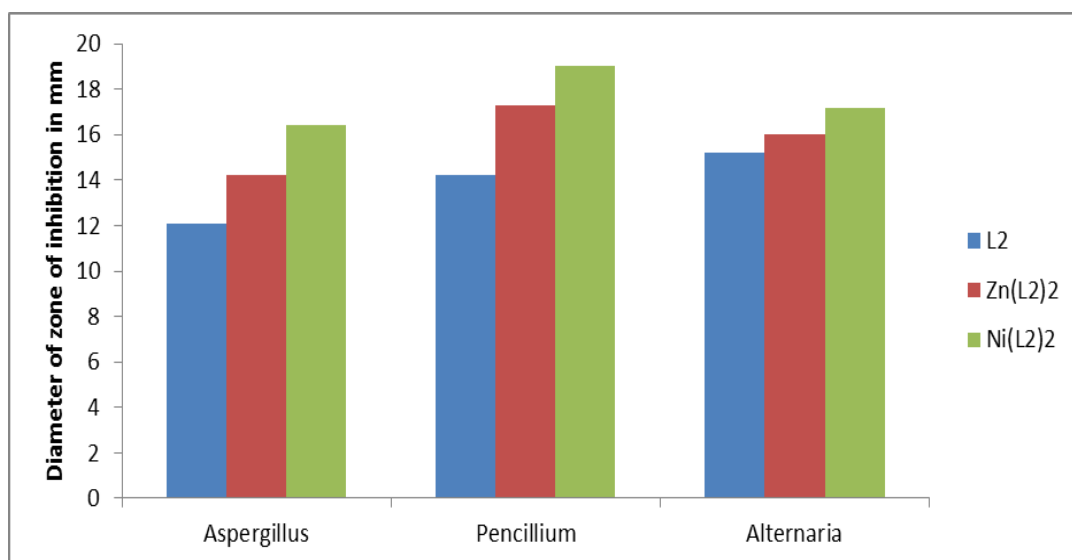
Fig. 1.5.1. Antifungal studies of 1d (L$_1$) and its Zn(II) & Ni(II) complexes.
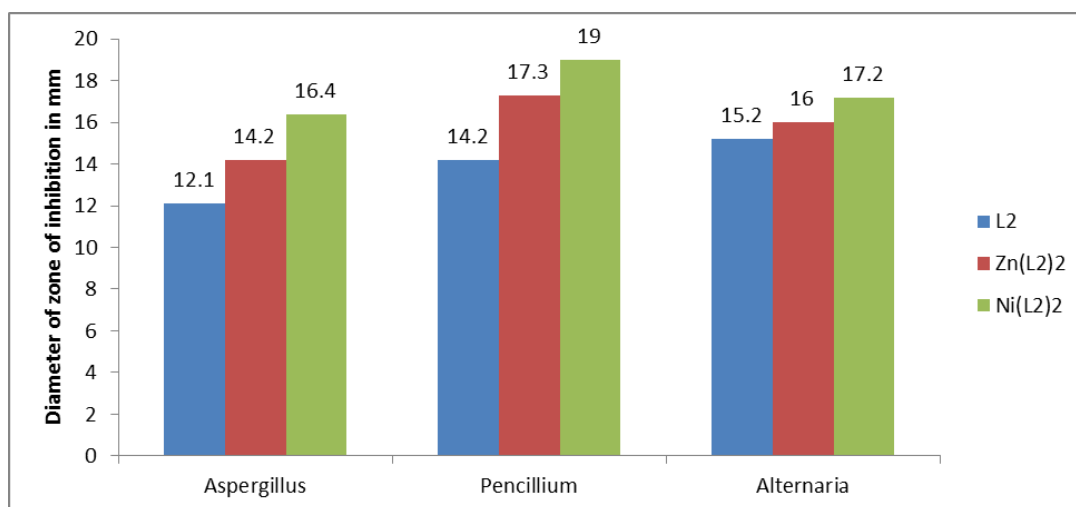
Fig. 1.5.2 Antifungal studies of 1e (L$_2$) and its Zn(II) & Ni(II) complexes.

The peak level of antifungal properties of the methyl derivative of analogues were observed at the concentration of 500μg/ml.From these results, it can be concluded that there is a positive response in the antifungal activities against higher concentration of the compounds.The activities of the compound, **1d** was observed to be similar values 7.9 &8.6 against Alternaria & Aspergillus but towards Pencillium it has shown an increased zone of inhibition ie.10.4 . Among the synthetic ligands,

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dimethyl derivative of ligand were found to be powerful antifungal agent and methyl derivative were least active compounds.Among the four complexes of ligands 1d &1e, most of them were found to be potent antifungal agents than the ligands, and the Ni(II) complexes of both shown more antifungal activity. The Zn (II) complexes of **1d**&**1e** were found to be least active. The Ni(II) complexes of (4E,6E)-4,6- bis (2,5-dimethyl benzylidene) cyclohexane -1,3 doine (**1e**) had considerable antifungal activity against all the three fungal cultures. It made an extreme zone of inhibition of 19mm against pencillium which is as good as with the zone of inhibition produced by the standard drug (21mm).

CHAPTER –II

SYNTHESIS, CHARACTERISATION AND BIOCHEMICAL ACTIVITIES OF (4E, 6E)- 4,6- BIS (CHLORO-ARYL) CYCLOHEXANE 1,3 DIONESAND THEIR METAL COMPLEXES

INTRODUCTION

This chapter deals with the preparation and characterization of three synthetic equivalents of curcuminoids with chloro substituted 1, 3 cyclohexandione. These complexes were prepared by the reaction of substituted benzaldehydes namely (2-chlorobenzaldehyde, 4- chloro benzaldehyde & 3,4- dichlorobenzaldehyde) with cyclohexane-1,3-dione-boric oxide complex. The solvent used was ethyl acetate and the condensation was carried out in the presence of tri-butyl borate and n-butyl amine as in **scheme 2.1.1**.The synthesized compounds were purified by column chromatography by using 4:1 chloroform:acetone mixture as eluent. For better purification it was recrystallized from hot benzene.
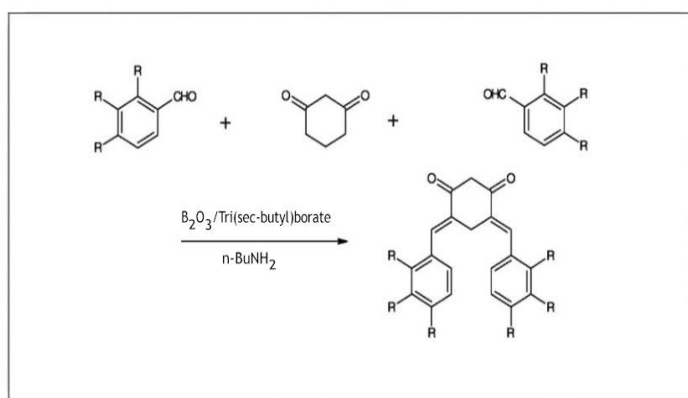
Scheme 2.1.1

Compound	Ar
2a	
2b	
2c	

SECTION –I

SYNTHESIS AND CHARACTERISATION OF (4E,6E)-4,6-BIS(CHLORO-ARYL) CYCLOHEXANE 1, 3 DIONES

The synthetic details of the compounds are given in **Table 2.1.1.**

Table 2.1.1 Synthetic details of ligand.
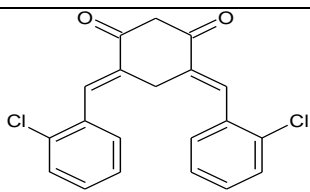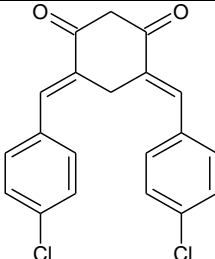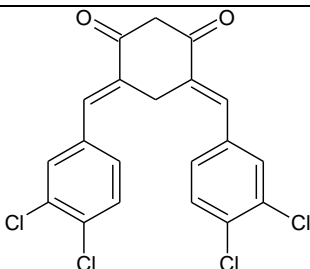
compounds	Aldehyde used	Structure of ligand	Systematic name	Yield%
2a	2-chloro benzaldehyde		(4E,6E)-4,6-bis(2-chlorobenzylidene)cyclohexane-1,3-dione	69
2b	4-chloro benzaldehyde		(4E,6E)-4,6-bis(4-chlorobenzylidene)cyclohexane-1,3 dione	74
2c	3,4 dichloro benzaldehyde		(4E,6E)-4,6-bis-(3,4dichlorobenzylidene)cyclohexane-1,3 dione	63

Table 2.1.2 Physical & spectral details of ligands.

compounds	MP.(^{0}C)	Elemental analysis		Electronic λ max (nm)
		C	H	
		Found/ (calculated)		
2a	104	67.31 (67.41)	3.01(3.93)	273 307
2b	101	67.07 (67.42)	3.03(3.94)	277 309
2c	123	55.48 (56.60)	2.31(2.83)	293 334

From the elemental percentage analysis and the information get from the molecular weight, assumed that the compound exists in [ML$_2$] stoichiometry. It was further confirmed by mass spectral analysis.

2.1.2 Characterization of ligands

The synthetic compounds were characterized by numerous spectral technique like UV,IR,^{1}H NMR,^{13}C NMR and mass spectral techniques. Detailed analysis is given below.

UV spectra

The synthesized dicarbonyl compounds showed two prominent types of transitions in electronic spectrum at about 310 nm and 270 nm assignable to n→π* and π→π* transitions respectively.

IR spectra

All the complexes have $\nu_{C=O}$ vibrations and showed the noticeable bands at about 1624 cm^{-1},1633 cm^{-1}, and 1635 cm^{-1}. Metal complexes influence the vibrations of carbonyl bands and from the stretching frequencies we can identify the kind of carbonyl group existing in the synthetic analogues. There are two factors which affect or influence the free carbonyl stretching frequencies -extended conjugation and H-bonding. Because of these two factors free carbonyl stretching frequencies reduced. From the extensive analysis of the spectra it has been found that the compound exists in the H-bonded form. This was further supported by the occurrence of broad band near the region of 3500-2500cm^{-1}. The existence of bands in the area 1577-1584 cm^{-1} are due to $\nu_{C=C}$ phenyl stretching,ν(C-C) alkenyl, ν_{as}(C-C-C) chelate ring, ν_s(C-C-C) chelate ring and β(C-H) chelate ring are responsible for

the presence of further peaks. The important IR spectral bands and their probable assignments are given in **Table 2.1.3** and spectrum is reproduced in **Fig.2.1.1**.

Table 2.1.3 IR spectral details of ligands.

Ligands			Probable assignments
2a	2b	2c	
1624	1633	1635	ν(C=O) cyclohexyl
1618	1626	1619	ν(C=O) cinnamonyl
1584	1581	1577	ν(C=C) phenyl
1534	1527	1531	ν(C-C) alkenyl
1480	1472	1479	ν_{as}(C-C-C) chelate ring
1321	1311	1326	ν_{s}(C-C-C) chelate ring
1126	1014	1018	β(C-H) chelate ring
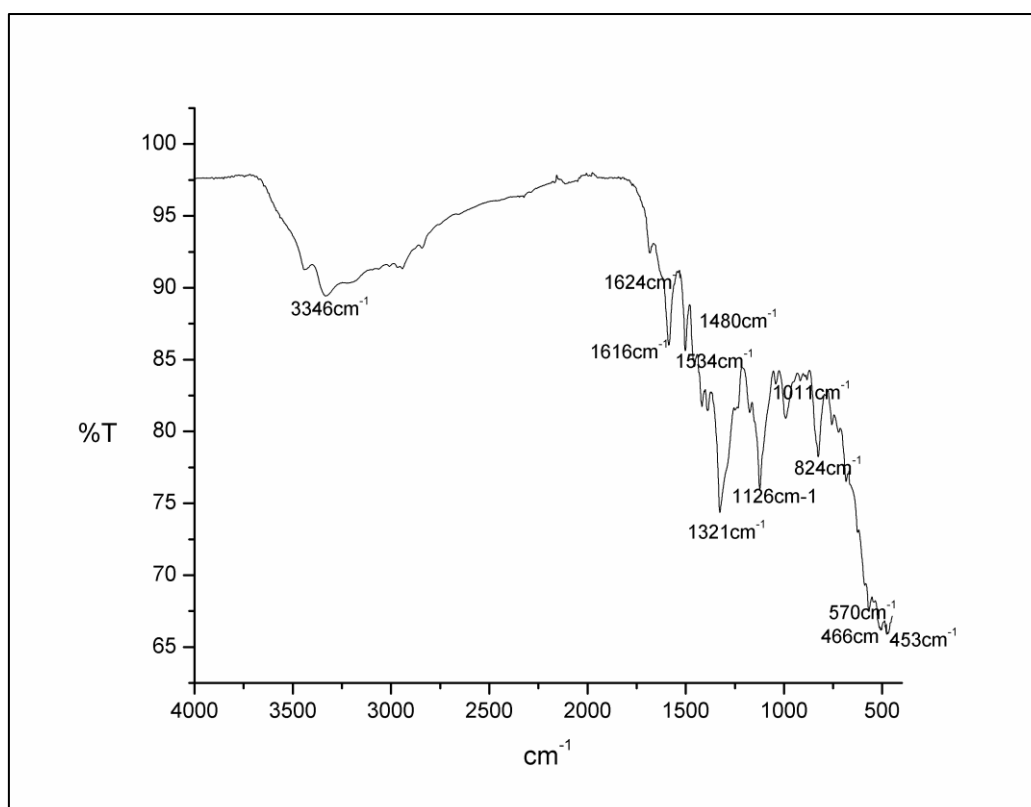
Fig 2.1.1 IR spectrum of 2a.

^{1}H NMR spectra

^{1}H NMR spectra of the compounds are in agreement with the intramolecularly hydrogen bonded structure of the compounds. All the compounds showed a signal at 16 ppm which is due to the intra molecular H-bonded proton. The methine protons displayed a signal atabout 5.7 ppm and at about 7.9 due to phenyl ones. The

absorptions which is in the range of 6.4-8.2 ppm correspondings to alkenyl proton. **Fig 2.1.2** represents the NMR spectrum of the synthetic analogues. Spectral details are given in **Table 2.1.4.**

Table 2.1.4 ^{1}H NMR spectral data.

Compounds	Chemical shift(δ ppm)			
	Enolic	Methine	Alkenyl	phenyl
2a	16.07	5.6	6.54-8.01	7.4-7.73
2b	16.04	5.7	6.5-8.31	7.167-7.87
2c	16.01	5.74	6.48-8.07	7.273-7.73

^{13}C NMR spectra

The dissimilar carbon atoms can be easily identified from the ^{13}CNMR spectra .The ^{13}CNMR spectra of chemically and magnetically dissimilar carbon atoms displays dissimilar peak. First carbon atom (C1) in 3c displays a peak at 108 ppm and it indicates the presence of keto–enol tautomerism. The second carbon atom (C2 carbonyl C) displays a peak at 197.73 ppm. The carbon atom which is attached to the chlorine atom shows a downfield shift as the chlorine atom is electronegative. The third and fourth (C3 & C4) carbon atoms display a peak at 138.58 and 138.06 ppm. The carbon atoms which are situated in the phenyl ring lies between 133-145 ppm. The details of the spectrum were given in **Table 2.1.5-2.1.7**
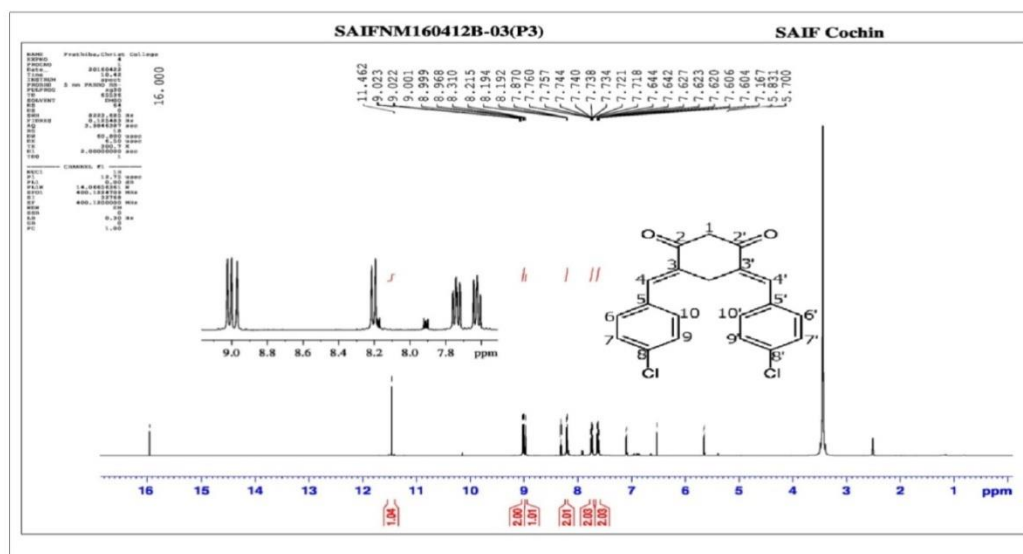
Fig 2.1.2 ^{1}H NMR spectrum of 2b.

Table 2.1.5 ^{13}C NMR spectral data of 2a (chemical shift in ppm).

C1	C2, C2'	C3, C3'	C4, C4'	C5, C5'
102.17	193	141.48	133.63	135.06
C6, C6'	C7, C7'	C8, C8'	C9, C9'	C10, C10'
128.59	129.38	139.22	132.38	129.54

Table 2.1.6 ^{13}C NMR spectral data of 2b (chemical shift in ppm).

C1	C2, C2'	C3, C3'	C4, C4'	C5, C5'
103.34	193.43	138.48	132.51	134.06
C6, C6'	C7, C7'	C8, C8'	C9, C9'	C10, C10'
135.57	130.38	127.22	129.78	129.62

Table 2.1.7 ^{13}C NMR spectral data of 2c(chemical shift in ppm).

C1	C2, C2'	C3,C3'	C4, C4'	C5, C5'
108.37	192.73	138.58	132.51	138.06
C6, C6'	C7, C7'	C8, C8'	C9, C9'	C10, C10'
134.37	129.38	137.22	128.78	127.62
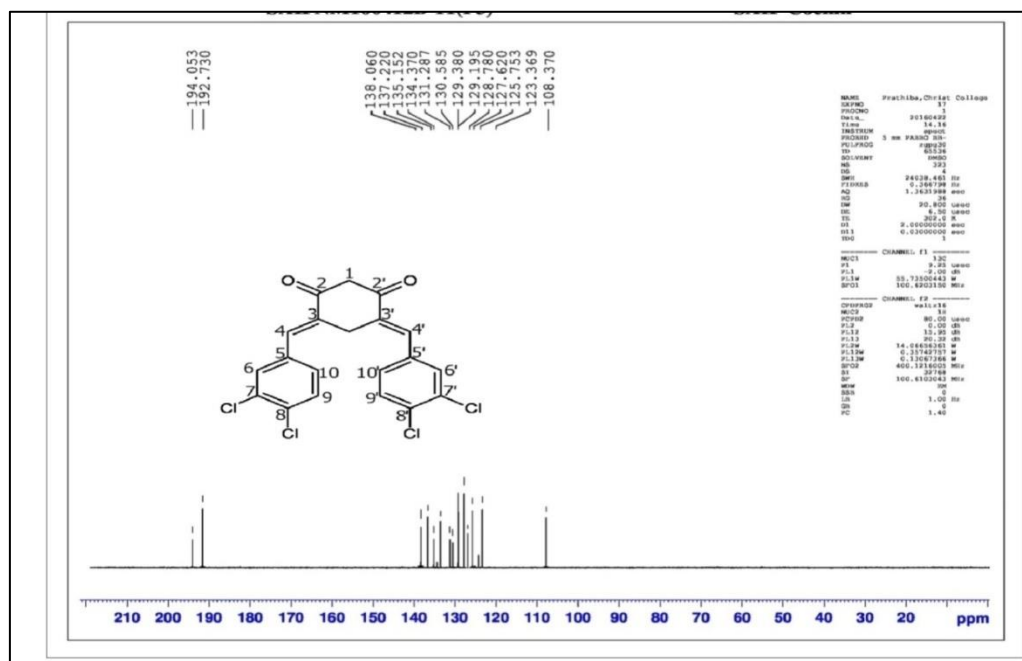
Fig 2.1.3 ^{13}C NMR spectrum of 2c.

Mass spectra

The mass spectral details of 2a, 2b and 2c are given below in **Table 2.1.8.** Mass spectrum of **2a** shows distinct molecular ion, M+ ion peak at m/z=335.9. The base peak in the spectrum is observed at m/z=189.1 and is due to [Ar-CH=CH$_2$]+ where(Ar=2-chlorophenyl). An intense peak observed at m/z=173.45 is due to [Ar-CH=CH-C=O]$^+$.Elimination of important groups like C$_2$H$_2$, CH$_2$=C=O, C=O,C$_2$H$_2$O, CH$_2$-CH=C=O from the molecular ion gives different fragments and the values are depicted in **Table 2.1.8**.The fragmentation patterns are given in **Scheme 2.1.2**.The mass spectrum of **2a** is given in **Fig.2.1.4**.
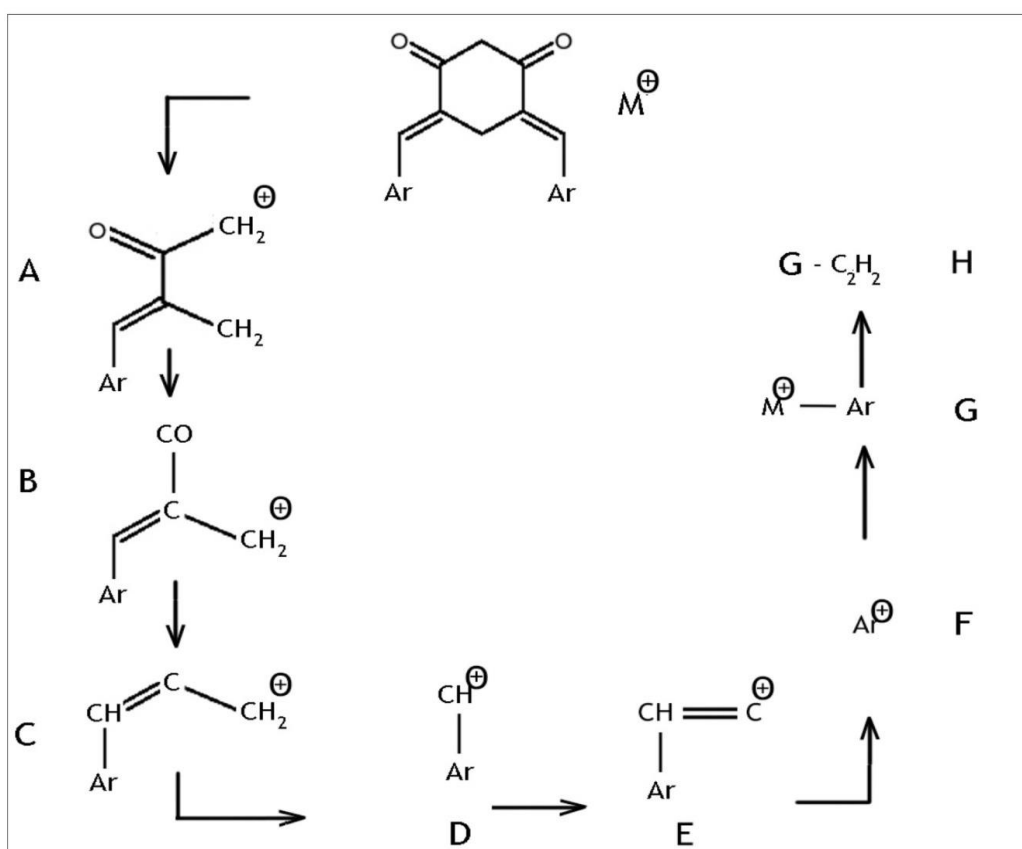
Table 2.1.8   Mass spectral fragments of 2a, 2b & 2c.

Fragment	Ligand	M+/ M+ 1 ion	A	B	C	D	E	F	G
Mass Pattern	2a	335	189	173	147	135	115.4	247.45	219.45
	2b	358	193	181	150	134	111	245	221.2
	2c	419	227	212	160	179	147	283.2	255.8

The mass spectrum of **2b** shows an intense molecular ion peak at m/z is358. Other prominent peaks in the spectrum are due to fragment ions.The peaks at m/z is181 and 193 are very prominent in the spectrum and are due to [Ar-CH=C(CH$_2$)]+ and [Ar-CH=C(C=O)CH$_2$]+respectively where Ar is 4-chlorophenyl. The mass spectrum of **2c** is reproduced in **Fig.2.1.5**.The molecular ion peak of **2c** is observed at m/z is 419.The base peak in the spectrum observed at m/z is160 can be assigned to the fragment ion [Ar-CH=C(C=O)CH$_2$]+,where Ar is 3,4-dichlorophenyl. Important peaks appeared in the spectrum of the compounds 2a, 2b and 2c can be conveniently accounted by the fragmentation pattern given in **Scheme 2.1.2.**

Thus all the spectral evidences such as UV,IR,NMR and mass spectra support the structure of the compounds as
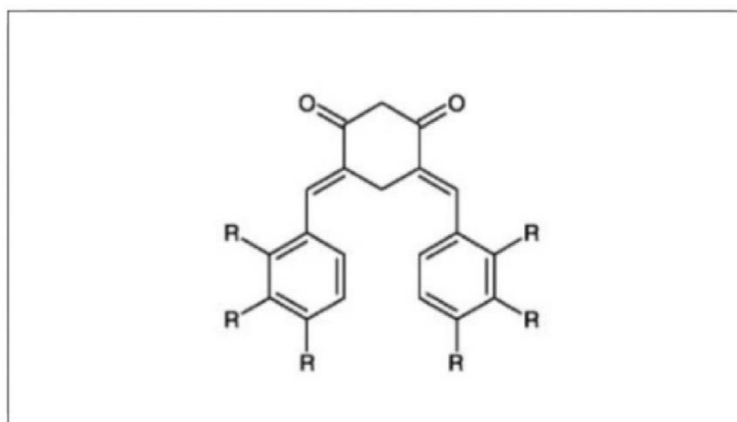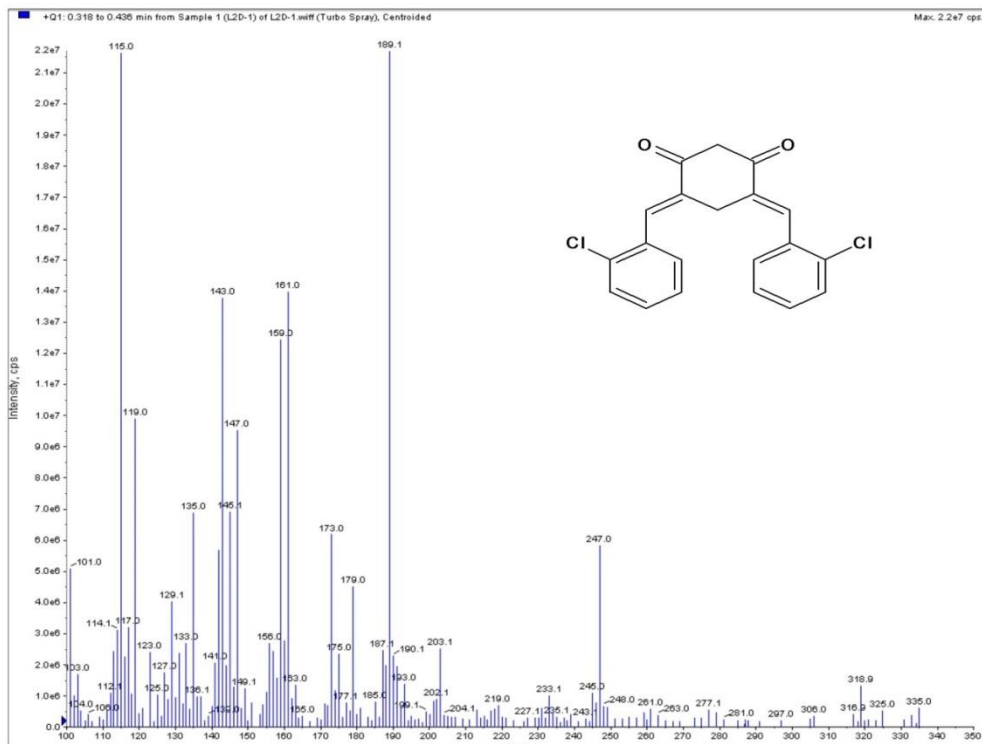
Fig.2.1.4 mass spectrum of 2a.
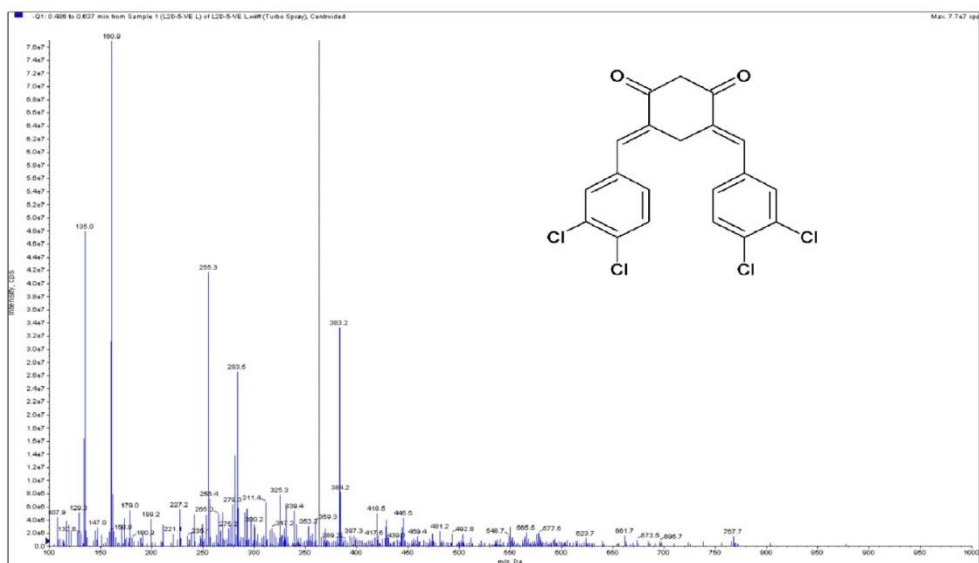
Fig 2.1.5 mass spectrum of 2c.

SECTION –II

SYNTHESIS AND CHARACTERISATION OF TRANSITION METAL COMPLEXES OF (4E,6E)-4,6- BIS(CHLORO-ARYL)CYCLOHEXANE- 1,3- DIONES

2.2.1 Synthesis of metal complexes

Copper(II),zinc(II)and nickel (II) complexes of**2a,2b and 2c** were synthesized by the following method. To a refluxing solution of the diketone (0.002mol) in methanol(25ml), an aqueous solution of metal salt such as copper (II) acetate, zinc(II) acetate and nickel(II) acetate (0.001mol) was added in order to prepare Cu(II), Zn(II) and Ni(II) complex respectively and the reaction mixture was refluxed for nearly 4 hr and cooled to room temperature. The precipitated complex was filtererd, washed with 1:1 methanol: water mixture and recrystallized from hot methanol.

2.2.2 Characterisation of metal complex

Physical and analytical datas of the complexes are given in **Table 2.2.1**-**2.2.3.**The data are in good aggrement with the [ML$_2$]stoichiometry of the complex.The nature of the metal complexes was confirmed from the UV,IR,NMR and mass spectral data of the complexes.

Table 2.2.1 Analytical and spectral data of metal complexes of 2a.

Metal	M.P.(^{0}C)	Elemental analysis(%) Found (calculated)			λmax	IR(cm^{-1})		
		C	H	M		(C=O)	(C-C-C)	(M-O)
Cu(II)	152	60.8 (61.7)	3.46 (3.61)	8.01 (8.17)	276 309	1594	1498	467 413
Ni(II)	157	51.74 (62.1)	3.19 (3.62)	7.37 (7.59)	278 311	1597	1505	466 423
Zn(II	155	60.32 (61.6)	3.49 (3.59)	8.17 (8.39)	272 314	1601	1517	454 426

Table 2.2.2 Analytical and spectral data of metal complexes of 2b.

Metal	M.P.(^{0}C)	Elemental analysis(%) Found (calculated)			λmax (nm)	IR(cm^{-1})		
		C	H	M		(C=O)	(C-C-C)	(M-O)
Cu(II)	166	61.8 (61.39)	3.66 (3.78)	8.21 (8.27)	278 311	1598	1512	433 410
Ni(II)	168	61.74 (62.52	3.29 (3.93)	7.36 (7.49)	271 321	1591	1492	446 418
Zn(II	167	60.94 (61.37)	3.59 (3.82)	8.37 (8.45)	273 319	1594	1490	475 415

Table 2.2.3 Analytical and spectral data of metal complexes of 2c.

Metal chelates	M.P. (^{0}C)	Elemental analysis(%) Found (calculated)			λmax (nm)	IR(cm^{-1})		
		C	H	M		(C=O)	(C-C-C)	(M-O)
Cu(II)	157	51.05 (52.45)	2.57 (2.622)	6.82 (6.94)	297 337	1598	1512	433 410
Ni(II)	165	52.03 (52.73)	2.37 (2.63)	6.17 (6.44)	298 339	1591	1492	446 428
Zn(II	169	51.59 (52.34)	2.48 (2.61)	6.93 (7.12)	297 338	1603	1513	444 416

Electronic spectra

The electronic spectra of the complexes showed a significant bathochromic shift in the $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transition of the free ligand.This clearly indicates the participation of carbonyl oxygens in metal complexation.

IR spectra

The absence of prominent bands in the region of 1800-1650cm^{-1} suggests the involvement of the dicarbonyl moiety of the curcuminoids incomplexation with metal ions.This is because metal ion complexation will reduce the stretching vibrations of the carbonyl groups.Further the broad band of the free ligand observed in the region 2500-3500cm^{-1} due to intramolecularly H-bonded carbonyl groups cannot be observed in the spectra of the complexes. This clearly indicates the removal of H-bonded proton by the metal ion. The IR spectra of Zn(II) complex of **2b** is showed in **Fig 2.2.1.**
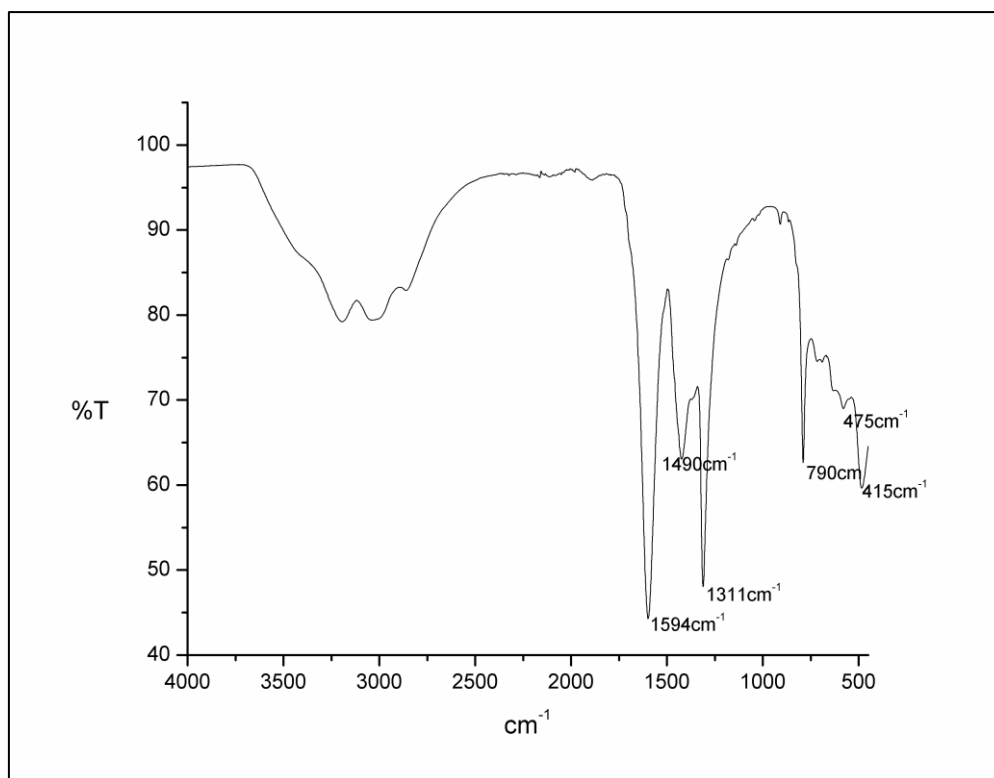
Fig.2.2.1 IR spectra of Zn (II) complex of 2b.

^{1}H NMR spectra

The peak at 16 ppm due to the enolic proton was absent in the spectra of complexes. This clearly indicates the replacement of enolic proton by metal ion. Whereas the other two protons namely phenyl and alkenyl do not take part in complex formation, so their peak doesn't show much changed in spectrum and methine signals showed a downfield shift. Thus in total both the ligand spectra and complex spectra were almost identical and the only difference is in the case of enolic proton. The ^{1}H NMR spectrum of Ni (II) complex of ligand **2b** is depicted in **Fig 2.2.2.**
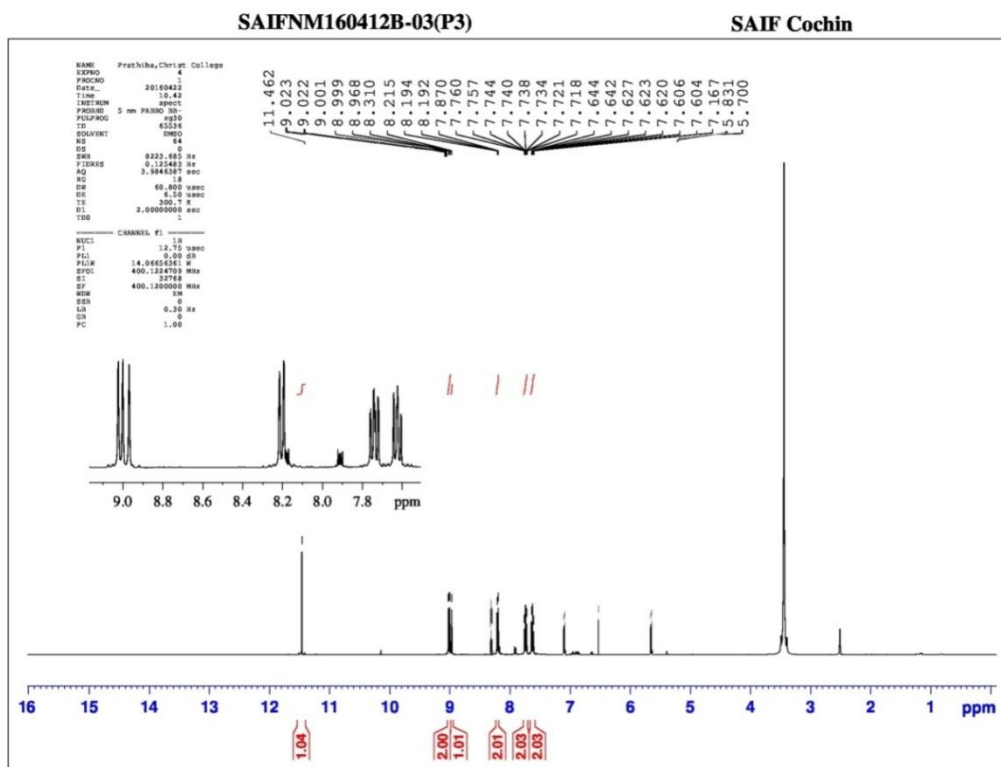
Fig.2.2.2 The NMR spectrum of Ni (II) complex of 2b.

Mass spectra

The suggested structure of complexes are in agreement with the observed mass spectra of complexes.The molecular ion peak gives idea about the stochiometry of the complex with metal ligand ratio 1:2. The probable fragmentation pattern of the complexes based on the observed peaks has been illustrated in **Scheme 2.2.1.** Mass spectral details of the metal chelates of **2a, 2b**&**2c** are given in Table 2.2.4. Mass spectrum of Zn(II) complex of **2a** is given in **Fig.2.2.3** and Cu(II) complex of **2b** is depicted in **Fig.2.2.4.**

Peaks corresponding to stepwise elimination of aryl groups are a characteristic feature of all the complexes. The molecular ion peak corresponding to $[ML_2]^+$ ion is present in the mass spectra of all complexes. The important peaks observed in the spectra can be identified from the fragmentation pattern given in **Scheme 2.2.1**. Smaller molecules are eliminated from the molecule to get a large number of peaks beyond the peaks discussed in the Scheme. Certain fragments rearrange to form stable cyclic species. Peaks due to $[ML]^+$,$[L]^+$ and fragments of $[L]^+$ are also detected in the spectrum.
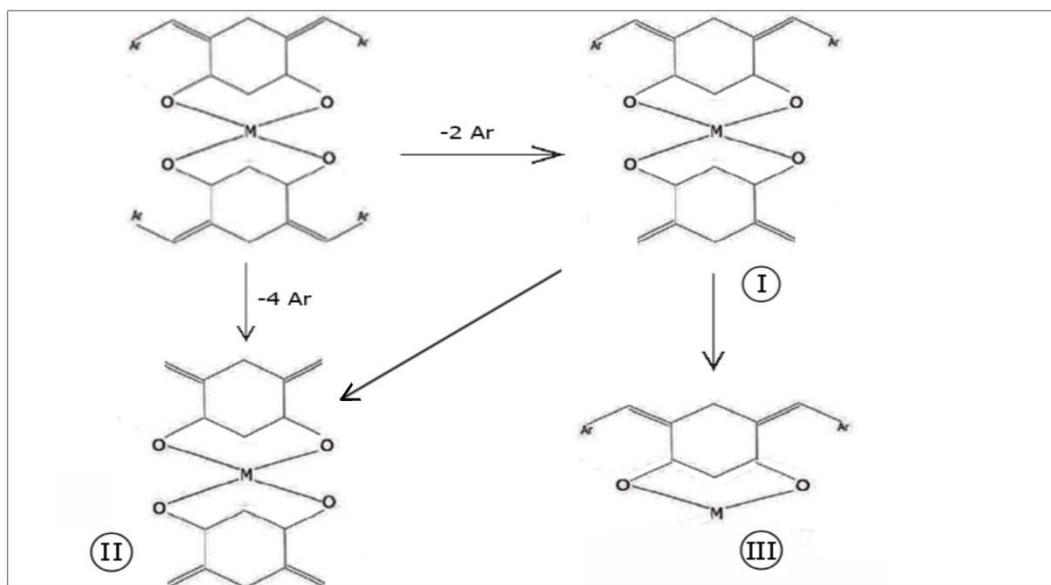
Scheme 2.2.1   Fragmentation pattern of metal complexes.

In the mass spectrum of Zn (II) complex of **2a**, the molecular ion peak is present at 775 which is not a very intense peak.The peak at 661 is due to the removal of 2Ar groups and the peak at 419 is due to the removal of 4Ar groups where Ar is 4-chlorophenyl group.The peak at 355 is due to the ligand. In the mass spectrum of Cu(II) complex of**2b** ,the molecular ion peak is present at 778 which is not a very intense peak.The peak at 489 is due to the removal of 2Ar groups and the peak at 427 is due to the removal of 4Ar groups where Ar-2-chlorophenyl group.The peak at 358 is due to the removal of a ligand group and oxygen from the molecular ion.Thus the observed UV,IR,^{1}H NMR and mass spectral data of the above mentioned complexes supports the scheme of synthesis as
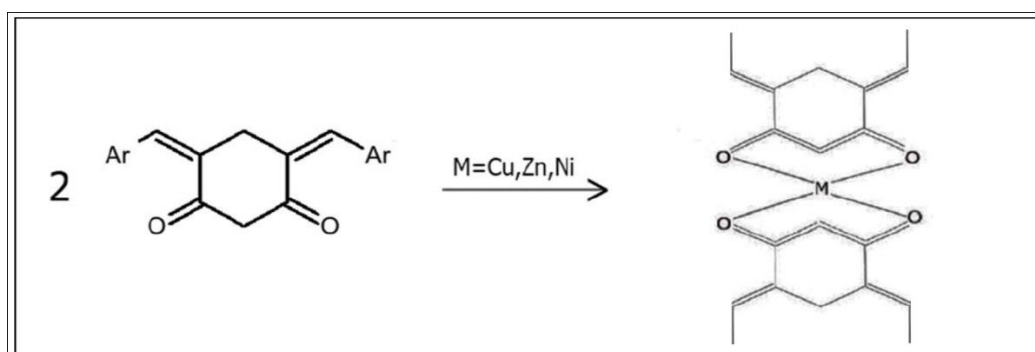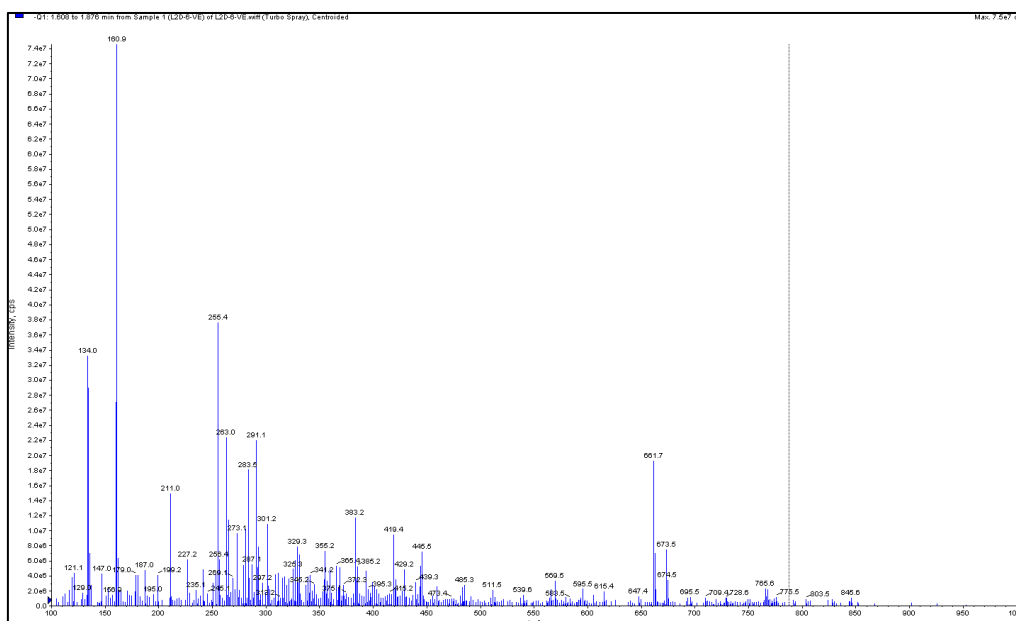
Fig.2.2.3The mass spectrum of Zn (II) complex of 2a.
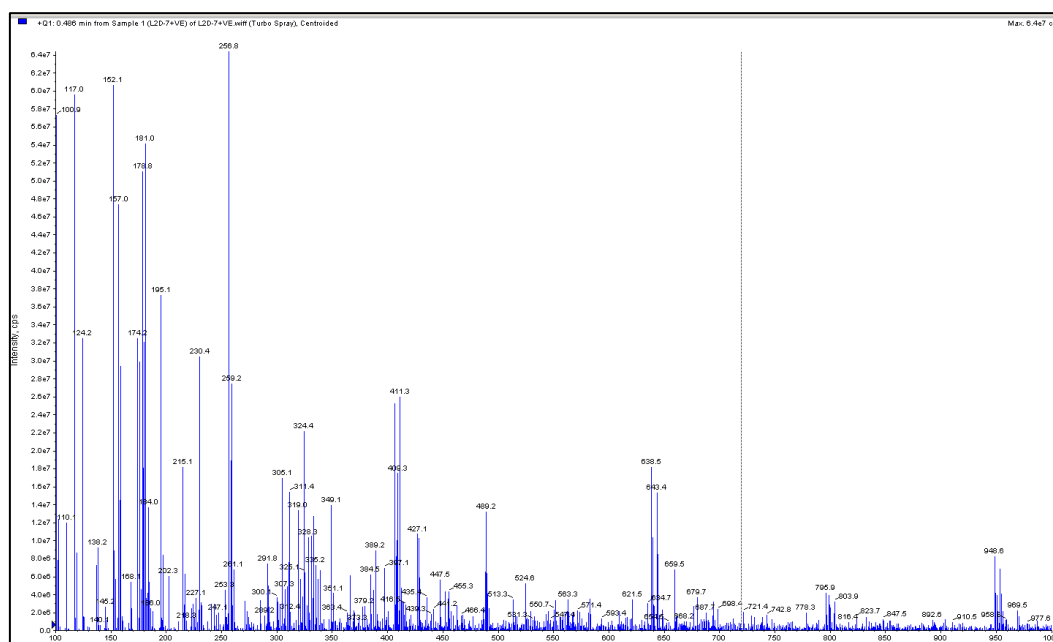
Fig.2.2.4 The mass spectrum of Cu(II) complex of 2c.

SECTION-III

CYTOTOXIC AND ANTITUMOUR ACTIVITIES OF (4E,6E)-4,6-BIS(CHLORO ARYL) CYCLOHEXANE- 1,3-DIONES AND THEIR METAL CHELATES

This section deals with the cytotoxic and antitumour activities of chloro aryl cyclohexane1,3 diones and their metal chelates. Both in vivo and in vitro cytotoxic studies were carried out are considered in section III. DLA & EAC cells were used for in vitro studies. In vivo studies conducted both by solid tumour and ascites tumour method and their results were compared with that of reference drug.

2.3.1 In vitro cytotoxic study of thecurcuminiod analogues and their metal chelates

Cytotoxic activity of curcuminiod analogues and their metal chelates Cu(II),Zn(II) and Ni(II) were assessed by determining the percentage viability of DLA and EAC cells using Trypan blue dye exclusion technique(Moldeus *et al,*1978).

2.3.2 In vitro studies of (4E,6E)-4,6-bis(2-chlorobenzylidene)cyclohexane- 1,3-dione (2a) and their metal complexes [Cu(II),Zn(II) & Ni(II)] against EAC & DLA cells.

In vitro cytotoxic test was conducted by the synthetic analogues of different concentrations against EAC.The percentage of cell death is a measure of cytotoxic character of the compounds. The results obtained are given in **Table 2.3.1** and represented graphically in **Fig 2.3.1.** and **Fig.2.3.2.**

Table 2.3.1 In vitro studies of 2a and their metal complexes to EAC & DLA cells.

Conc.	Percentage cell death to EAC				Percentage cell death to DLA			
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$
200	27	78	65	49	25	75	63	47
100	23	67	51	34	21	64	49	33
50	16	48	39	21	11	43	35	19
20	9	2	21	19	8	25	19	17
10	4	19	13	11	5	13	11	9
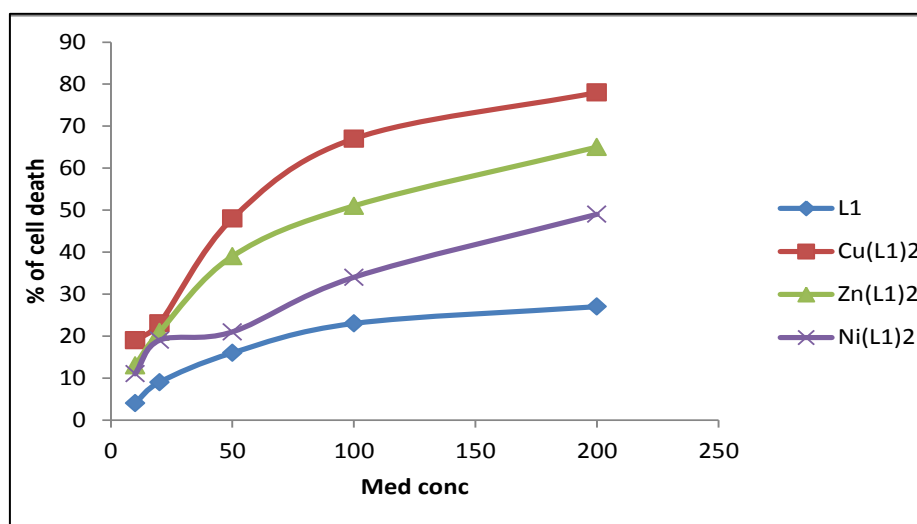
Fig 2.3.1 In vitro studies of 2a and their metal complexes towards EAC cells.

The observed data clearly indicated the metal complexation drastically increased the cytotoxic activity of the curcuminiods. In effect, Cu(II) complex of test compound produces 78% of cell death to EAC cells. Amongst the metal complexes, Ni complex displays minimal activity towards EAC. The cytotoxic nature of test compounds follow the order Cu(II)>Zn(II)>Ni(II). Associating(4E,6E)-4,6-bis(2-chlorobenzylidene)cyclohexane-1,3 dione (2a) and its metal complexes ,it can be seen that all the metal chelates are more cytotoxic than the ligand. Amongst the metal complexes, Cu (II) showed greater cytotoxicity to DLA cells. The activity of

copper complex was nearly four times than that of the ligand.The cytotoxic activities follow the order Cu (II)>Zn (II)>Ni (II). The cytotoxic effects of chelates depend on the chelate geometry and thermal stability.The outcome of the study in terms of percentage of cell death is depicted diagrammatically in **Fig 2.3.2.**
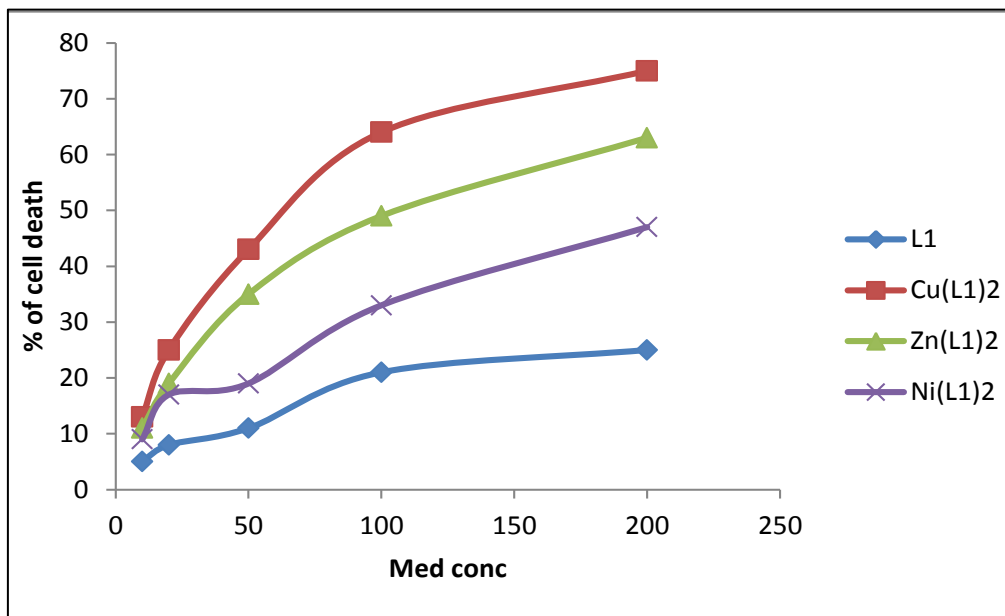
Fig 2.3.2 In vitro studies of 2a and their metal complexes against DLA cells

2.3.4 In vitro cytotoxic studies of 2b and their metal complexes against EAC & DLA cells

In vitro cytotoxic studies of **2b** and their metal complexes against EAC & DLA cells are given in **Table2.3.2** and also illustrated in**Fig 2.3.3 & 2.3.4** respectively.

Table 2.3.2 In vitro studies of 2b (L2) and their metal complexes against EAC & DLA

Conc.	Percentage cell death to EAC				Percentage cell death to DLA			
	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$
200	21	73	59	44	19	71	57	43
100	19	62	44	32	18	59	43	31
50	8	42	31	21	7	38	29	20
20	7	23	17	16	3	20	16	15
10	3	13	8	7	2	12	7	6
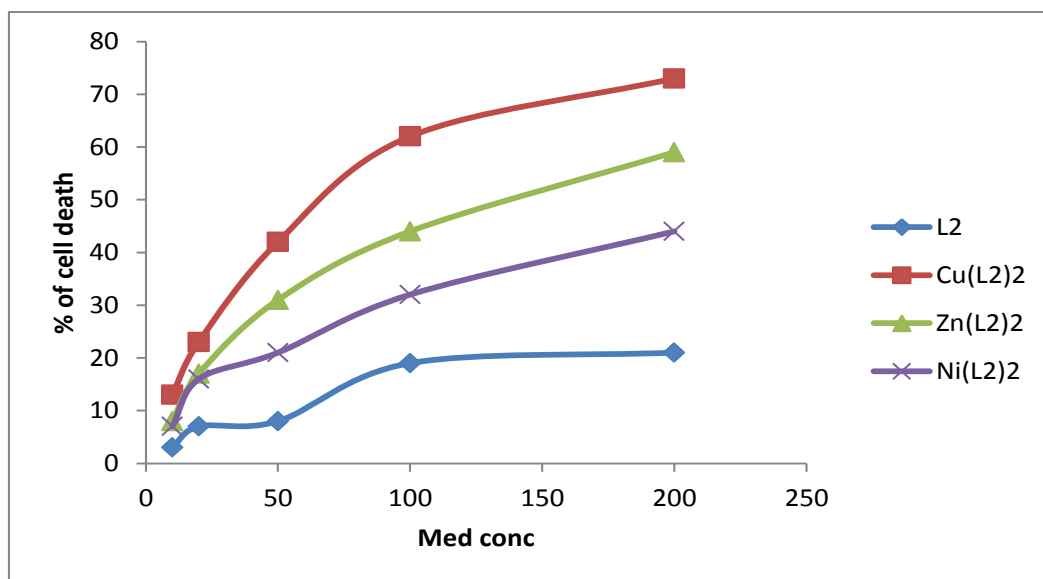
Fig 2.3.3 In vitro cytotoxic studies of 2b and their metal complexes against EAC.
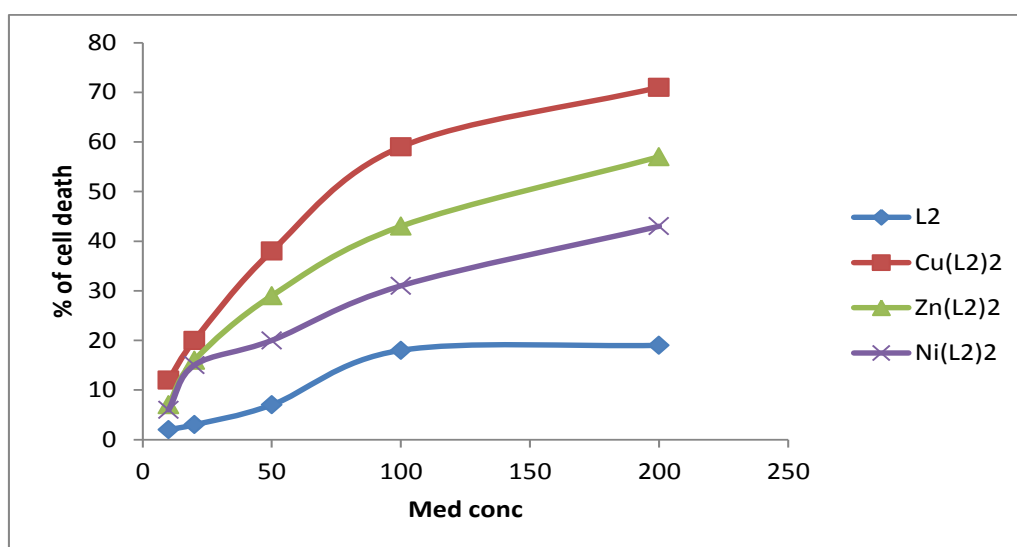
Fig 2.3.4 In vitro cytotoxic studies of 2b and its metal complexes against DLA.

The curcuminiod analogue **2b** with chloro substitution in the para position of the phenyl ring appeared to be less active compound than the ortho phenyl ring system. However, the metal complexes Cu(II) & Zn(II) of the compounds showed pronounced effect in cytotoxicity.

2.3.5 In vitro cytotoxic studies of 2c & its metal complexes to EAC & DLA cells.

In vitro cytotoxic test was conducted by the curcuminiod analogues of different concentrations against EAC& DLA. The results of the study towards EAC &DLA are specified in **Table 2.3.3** & diagrammatically represented in**Fig 2.3.5 & Fig 2.3.6.**

Table 2.3.3 In vitro studies of 2c (L3) and its metal complexes to EAC & DLA.

Conc.	%cell death to EAC				%cell death to DLA			
	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$Ni(L_3)_2$	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$Ni(L_3)_2$
200	19	48	34	31	14	44	29	28
100	14	33	22	18	7	27	17	15
50	10	19	17	14	4	17	12	11
20	5	10	9	6	2	8	5	3
10	1	3	2	0	0	1	1	0

The data showed that though 2c and its metal chelates couldnot prevent the development of EAC cells. They were reasonably inactive at minor concentrations.
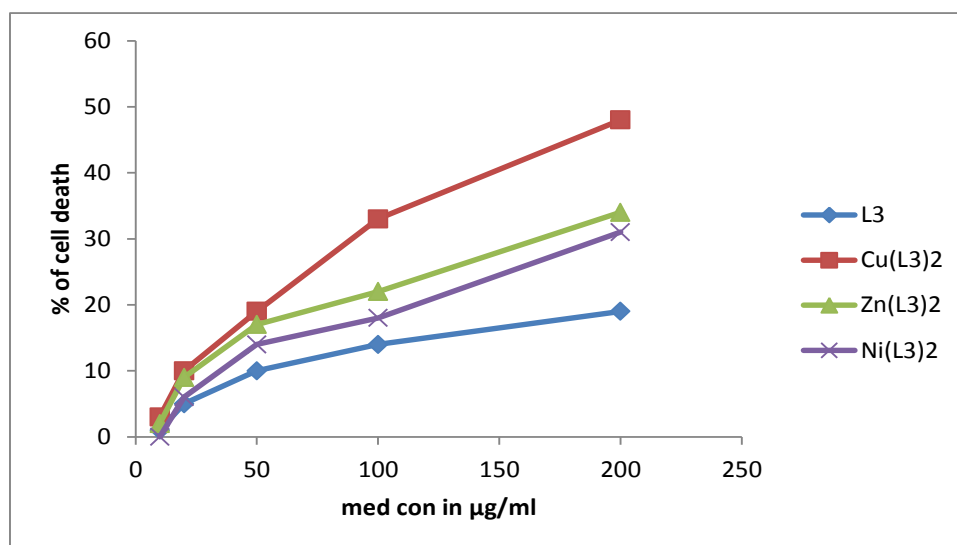
Fig 2.3.5 In vitro studies of 2c and their metal complexes towards EAC.

The disubstituted ligand and its Ni(II) complex showed a percentage of cell death zero at 10μg/ml. In all these cases very low level activity observed at lower concentration ie. at 10μg/ml but as the concentration increases gradually there is significant change in the cytotoxicity. All the metal complexes possess minimum level of cytotoxic activity, among which copper complex possess the maximum.
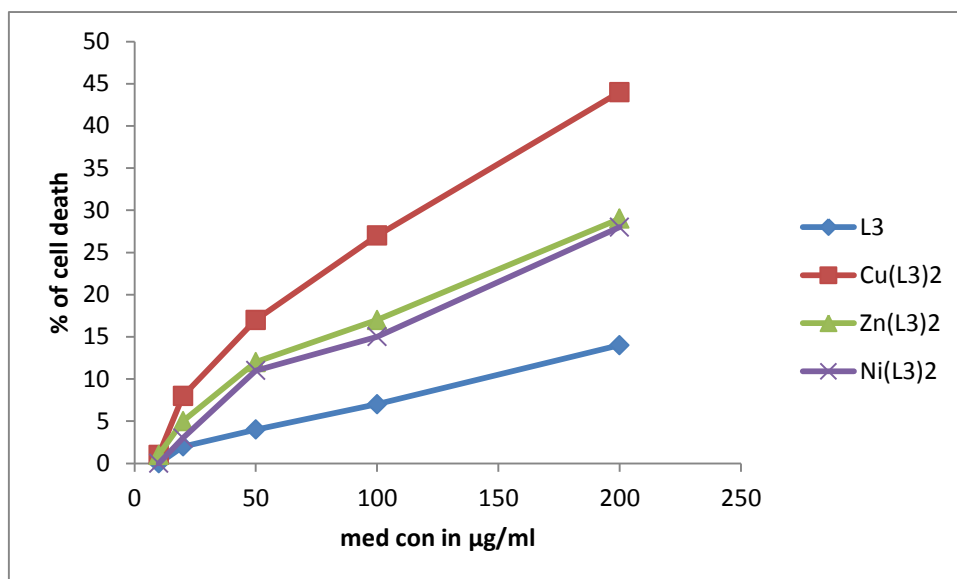
Fig 2.3.6 In vitro studies of 2c and their metal complexes towards DLA.

Conclusion

A relative study of in vitro cytotoxicity of chloro curcuminiods 2a, 2b and 2c were done. It should be concluded that **2a** showed maximum activity against EAC & DLA and least activity in the case of **2c**. This may be due to the steric factor.

IN VIVO ANTITUMOUR ACTIVITIES

The results of 2- chloro substituted curcuminiod analogues and their Cu(II) & Zn(II) complexes on the existence percentage of tumour carrying creatures were examined. Albino mice weighing 18-25 kg were used for the study purpose. Ascites tumour was made by introducing viable EAC cells in 0.1 ml of PBS into the peritoneal cavity. Out of 11 groups, one group was kept as control group which wasn't treated with any drug. It was labeled as group 1.Another group was treated with the standard drug, cyclophosphamide and it was labeled as group 2. Three groups were also selected, in which the ligand **2a** was applied having concentrations 20μg/ml,10μg/mland 5μg/ml and they were labeled as group 3,4 & 5 respectively. The Zn(II) complex and Cu(II) complex of 2a was used in the treatment of another 6 groups and they were labelled as group 6,7 & 8 and 9 to 11 respectively.

2.3.6 Effect of 2a and their Cu (II) & Zn(II) complexes on ascites tumour.

Al the synthetic analogues having different concentrations made intensification in the life span of ascites tumour carrying mice. The results are shown below in Table **2.3.4.**

Table 2.3.4 Effect of 2a and their Zn(II) & Cu(II) complexes on ascites tumour

	Animal group	Concentration μg/ml	No. of animals with tumour	No. of days survived	% ILS
1.	Control		5/5	17.3±1.10	
2.	Standard		5/5	30.6±0.489	76.87
3.	L_1	20	5/5	21.0±1.05	21.38
4.	L_1	10	5/5	19.8±1.74	14.45
5.	L_1	5	5/5	18.5±1.74	6.93
6.	$Cu(L_1)_2$	20	5/5	25.6±1.36	47.97
7.	$Cu(L_1)_2$	10	5/5	21±1.01	21.38
8.	$Cu(L_1)_2$	5	5/5	19.9±1.43	15.02
9	$Zn(L_1)_2$	20	5/5	22.5±2.4	30.05
10	$Zn(L_1)_2$	10	5/5	19.9±3.7	15.02
11	$Zn(L_1)_2$	5	5/5	18.6±1.50	7.51

The synthetic analogues were given for treatment in concentrations 5, 10,20μg/ml. The survival of animals by the control group is 17.3 and that of standard group is 30.6. Here the standard drug used is cyclophosphamide. From the result it can be shown that the life span of mices in the standard group increases compared to the control group. The ligand (4E,6E)-4,6- bis(2-chlorobenzylidene) cyclohexane -1,3- dione made only 21.38 % ILS at a concentration of 20μg/ml. But

at the same concentration, its copper complex retains an increased life span of 47.97%.That is metal chelation produces greater activity towards the reduction of ascites tumour.The result obtained by the metal chelated complex is comparable with the result that obtained by the standard drug.Among the metal complexes copper complex was most in effect to increase the life period of tumour burden mice. Plasma copper concentration increases in neoplastic and autoimmune diseases as an immune mediated physiological response to the disease state. Treatment with copper complexes is a therapeutic support of this increase in plasma copper and the attendant distribution of copper to affected tissues to enable de-novo synthesis of copper dependent enzymes required to bring about remission by reestablishing normal tissue function.

ANTIBACTERIAL STUDIES OF (4E,6E)-4, 6-BIS (CHLOROARYL) CYCLOHEXANE- 1,3 –DIONE AND THEIR METAL CHELATES

2.4.1 Antibacterial studies of 2a and 2b and their metal chelates

All the synthetic analogues and their metal complexes were assessed for their antibacterial activities. Antibacterial study was conducted by agar well diffusion method and the bacterial strains used were Escherichia Coli, Klebisella P neumoniae & Bacillis Subtilis. The test compounds have shown marginal to outstanding potency in the invitro antibacterial study.There is a comparable antibacterial activity between curcumine analogues and the standard drug streptomycin (**Table 2.4.1).** The antibacterial activity was stated in terms of diameter of zone of inhibition in mm.

Table 2.4.1 Antibacterial studies of 2a ,2b and their metal chelates.

Bacteria	Diameter of zone of inhibition in mm							
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$
E Coli	12.2	16.5	12	14	12	17	13	16
Klebsiella	10	14	11	12	11	15	12	14
Bacillus	3.5	7.5	5	6	5	10.5	6	9.5
Standard	20	20	20	20	20	20	20	20
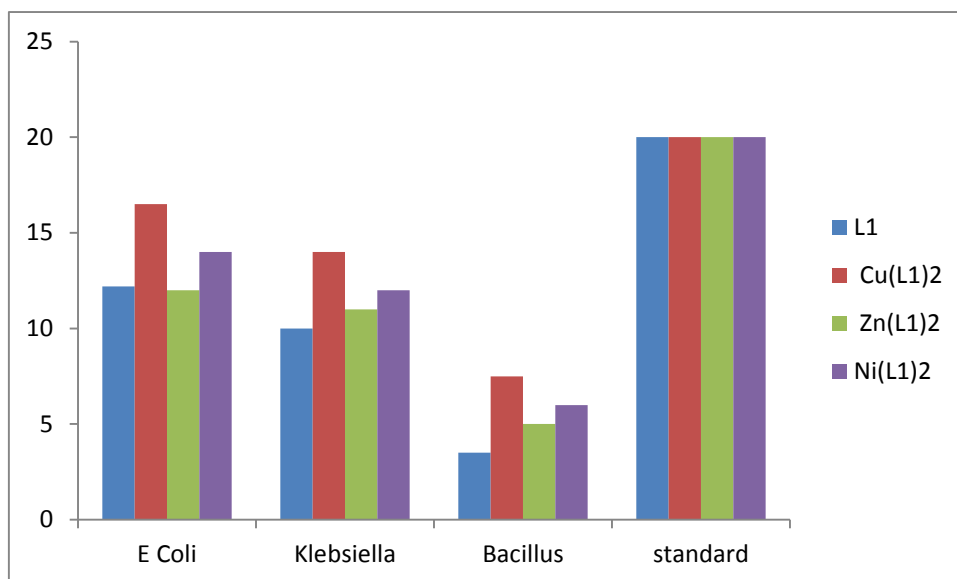
Fig 2.4.1 Antibacterial studies of 2a and their metal complexes.
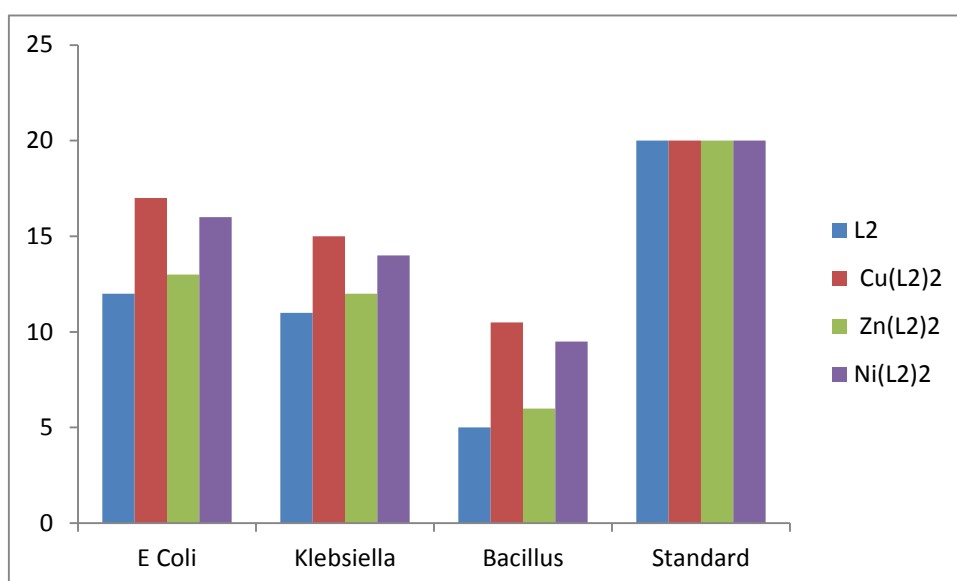
Fig 2.4.2 Antibacterial studies of 2b and their metal chelates.

The ligands **2a & 2b** have shown an increased activity against E. coli bacteria whereas minimal and moderate activities have been observed in Bacillus Subtilis and Klebsiella Pneumoniae respectively.Among the metal complexes copper complexes keep better activity than the other complexes and follows the order Cu (II)>Ni(II)>Zn(II). The copper and nickel complexes of (4E,6E)-4,6-bis(4-chlorobenzylidene) cyclohexane- 1,3- dione had shown enhanced activity and made a zone of inhibition of 17mm and 16mm respectively which is as good as with the diameter of zone of inhibition created by standard drug streptomycin ie:20mm.

2.4.2 Antibacterial studies of (4E,6E)-4,6- bis (3,4- dichloro benzylidene) cyclohexane-1,3- dione and their metal chelates

The investigation results are given below in **Table 2.4.2.**On comparing the acitivity studies of **2c** it has been found that, dichloro substituted ligand displayed less antibacterial activity than **2a & 2b**. The ligand namely (4E,6E)-4,6-bis (3,4dichlorobenzylidene) cyclohexane-1,3- dione & its metal complexes were most dynamic towards E. coli bacterial strains and created a zone of inhibition of 14 mm whereas it showed 7.5mm and 12.5mm against Bacillus Subtilis and Klebsiella respectively. Metal chelation causes an increase in the power of antibacterial activity.In the current study the antibacterial effect of metal complexes charts as Cu(II)>Ni(II)>Zn(II)as it enhances chelation .It penetrates more to the lipid membrane and increases hydrophilic and lipophilic character of metal ion.

Table 2.4.2 Antibacterial studies of 2c and their metal chelates.

Bacteria	Zone of inhibition in mm			
	L_3	$Ni(L_3)_2$	$Zn(L_3)_2$	$Cu(L_3)_2$
E Coli	10	12.5	10.5	14
Klebsiella	6.5	10.3	8	12.5
Bacillus	3.5	7	4	7.5
standard	20	20	20	20
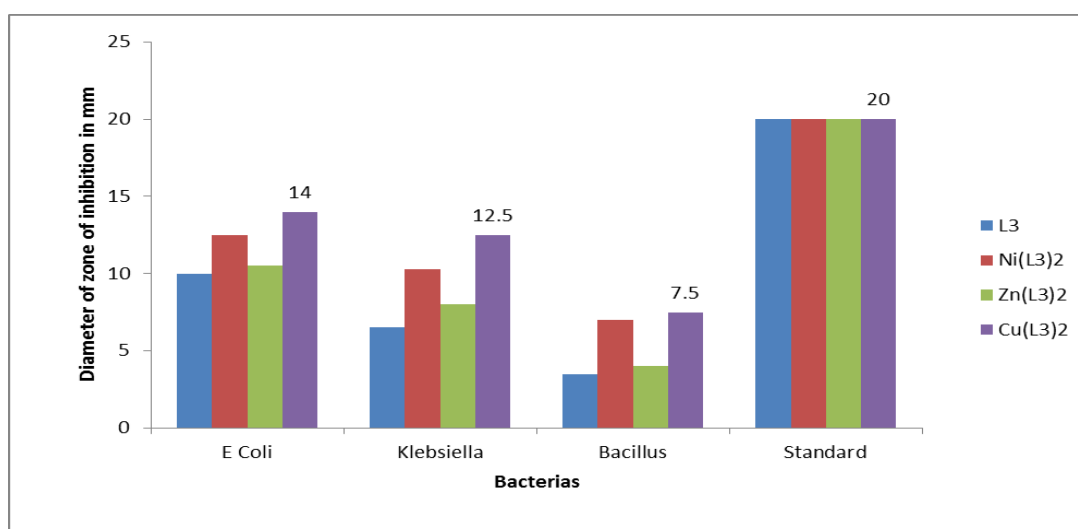
Fig. 2.4.3 Antibacterial studies of 2c and their metal chelates.

SECTION –V

ANTIFUNGAL STUDIES OF (4E,6E)-4,6- BIS (CHLOROARYL)CYCLOHEXANE- 1,3- DIONE AND THEIR METAL CHELATES

2.5.1 Antifungal studies of chloro analogues and their metal chelates.

The halogen derivative of curcumine analogues and their metal chelates were studied for their antifungal character. To study the antifungal properties of test compounds three fungal cultures were used and the analysis was following Kirby Baurer disc plate technique. The curcuminiod ligands and their metal complexes were prepared in DMSO with variable concentrations to evaluate their property against Alternaria Alternate, Aspergillius Niger and Pencillium Chrysogenum. For the antifungal study standard drug used was fluconazole.A control disc was retained without any application test compounds. The analogues which are operative upon fungal cultures, development of fungus were inhibited as zone. By measuring the diameter of zone of inhibition in mm antifungal activities were found. The results of the studies are given in **Table 2.5.1, 2.5.2 and 2.5.3.** Among the synthetic ligands, ortho derivative of ligand were found to be powerful antifungal agent and para derivative were least active compounds. Among the four complexes of ligands **2a &2b**, most of them were found to be potent antifungal agents than the ligands, and the Zn(II) complexes of both shown more antifungal activity.

Table 2.5.1 Antifungal activities of 2a (L_1) and its Zn(II) & Ni(II) complexes.

Fungi	Diameter of zone of inhibition in mm								
	L_1			$Zn(L_1)_2$			$Ni(L_1)_2$		
	100 μg	250 μg	500 μg	100 μg	250 μg	500 μg	100 μg	250 μg	500 μg
Aspergilus	10.5	12	15	13	16	18.5	11	15	17
Pencillium	11	13	16	13.5	16	19	11.5	15	17.5
Alternaria	11	14	17	14	17	19.5	13	16	18

Table 2.5.2 Antifungal activities of 2b (L_2) and its Zn(II) & Ni(II) complexes.

Fungi	Diameter of zone of inhibition in mm								
	L_2			$Zn(L_2)_2$			$Ni(L_2)_2$		
	100 µg	250 µg	500 µg	100 µg	250 µg	500 µg	100 µg	250 µg	500 µg
Aspergilus	8	9.5	11	13.5	17	18.5	10.1	11.7	12.5
Pencillium	11.5	13	11.2	14	16	19	11	11.7	12.5
Alternaria	12	14	12.2	14.5	17	19.5	12.1	12.3	12.7

Table 2.5.3 Antifungal activities of 2c (L_3) and its Zn(II) & Ni(II) complexes.

Fungi	Diameter of zone of inhibition in mm								
	L_3			$Zn(L_3)_2$			$Ni(L_3)_2$		
	100 µg	250 µg	500 µg	100 µg	250 µg	500 µg	100 µg	250 µg	500 µg
Aspergilus	12.5	15	18	17.3	20	21	16.4	18.3	19.1
Pencillium	12	14	17	14.4	17	19	13.7	16	18.2
Alternaria	13	16	18	15.6	19	19	14.8	18.2	18.7

The ligand **2c** and its Zn(II) complex indicated extraordinary antifungal character compared to its mono chloro derivative. The antifungal effects of dichloro derivative were stronger than that of mono chloro derivative. It is because of existence of two chloro group that might enhance lipophilicity of the mother nucleus, which made it easier for the molecular to enter into the cell membrane of fungi to inhibit its growth. Compared with the reference drug the igand and its metal complex showed 75 & 93% of activity respectively towards the test fungi. The Zn (II) complex discloses exceptional antifungal character.
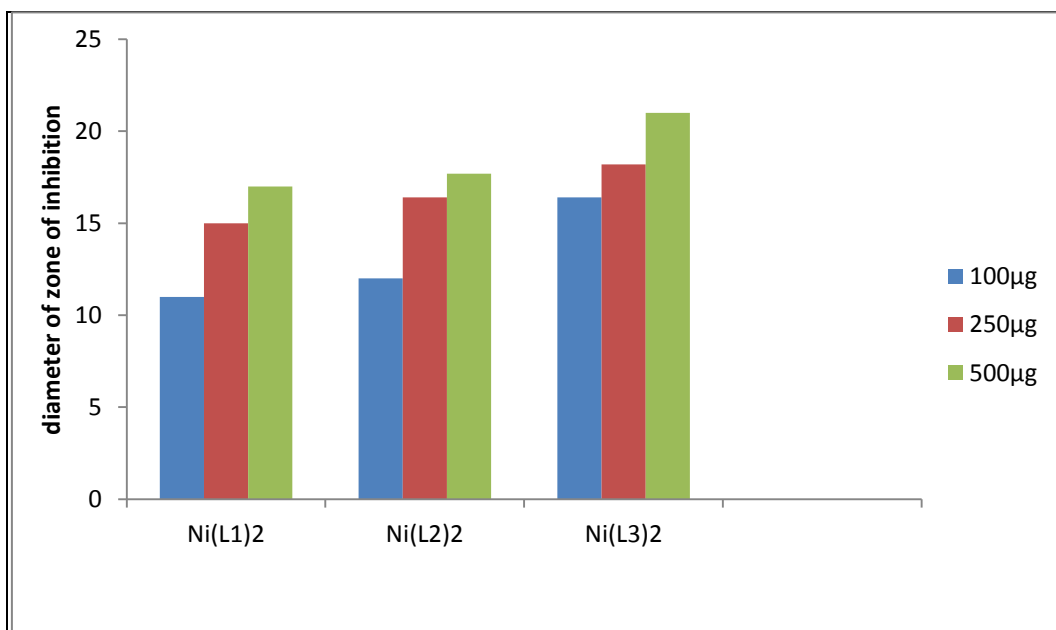
Fig 2.5.1 Ni (II) complexes of chloro analogues against Aspergillus.
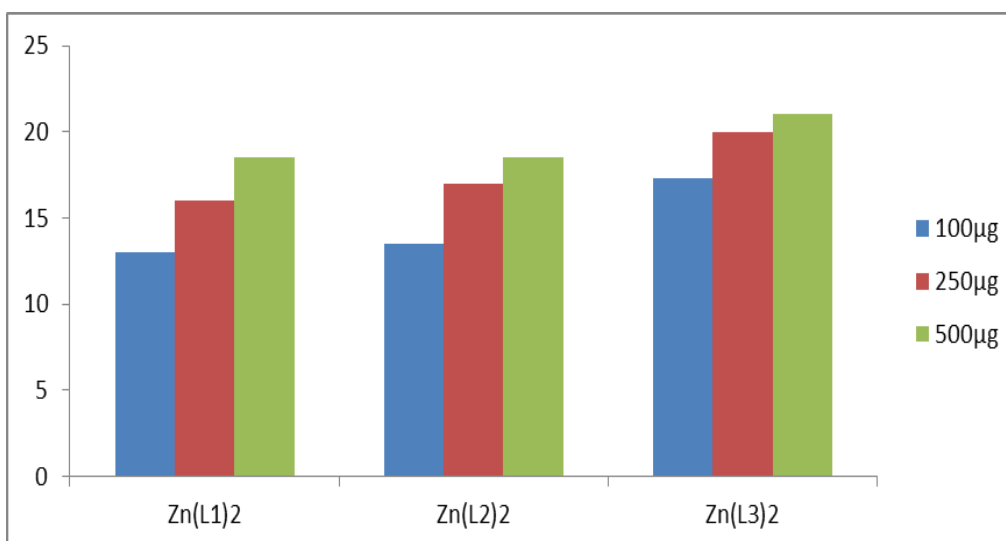
Fig 2.5.2 Zn (II) complexes of chloro analogues against Aspergillus.

CHAPTER –III

SYNTHESIS, CHARACTERISATION AND BIOLOGICAL ACTIVITIES OF (4E,6E)-4,6-BIS(THIOPHENE-ARYL)CYCLOHEXANE-1,3- DIONES AND THEIR TRANSITION METAL COMPLEXES.

INTRODUCTION

Heterocyclic products of curcumin roused a significant consideration in their biological activities. It was expected that structural analogues of curcumin in which phenolic moiety is substituted by heterocyclic ring would exhibited novel molecular templates with remarkable biological activities in animal models. So all efforts were made to obtain synthetic analogues of curcumin in which hetero rings were attached to 1, 3- diketone system through an olefinic linkage.A series of (4E,6E)-4,6-bis(aryl-thiophene)cyclohexane-1,3- diones were synthesized by the condensation of heterocyclic aldehydes with cyclohexane1,3 diones as in **scheme 3.1.1.** This chapter is divided into five sections.
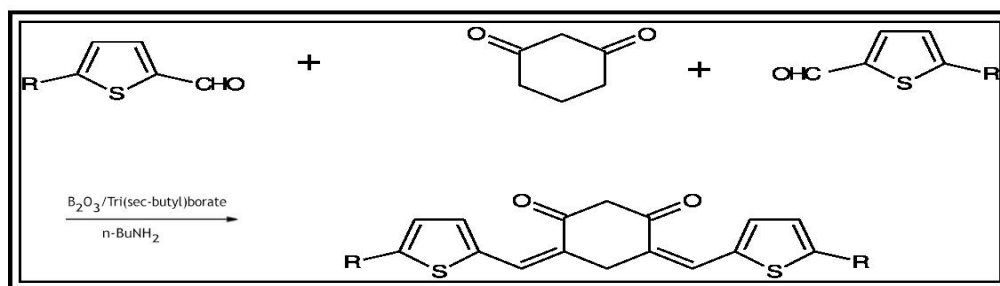
Compound	Ar
3a	
3b	

SYNTHESIS AND CHARACTERISATION OF THIOPHENE BASED CYCLOHEXANE -1, 3- DIONES

3.1.1 Synthesis of ligands 3a and 3b

The first section deals with the synthesis and characterization of thiophenyl substituted synthetic curcuminoid analogues. These are prepared by the reaction of hetrocyclic aldehydes (thiophene-2-carboxaldehyde and 5-methyl thiophene -2-carboxaldehyde) with cyclohexane-1,3 dione-boric oxide complex. The solvent used here was ethyl acetate and the condensation is carried out in the presence of Tributyl Borate and n-Butyl amine. The synthesized compounds were purified by column chromatography using 4:1 chloroform: acetone mixture as eluent. For better purification it was recrystallized from hot benzene. The synthetic details are given in **Table 3.1.1.**
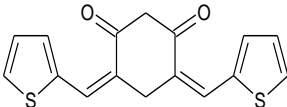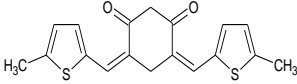
Ligand	Aldehyde used	Structure of ligand	Systematic name	Yield %
3a	Thiophene 2-aldehyde		(4E,6E)-4,6-bis(thiophen-2-ylmethylidene)cyclohexane-1,3-dione	67
3b	5-methyl thiophene -2-aldehyde		(4E,6E)-4,6-bis(5methylthiophen-2-ylmethylidene)cyclohexane-1,3-dione	64

Table 3.1.1 Synthetic details of ligand.

Table 3.1.2 Analytical and electronic spectral data of ligand 3a &3b.

compounds	M.P(^{0}C)	Elemental analysis		Molecularweight	Electronic λ max (nm)
		C	H		
		Found(calculated)			
3a	119	63.82 (64.00)	3.56 (4.00)	300 (296)	264 360
3b	166	65.16 (65.85)	4.01 (4.87)	328 (327)	273 367

3.1.2 Characterisation of ligands: Electronic spectra& IR spectra.

When the ligand **3a &3b** compared to each other, the ligand **3b** showed a bathochromic shift in transitions. They exhibited two wide-ranging band at 273nm and 367nm respectively owing to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition. The IR spectra convey the characteristics of carbonyl group,if it is in the free or H-bonded arrangement. The spectral details are given in **Table 3.1.3.**

Table 3.1.3 IR spectral data of ligand 3a &3b.

Ligands		Probable assignments
3a	3b	
1647	1660	ν(C=O) cyclohexyl
1588	1581	ν(C=C) thiophenyl
1535	1551	ν(C-C) alkenyl
1506	1520	ν_{as}(C-C-C) chelate ring
1444	1410	ν_s(C-C-C) chelate ring
1136	1163	β(C-H) chelate ring

From the spectral data it is clear that the keto group is in the H-bonded form because, if it was in the free form its stretching frequency will be in the range of 1700-1800cm^{-1}. The factors which cause the lowering of stretching frequency of carbonyl group are H-bonding and resonance. The ligand display broad band about the 2500-3500cm^{-1} and the OH stretching vibration accounts it. ν(C=C) vibrations of the heterocyclic thiophenyl group detected at 1580-1590cm^{-1}. ν(C-C) alkenyl, ν_{as}(C-C-C) chelate ring, ν_{s}(C-C-C) chelate ring and β(C-H) chelate ring are responsible for the presence of further peaks The IR spectra for **3a & 3b** were specified below. The IR spectrum of **3a** is given in **Fig.3.1.1 and 3b** in **Fig.3.1.2.**
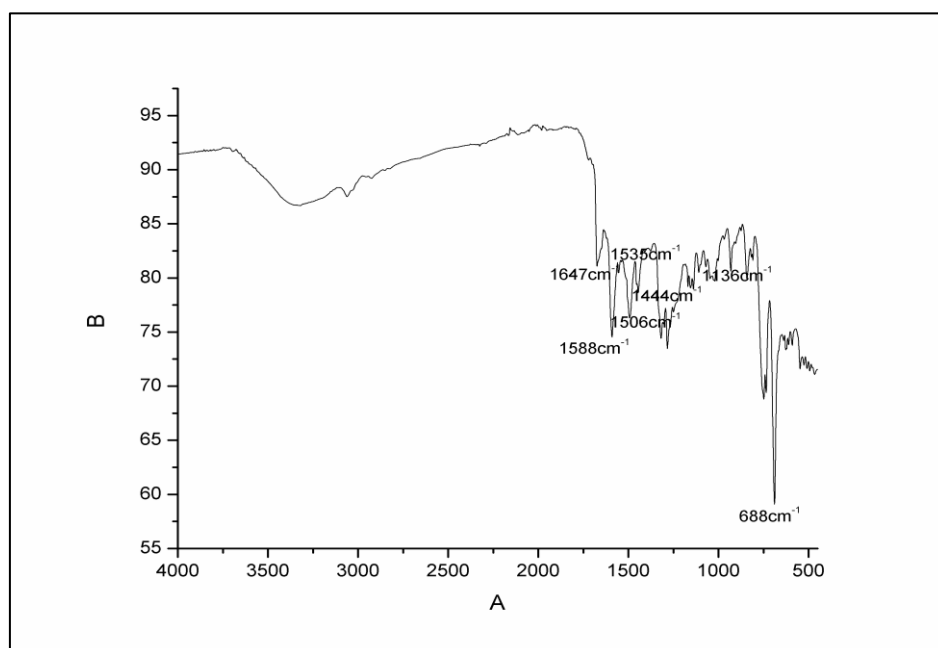
Fig.3.1.1 IR spectrum of 3a.
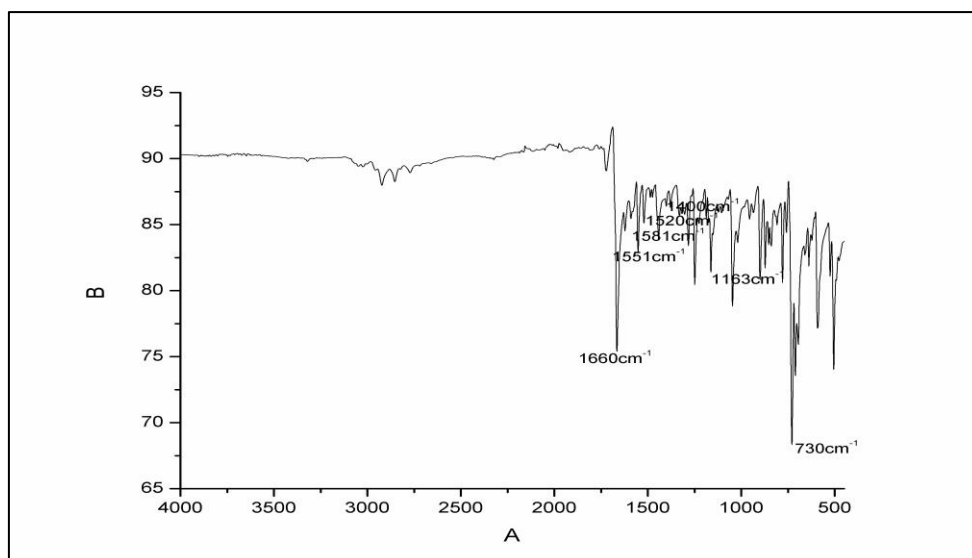
Fig 3.1.2 IR spectrum of 3b.

^{1}H NMR spectra

The ligands **3a** and **3b** show particular peaks equivalent to enolic,methane,alkenyl and thiophenyl protons.(Table.3.1.4) and they displayed a one proton singlet at 16 ppm, and it is due to the the presence of enolic proton. The methine proton which is also H-bonded and exhibit a peak of 5.7 ppm.

Table 3.1.4 Characteristic ^{1}H NMR spectral data of ligands 3a &3b.

Compounds	Chemical shift(δ ppm)				
	Enolic	**Methine**	**Alkenyl**	**Thiophenyl**	**Methyl**
3a	16.2	5.4	6.4-7.9	7.08-7.7	-
3b	16.24	5.7	6.5-7.3	7.01-7.9	2.6

The signals which are also seen in spectra are heterocyclic protons present in the range δ 7.08-7.9 ppm and alkenyl protons in the range of 6.4-7.3 ppm. The methyl protons of **3b** exhibited an additional peak at 2.6 ppm. The ^{1}H NMR spectra of **3b** is depicted in**Fig.3.1.3.**
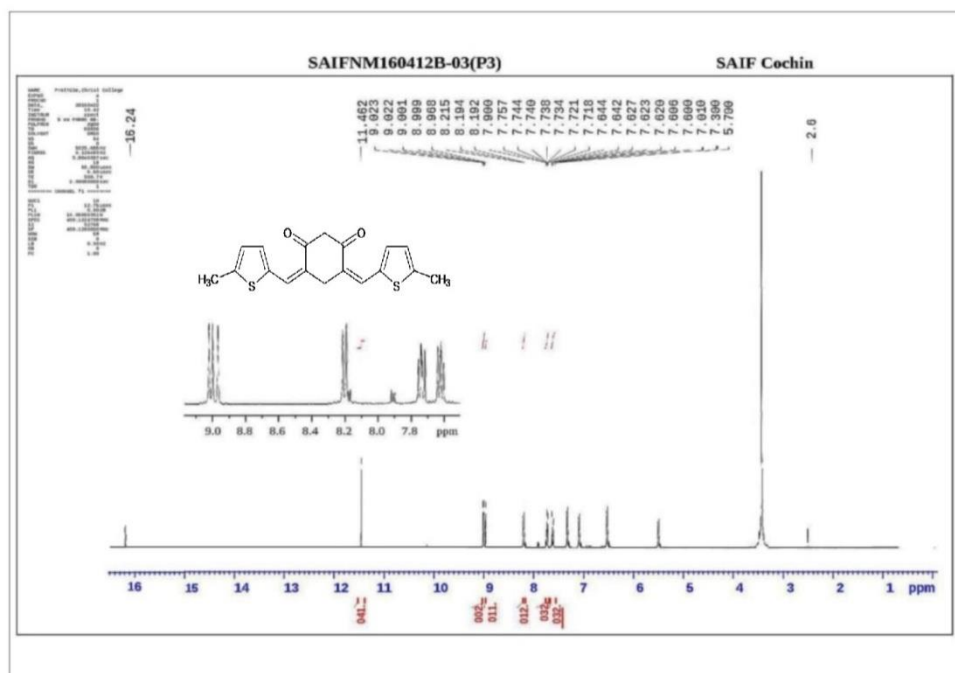
Fig 3.1.3 ^{1}H NMR spectrum of 3b.

^{13}C NMR spectra

The ^{13}C NMR spectra data of **3a** and **3b** are given in **Table 3.1.5 & 3.1.6.**

Table 3.1.5 ^{13}C NMR spectral data of 3a (chemical shift in ppm).

C1	C2,C2'	C3,C3'	C4,C4'
104.57	187	143.48	127.63
C5,C5'	C6,C6'	C7,C7'	C8,C8'
145.59	132.38	131.22	128.22

The peak at 104.57 ppm corresponds to methane. Generally CH$_2$ carbon which is be near to two carbonyl groups gave a peak of 55 ppm. But there is a chance of H-bonding, so methane group converted to an alkenyl carbon.So a downward shift is observed in C1 case. This compound has a carbonyl functional group, and these carbonyl carbons have the highest chemical shift value than the other carbon atoms and showed a peak of 187 ppm in **3a** and in **3b**. Heterocyclic thiophenyl group exhibit a peak at 127.63 in **3a** and in**3b**. Compared to 3a, 3b showed an additional peak at 20 ppm which was due to the methyl group present in it.

Table 3.1.6 ^{13}C NMR spectral details of 3b(chemical shift in ppm).

C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'
103.54	187.11	137.73	126.72	136.91
C6,C6'	C7,C7'	C8,C8'	C9,C9'	
133.55	129.93	123.22	20.98,19.91	
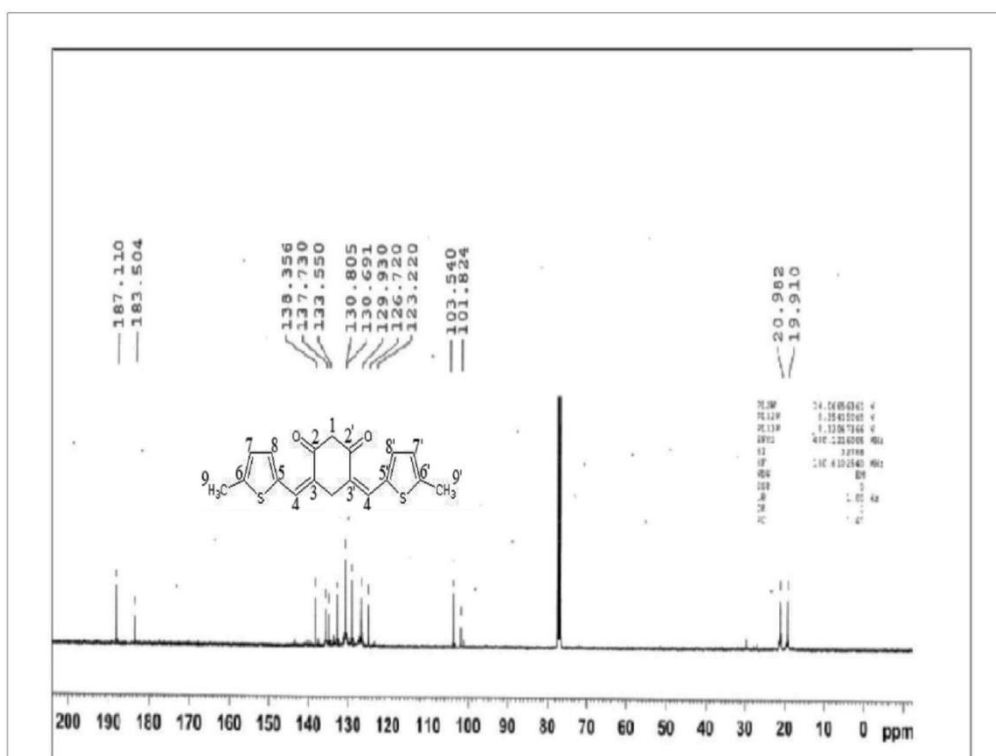
Fig 3.1.4 C^{13} NMR spectrum of 3b.

Mass spectral details

The mass spectrum of **3a** & **3b** are represented in **Fig.3.1.5 &3.1.6** respectively. The mass spectrum of **3a** shows a molecular ion (P+1) peak at 301 and a base peak (most intense peak) at 150 which is due to fragment (structure B) Ar-CH=C(CO)CH$_2$ in **Scheme 3.1.1**. Elimination of different moeity from the compound in accordance with the scheme, give rise to slighter fragments. In the mass spectrum of **3a** several small fragments are observed apart from the fragmental pattern described in **Scheme 3.1.1.**
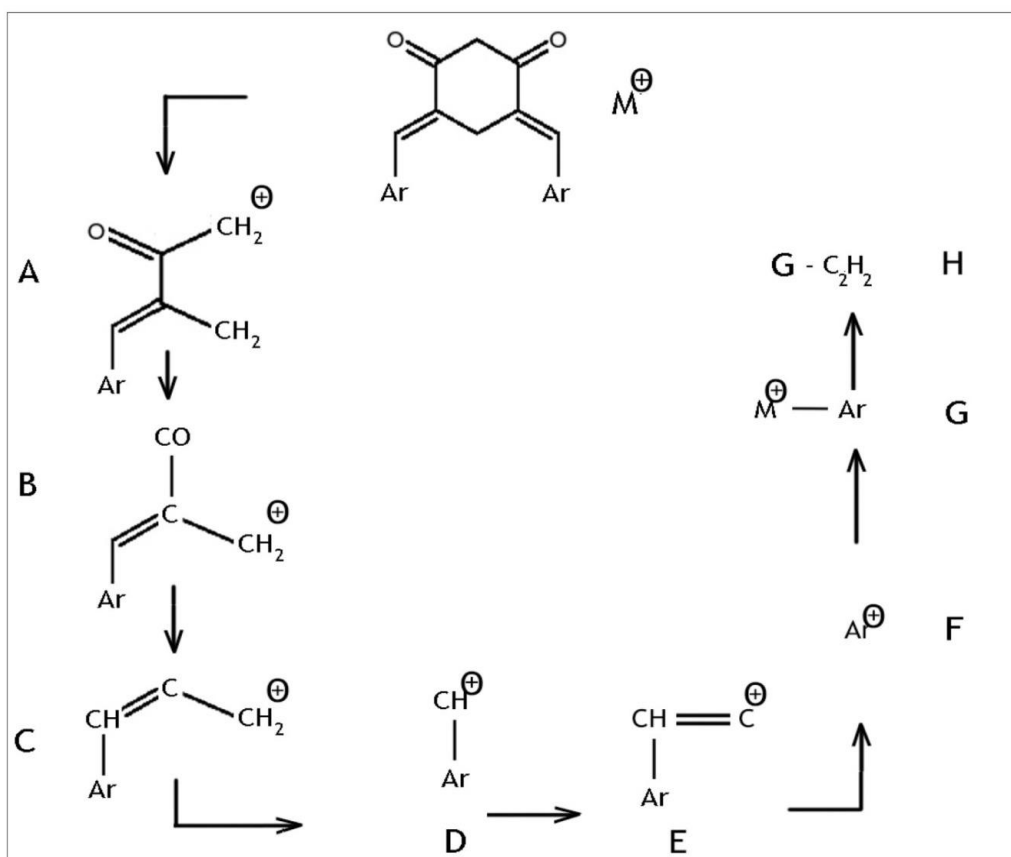
Scheme 3.1.1.

Table 3.1.7   Mass spectral fragmental pattern of 3a & 3c.

Fragment	Ligand	M+/ (M+1)/ (M+2) ion	I	II	III	IV	V	VI	VII	VIII
Mass pattern	3a	301	164	150	122	96	108	83	217	191
	3b	328	178	164	136	110	122	97	231	205

The mass spectrum for **3b** shows a much less intense molecular ion(P+1) peak at 328.The fragment peak at 164 due to B (Ar-CH=C(C=O)CH$_2$)$^+$ is the most intense in the mass spectrum.The next intense peak is observed at m/z 110 which is due to fragment C(Ar-CH$^+$).All other peaks in the spectrum can be explained from the fragmentation pattern given in scheme. The mass spectrum of **3b** is given in **Fig.3.1.6.**
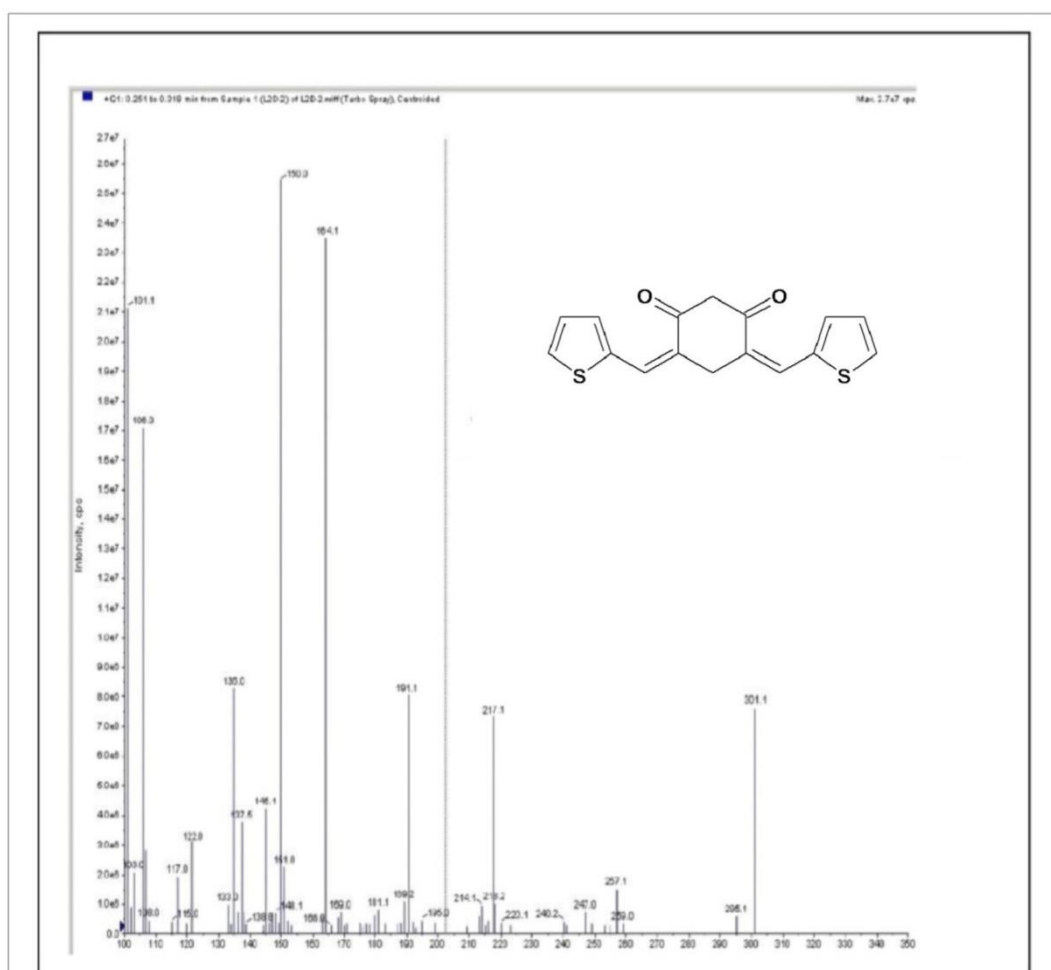
Fig 3.1.5 Mass spectrum of 3a.
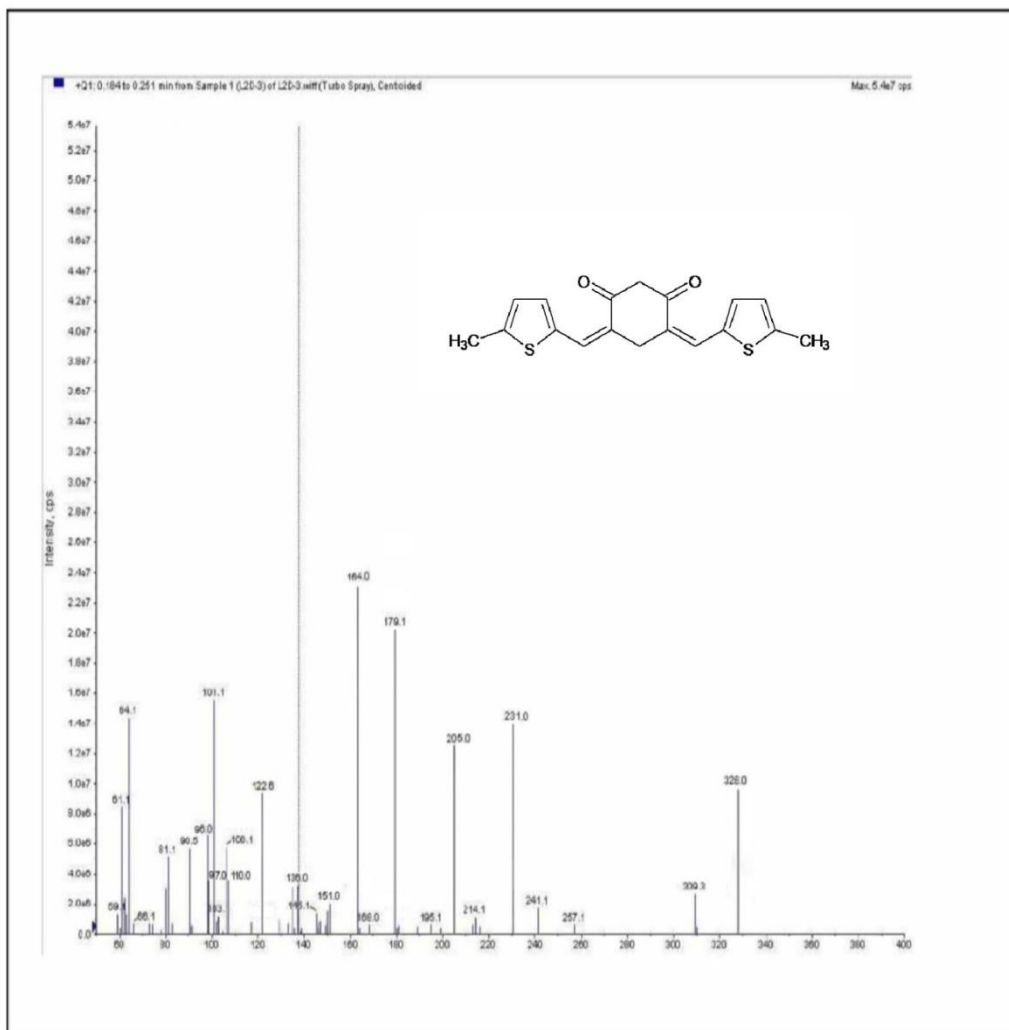
Fig 3.1.6 Mass spectrum of 3b.

All the the characterization by UV, IR, NMR, mass spectra and TG analysis support the structure of the compounds as
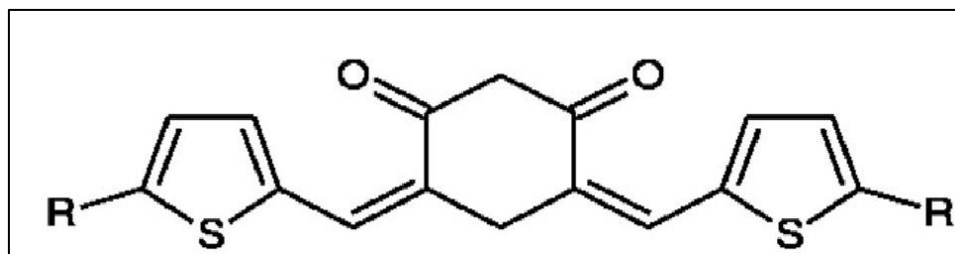
Thermogravimetric analysis

Thermogravimetric analysis of (4E,6E)-4,6-bis(aryl)cyclohexane-1,3-diones (**Table 3.1.8**) provides some significant results about the configuration of molecules. The compound **3a** displays a one stage decomposition array as shown in Fig.3.1.8. The compound is steady up to a temperature of 200°C and then decomposition begins slowly with a sharp drop in the mass up to a temperature of 410°C. The peak temperature in DTG is found at 274°C and the sample was heatedfrom 40^{0}C to a temperature of 730°C.

Table 3.1.8   Thermogravimetric studies of 3a & 3b.

Compound (mol. mass)	Temp. range in TG (^{o}C)	Peak Temp. (^{o}C)	Mass loss %		Pyrolysis %
			TG	Theoretical	
3a (300)	200-410	274	60.04	63.13	71.62
3b (328)	190-380	312	57.52	58.82	74.59
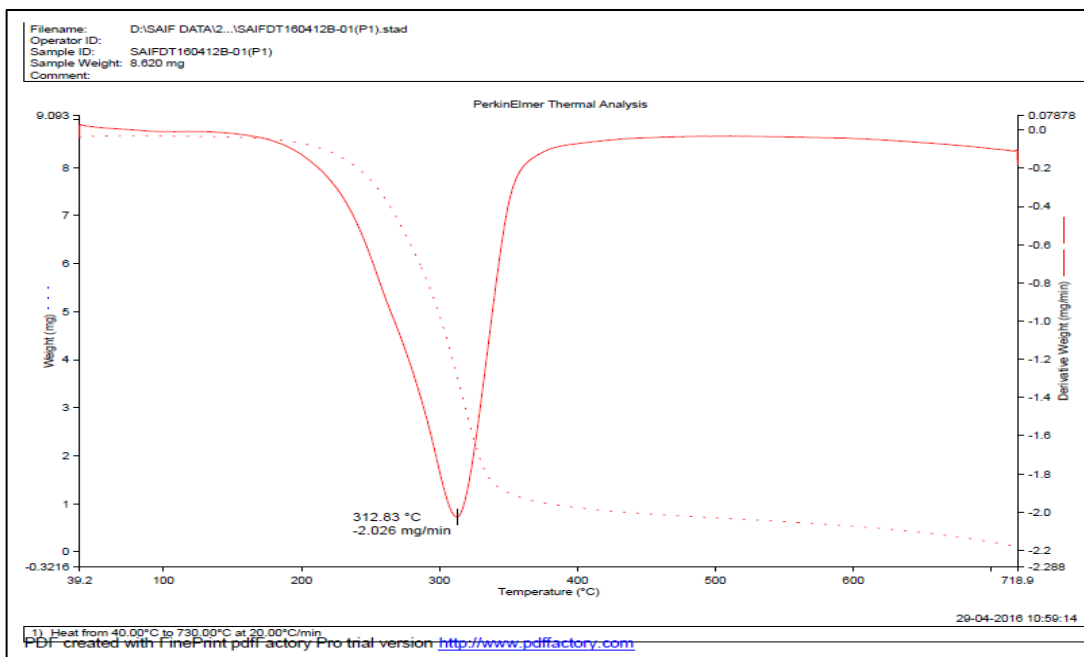
Fig.3.1.7   Thermogravimetric studies of 3a.
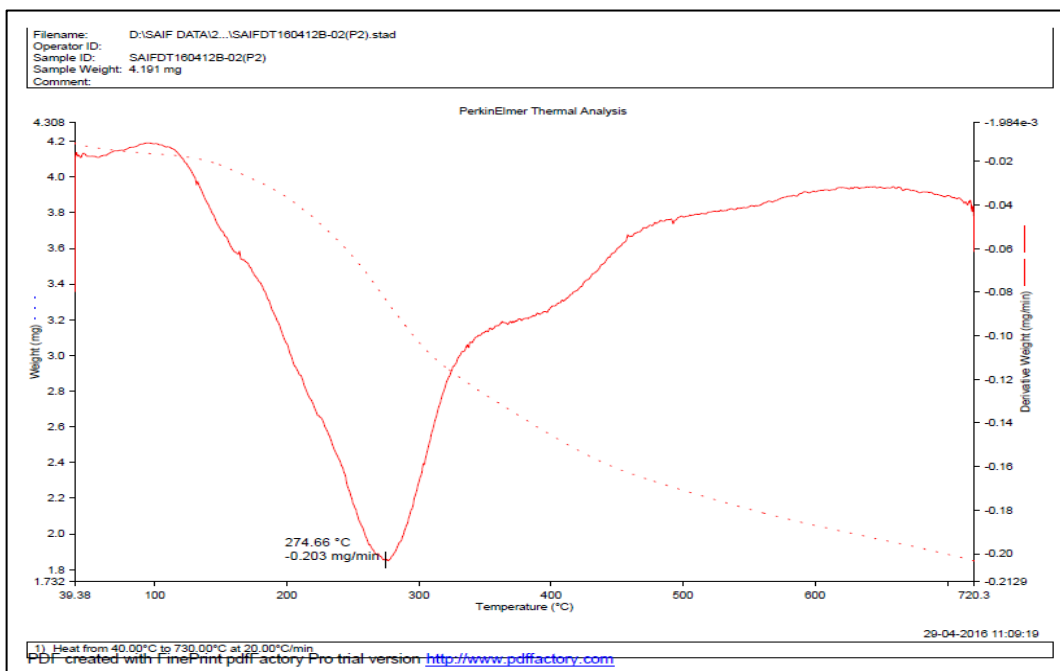
Fig.3.1.8 Thermogravimetric studies of 3b.

115

SYNTHESIS AND CHARACTERISATION OF TRANSITION METAL CHELATES THIOPHENE DERIVATIVE CYCLOHEXANE -1,3 DIONES

3.2.1 Synthesis of metal complexes of (4E,6E)– 4,6 -bis(aryl) cyclohexane 1,3 diones

Metal complexes of compounds **3a** and **3b** were synthesized by refluxing solution of diketone (0.002mol) in methanol (25ml), an aqueous solution of metal salt like copper (II) acetate, zinc (II) acetate and nickel (II) acetate (25 ml, 0.001mol) was added and the reaction mixture was refluxed for nearly 4 hr and cooled to room temperature. The precipitated complex recrystallized from hot methanol.

3.2.2 Characterization of metal complexes of ligands 3a and 3b

The physical and analytical datas are given in **Table 3.2.1** and **Table 3.2.2** respectively.

Table 3.2.1 Analytical and spectral data of metal complexes of 3a

Metal	M.P. (^{0}C)	Elemental analysis Found/(Calculated)			Electronic λ max (nm)	IR frequencies(cm^{-1})		
		C	H	M		(C=O)	(C-C-C)	(M-O)
Cu(II)	148	56.44 (57.87)	3.29 (3.6)	9.11 (9.7)	265 364	1616	1534	466 453
Zn(II)	153	57.71 (58.29)	3.49 (3.4)	8.77 (8.9)	268 363	1513	1527	470 443
Ni(II)	157	56.81 (57.71)	3.49 (3.60)	9.17 (9.2)	261 366	1588	1521	467 456

3.2.3 Spectral details

Electronic spectra

After the complexation when the electronic spectrum of the complex was compared with that of ligand there was not much difference in the absorption peaks. That is the complex formation did not alter the structure of the ligands. The association of carbonyl moiety in chelate formation can be detected from bathochromic shift of 265nm (π-π^*) and 364nm (n-π^*) of absorption maxima to longer wavelength.

Table 3.2.2 Analytical and spectral data of metal complexes of 3b.

Metal	M.P (^{0}C)	Elemental analysis(%) Found/(calculated)			Electronic λmax (nm)	IR frequencies (cm$^{-1)}$		
		C	H	M		(C=O)	(C-C-C)	(M-O)
Cu(II)	191	59.02 (60.3)	4.31 (4.47)	8.12 (8.81)	271 374	1601	1521	470 450
Ni(II)	194	59.97 (60.50)	4.03 (4.48)	7.97 (8.21)	273 371	1598	1527	461 447
Zn(II)	197	58.83 (59.88)	4.22 (4.43)	8.96 (9.06)	275 377	1583	1518	467 451

IR spectra

When a complex is formed from free ligand its enolic proton is lost and it is replaced by metal ion. There is so much evidence for the participation of carbonyl carbon in complex formation .First one was the stretching bands of H- bonded carbonyl peak occurring in the region of 1641cm^{-1} in 3a and 1660cm^{-1} in 3b. It is lowered to

1616cm^{-1} in 3a and 1601cm^{-1} in **3b**. The second reason is that there is a medium intensity bands of metal oxygen bond depicted in the region of 420-480cm^{-1}.
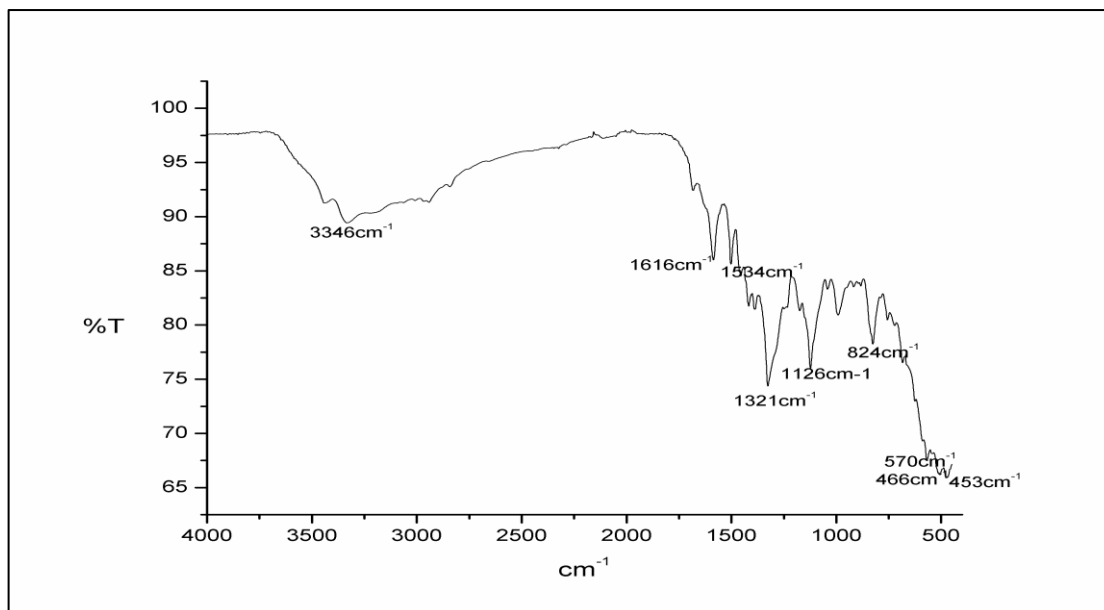
Fig.3.2.1 IR spectrum of Cu (II) complex of 3a.
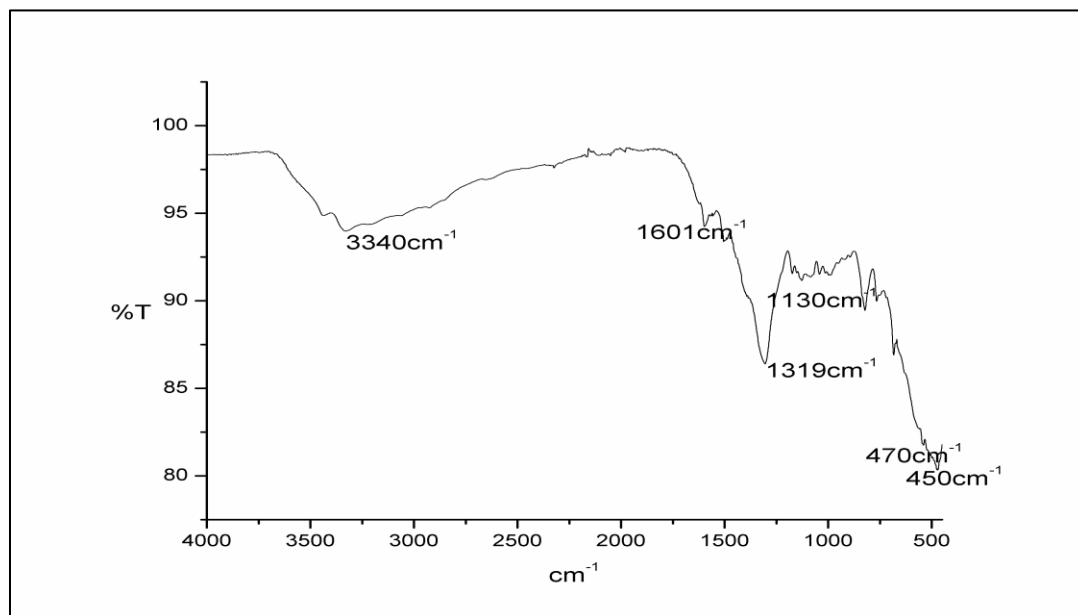
Fig. 3.2.2 IR spectrum of 3b.

NMR spectral details

Enolic proton in the ligand is supposed to be responsible for the peak at 16 ppm, but after the complex formation it is completely absent from the spectrum. The main reason for this is the replacement of enolic proton by a transition metal. Whereas the other two protons namely phenyl and alkenyl do not take part in complex formation, so their peak doesn't show much changed in spectrum and also methine signals showed a downfield shift. Thus in total both the ligand spectra and complex spectra were almost identical and the only difference is in the case of enolic proton. The ^{1}H NMR spectra of Zn (II) complex of ligand **3a** is depicted in **Fig 3.2.3.**
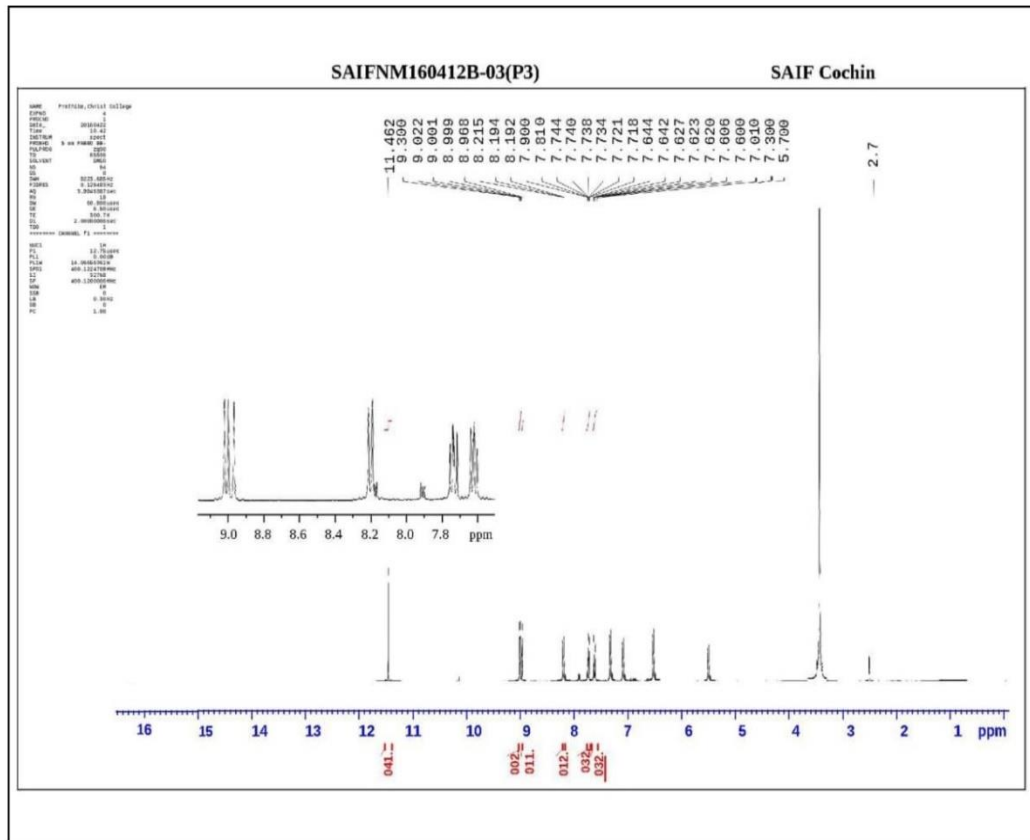
Fig. 3.2.3 ^{1}H NMR spectrum of Zn(II) complex of 3b.

Mass spectra

In their mass spectra, all the complexes showed relatively intense peaks at m/z corresponding to [ML$_2$] stoichiometry, where M is metal and L is ligand. Mass spectral fragments are another important tool in elucidating the structure of metal complexes. In all the cases [ML$_2$]+ ion peak, the molecular ion peak is found. The mass spectral analysis shows that stepwise removal of aryl groups is a characteristic feature of all the complexes. Smaller molecules like O, OH, CH etc. are also eliminated.Peaks due to [ML$_2$]$^+$,[L]$^+$ and fragments of [L]$^+$ are also detected in the spectrum. The fragmental patterns of the metal chelates **3a** &**3b** can be identified from **Scheme 3.2.1.** which is given below.
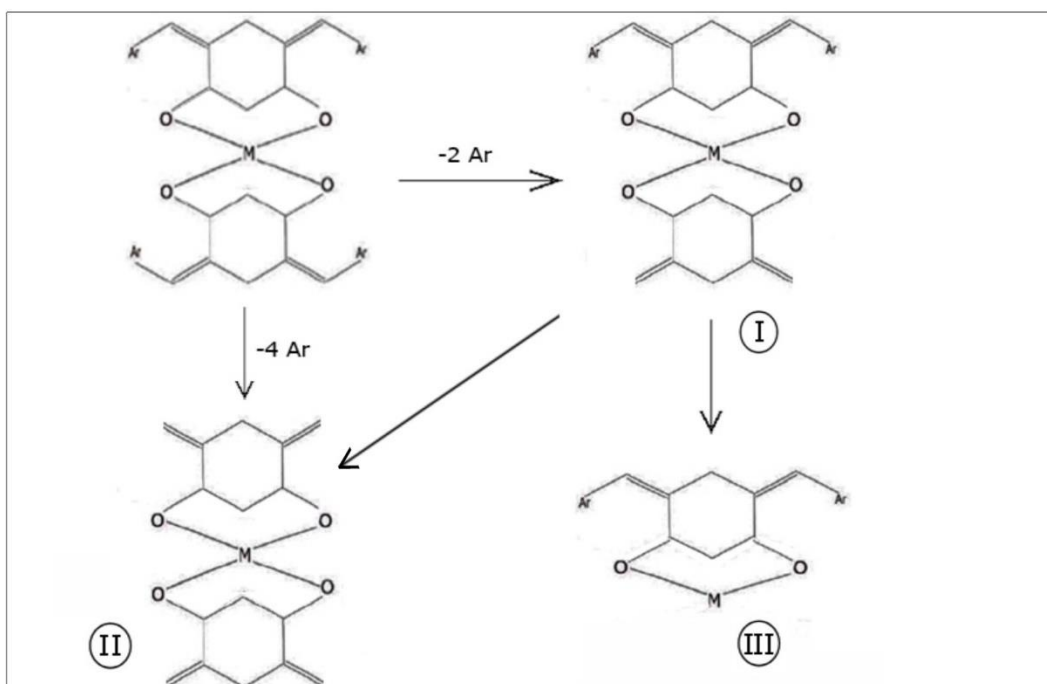
Scheme   3.2.1.

In the mass spectrum of Zn(II) complex of (4E,6E) -4,6-bis (5-methyl thiophene-2-yl methylidene) cyclohexane -1,3 dione (**3b)** a less intense M+ peak is observed at 719. The peak at 499 is due to the fragment ion formed by the removal of 2 Ar groups from molecular ion (Ar is 5-methylthiophenyl).The peak due to the ligand is observed at 326. The peak at 283 is due to the removal of one S and CH group from ligand and the base peak at 238 is due to the removal of the second S and CH group.This is depicted in **Fig 3.2.4.**

The observed UV, IR, ^{1}H NMR and mass spectral data of the metal complexes strongly supports the scheme of reaction as
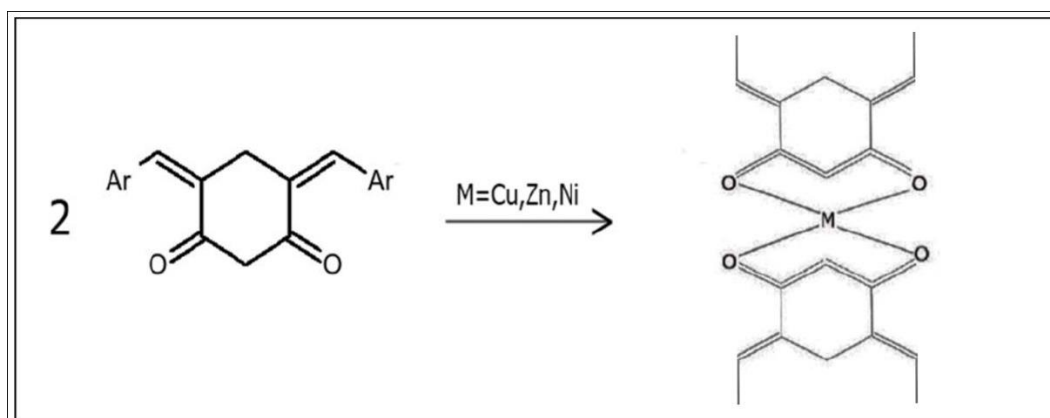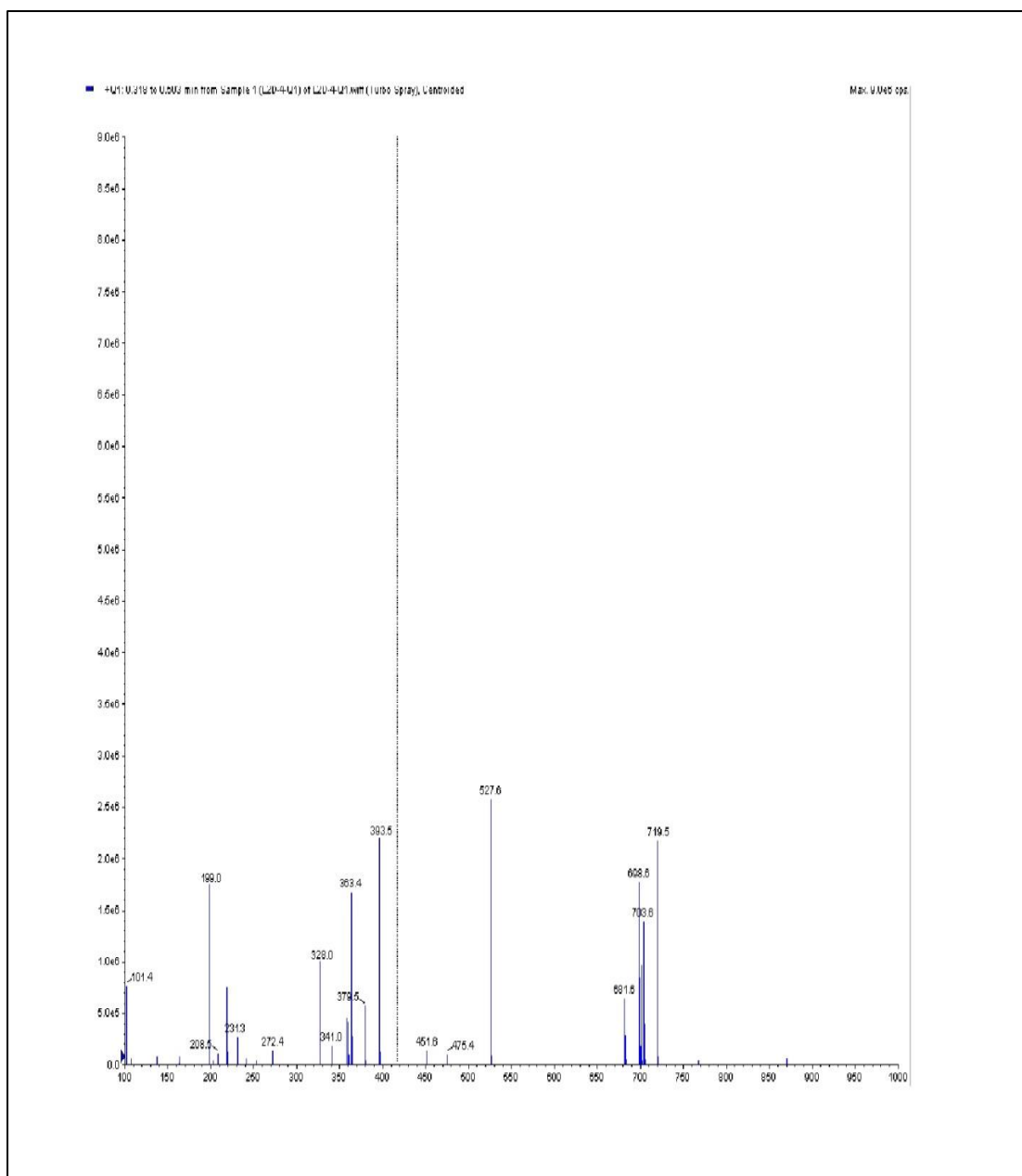
Fig. 3.2.4 Mass spectrum of Zn(II) complex of 3b.

SECTION – III

INVIVO AND INVITRO CYTOTOXIC AND ANTITUMOUR ACTIVITIES OF (4E,6E) -4,6-BIS (THIOPHENE-2-YL METHYLIDENE)CYCLOHEXANE -1,3 DIONE AND (4E,6E) -4,6-BIS (4-METHYL THIOPHENE-2-YL METHYLIDENE)CYCLOHEXANE -1,3 DIONE AND THEIR TRANSITION METAL COMPLEXES.

3.3.1 Cytotoxic studies.

Section III deals with the cytotoxic and antitumor activities of **3a** and **3b** and their metal chelates of Cu (II), Zn (II) &Ni (II) complexes. The curcuminiod analogues were tested for invitro cytotoxicity by means of Daltons Lymphoma Ascites (DLA) &Ehrlich's Ascites Carcinoma (EAC). The antitumor activity of test compounds was conducted both in ascites form and solid form of tumour and the result is compared with that of standard anticancer drug cyclophosphamide.

3.3.2 In vitro cytotoxic studies of 3a and their metal chelates against EAC cells

In vitro cytotoxic test was conducted by the curcuminiod analogues of different concentrations against EAC. The outcome of the study is specified in **Table 3.3.1** and represented graphically in **Fig 3.3.1.** and **Fig 3.3.2.**

Table 3.3.1 In vitro studies of 3a and their metal chelates against EAC & DLA

Concentration of drug	%cell death EAC				%cell death DLA			
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$
200	42	93	86	77	39	90	81	73
100	34	79	77	64	24	77	73	64
50	21	67	57	44	13	63	57	46
20	11	44	41	39	9	47	41	36
10	7	36	29	19	4	33	29	21
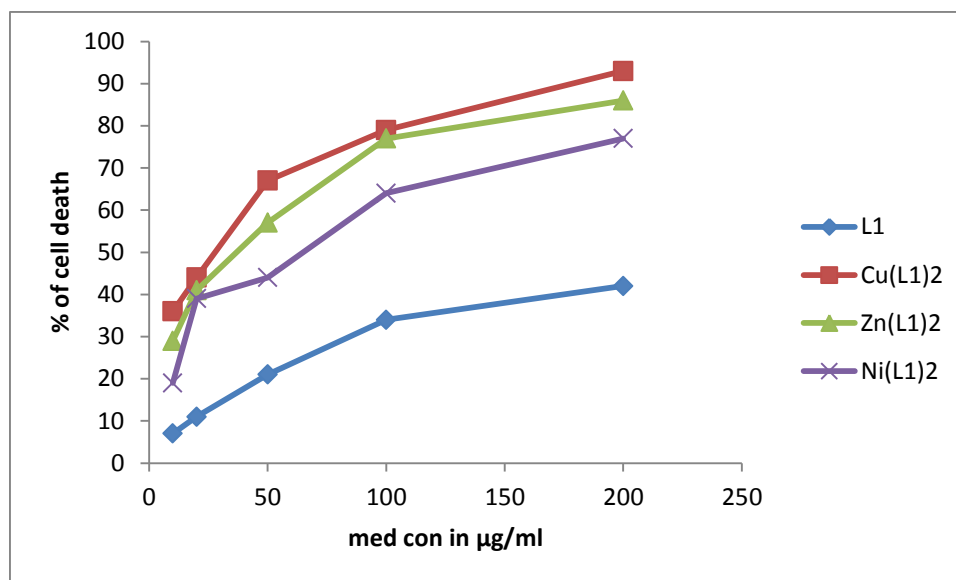
Fig.3.3.1 In vitro cytotoxic studies of 3a and their metal chelates against EAC cells.

At a particular concentration say 200μg/ml the test ligand made 42% cell death against EAC. It is proven that cytotoxic activity clearly depends on the concentration of the compounds, as it showed at lesser concentration it displays minimum level of activity. It could be concluded from this result that metal chelation increases cytotoxic activity of any ligand. Metal complexes showed reasonable activity even in their lower concentrations. Like the ligand metal complex also showed greater activity at higher concentration. In effect, Cu (II) complex of test compound produce 93% of cell death to EAC cells. Amongst the metal complexes, Ni complex display minimal activity towards EAC.

3.3.3 In vitro cytotoxic studies of 3aand their metal complexes towards DLA cells.

Associating (4E,6E)-4,6 bis (thiophene-2-yl methylidene)cyclohexane -1,3 dione(3a) and its metal complexes ,it can see that all the metal chelates are more cytotoxic than the ligand. Amongst the metal complexes,Cu(II) shown greater cytotoxicity to DLA cells. All compounds possessed extreme activity at higher concentations namely 200μg/ml. When the concentration of compounds increases the % of cell death also increases.That is concentration controls activity of reaction.For a particular concentration namely 200μg/ml the % of cytotoxicity of (4E,6E)-4,6 bis(thiophene-2-yl methylidene)cyclohexane -1,3 dione(3a) and its Cu(II),Zn(II), & Ni(II) complexes

were 39,90,81,& 74 correspondingly. The activity of copper complex was nearly four times than that of the ligand.
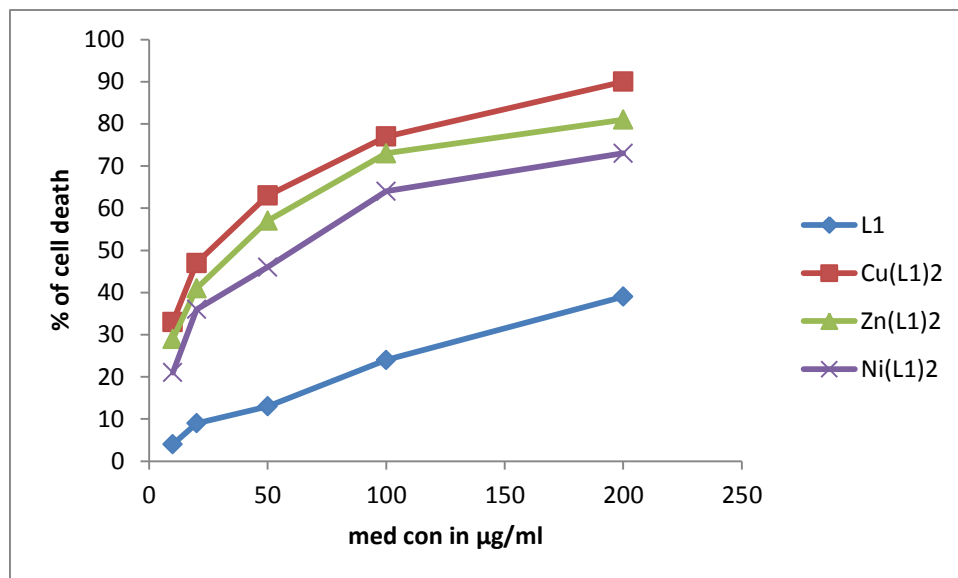
Fig.3.3.2 In vitro cytotoxic studies of 3a and their metal chelates against DLA.

3.3.4 In vitro cytotoxic studies of (4E,6E) -4,6-bis (5-methyl thiophene-2-yl methylidene) cyclohexane -1,3 dione (3b) and their metal complexes towards EAC

The invivo cytotoxic activities of synthetic diketones were analysed with EAC cell lines. The remarks were given in **Table 3.3.2** and sketchily in **Fig 3.3.3.** The diketone (3b) which possess a methyl group related to the diketone (3a) showed less significant activity with EAC cells. But here also the metal chelation considerably increases the cytotoxicity activity of compounds. But the methyl substituted complexes was found to be less effective than the other complexes. It was observed that the activity of copper complex was approximately 77% for methyl substituted diketones and not as much of copper complex of **3a.**

Table 3.3.2 In vitro studies of 3b and their metal chelates against EAC & DLA

Concentration of drug µg/ml	%cell death to EAC				%cell death to DLA			
	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$
200	38	77	73	71	33	76	71	63
100	23	75	62	59	20	63	61	53
50	12	61	47	41	11	57	43	42
20	8	44	33	24	7	31	28	21
10	3	29	16	9	2	16	13	7
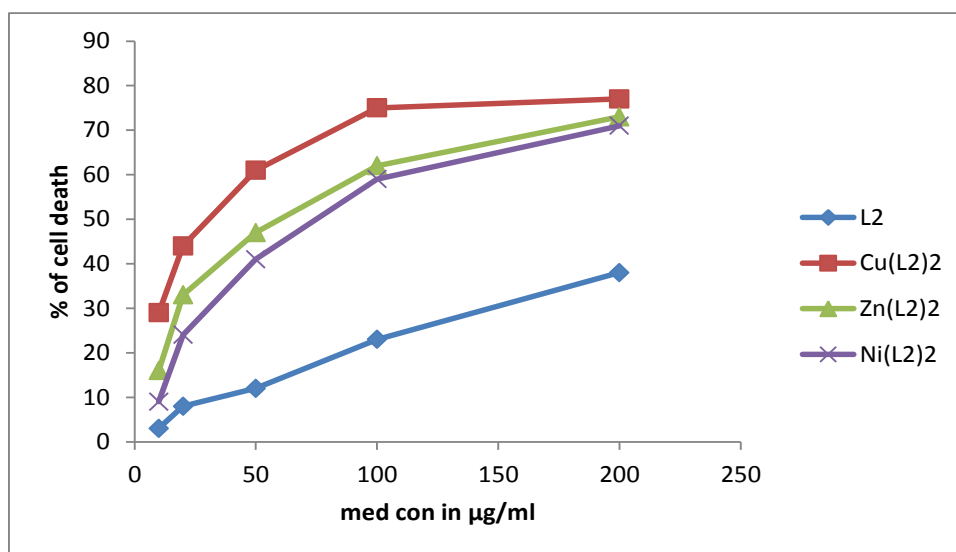
Fig 3.3.3 In vitro cytotoxic studies of 3b and their metal chelates against EAC.

3.3.5 In vitro cytotoxic studies of 3b and their metal chelates against DLA cells

Among the eight tested compounds, methyl substituted analogues (3b) has nominal activity against DLA cells compared to ligand 3a and it were shown in **Table 3.3.2** and depicted graphically in **Fig 3.3.4.**In all these cases very low level activity observed at lower concentration ie. At 10µg/ml but as the concentration increases gradually there is significant change in the cytotoxicity.
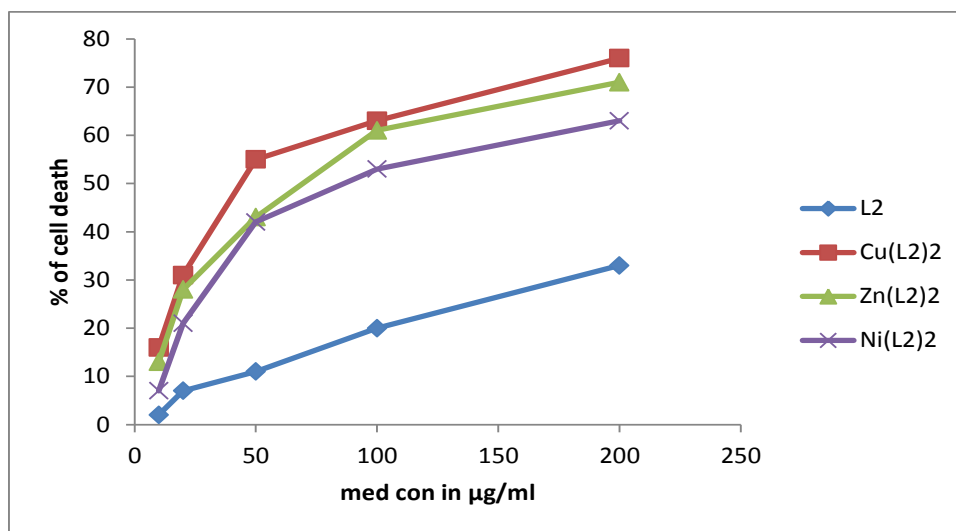
Fig. 3.3.4 In vitro cytotoxic studies of 3b and their metal chelates against DLA.

Inference

Relative study of test compounds, indicate that the methyl associated compounds show less activity than the unsubstituted heterocyclic analogues.Cu (II) complex of ligand **3a** were found to be effective in cytotoxic studies.

IN VIVO ANTITUMOUR STUDIES

The result of **3a, 3b** and their Zn(II) complexes on the existence percentage of ascites tumour carrying creatures were examined. Albino mice weighing 18-25 kg were used for the examination. Six animals formed a study group and there were 14 such groups. Ascites tumour was made in these animals by introducing viable EAC cells in 0.1 ml of PBS into the peritoneal cavity. Out of 14 groups, one group was kept as control group which wasn't treated with any drug. It was labeledas group 1.another group was treated with the standard drug,cyclophosphamide and it was labeled as group 2. Three groups were also selected, in which the ligand **3a** was applied having concentrations 20μg/ml,10μg/mland 5μg/ml and they were labeled as group 3,4 & 5 respectively. The Zn(II) complex of **3a** having concentrations 20μg/ml,10μg/mland 5μg/ml was used in the treatment of another three groups and they were named as group 6,7 & 8 respectively.

3.3.6 Effect of 3a and the Zn (II) complex on ascites tumour: The results are shown below in **Table 3.3.3** and graphically on **Fig 3.3.5**
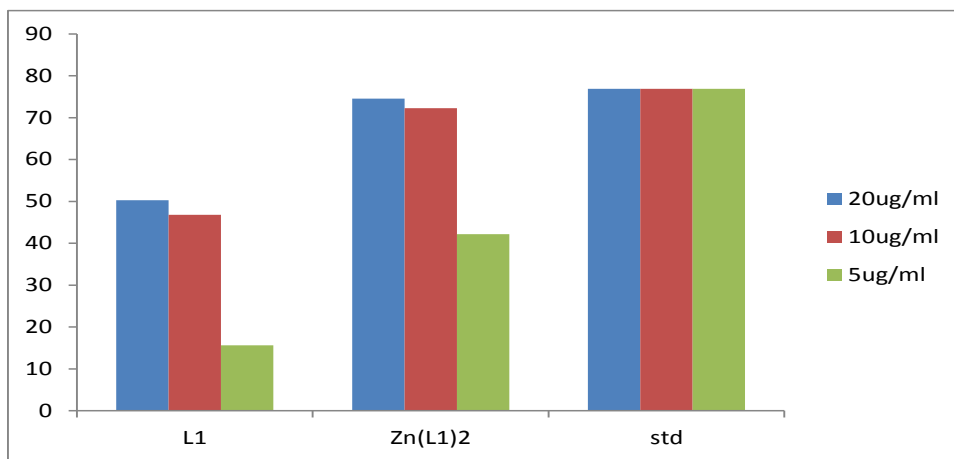
Fig 3.3.5 The % ILS with different concentration of 3a and their Zn (II) metal complex.

Table 3.3.3 Effect of 3a and their Zn(II) complexes on ascites tumour reduction

	Animal group	Concentration μg/ml	No. of animals with tumour	No. of days survived	% ILS
1.	Control		5/5	17.3 ± 1.10	
2.	Standard		5/5	30.6 ± 0.489	76.87
3.	L_1	20	5/5	26.0 ± 04	50.28
4.	L_1	10	5/5	25.4 ± 3.76	46.82
5.	L_1	5	5/5	20.0 ± 5.09	15.60
6.	$Zn(L_1)_2$	20	5/5	30.2 ± 1.04	74.56
7.	$Zn(L_1)_2$	10	5/5	29.8 ± 3.5	72.25
8.	$Zn(L_1)_2$	5	5/5	$24.6.0\pm1.78$	42.19

3.3.7 Effect of 3b and their Zn (II) metal complexes on ascites tumour reduction

All the synthetic analogues having different concentrations made intensification in the life span of ascites tumour carrying mice. The results are shown below in **Table 3.3.4.** and graphically in **Fig. 3.3.6.** The entire text compounds show greater activity at higher concentrations and all the metal complexes which differ in their concentrations act as good drug as they significantly reduce ascites tumour and life span exceeds, and the action comparable with that of standard drug. The death pattern of mice by applying drugs **3a** and **3b** noted and was found to be the unsubstituted thiophene ligand was most effective.

Table 3.3.4 Effect of 3b and their Zn(II) complexes on ascites tumour reduction

	Animal group	Concentration μg/ml	No. of animals with tumour	No. of days survived	% ILS
1.	Control		5/5	17.3 ± 1.10	
2.	Standard		5/5	30.6 ± 0.489	76.87
3.	L_2	20	5/5	26.01 ± 3.7	50.34
4.	L_2	10	5/5	23.4 ± 8.26	35.26
5.	L_2	5	5/5	22.0 ± 2.34	27.16
6.	$Zn(L_2)_2$	20	5/5	29.7 ± 3.04	71.67
7.	$Zn(L_2)_2$	10	5/5	27.8 ± 5.16	60.69
8.	$Zn(L_2)_2$	5	5/5	26.0 ± 3.78	50.28

3.3.8 Effect of synthetic compounds on solid tumour development.

Albino mice were used for solid tumour study by applying a number of synthetic analogues of different concentration. For the study, the right hind limb was selected and tumour was induced by injecting DLA cells. After one day, drug application was initialized and it was continued for a period of 10 days without any break. In order to

examine the effect of the drug, the diameter of the solid tumour was measured using vernier calipers and its volume was calculated. This process was conducted twice a week and a total of 11 readings were obtained. For the current study mice were grouped into 6. One group was preserved only with DLA cells and named as control group. (Group 1). Here the reference group is 2 and it was treated with standard drug namely cyclophosphamide. Group 3 to 6 constitute the test compounds.

Table 3.3.5 Effect of 3a & 3b on solid tumour.

analogues	Tumour volume
Control group	$5.872cm^3$
3a(L_1)	$3.11cm^3$
3b(L_2)	$3.97cm^3$
Zn(L_1)$_2$	$2.21cm^3$
Zn(L_2)$_2$	$2.97cm^3$
Std.group	$1.873cm^3$

It was observed that the solid tumour volume was significantly reduced with the application of all compounds. A significant reduction in the volume of tumour was noticed in the group which was treated with Zinc complex when compared to that of ligand. The tumour volume of the control group was measured as5.872 cm^3, and it were 3.11 cm^3 for **3a** &3.97 cm^3 for **3b** on the 31 ^{rst}day of the experiment. And it is observed that the tumour volume was much lesser in the ligand treated group when compared to the control group. Relating with that of control group, the ligands made a decrease in volume of $1.227cm^3$ and $0.553cm^3$correspondingly.It was observed that **3a** produces more significant reduction in the tumour volume than **3b**. The copper complexes of **3a** and **3b** were showed a tumour volume of $3.53cm^3$ and $3.01cm^3$ in that order. The decrease in tumour volume was $1.643cm^3$and $2.063cm^3$ respectively with respect to control group. Among the metal complexes, the copper complex of **3a** produced greater tumour volume reduction. Plasma copper concentration increases in neoplastic and autoimmune diseases as an immune mediated physiological response to the disease state. Treatment with copper complexes is a therapeutic support of this increase in plasma copper and the attendant distribution of copper to affected tissues to enable de-novo synthesis of copper dependent enzymes required to bring about remission by reestablishing normal tissue function.

ANTIBACTERIAL STUDY OF THIOPHENE DERIVATIVE OF CYCLOHEXANE -1,3 DIONE AND THEIR TRANSITION METAL COMPLEXES.

Antibacterial study of (4E,6E)-4,6-bis(aryl)cyclohexane-1,3-diones and their transition metal complexes Cu(II),Zn(II) & Ni(II)

Agar well diffusion method was adopted for antibacterial study of **3a** and **3b**and their transition metal complexes Cu(II), Zn(II) & Ni(II). The antibacterial studies of analogues were conducted with three bacterial stains namely Escherichia Coli, Klebsiella Pneumoniae & Bacillus Subtilis. The unsubstituted heterocyclic ligand was supreme active than the methyl substituted ligand and the result obtained has a comparable data to that of the reference drug. From the observed data it can be concluded that metal coordination considerably strengthens the antibacterial power of compounds. If greater is the zone of inhibition which is usually stated in mm greater, is the antibacterial activity.

3.4.1 Antibacterial studies of and metal chelates Zn(II),Cu(II) & Ni(II)

The unsubstituted thiophene diketone revealed, equivalent antibacterial activity to Bacillus Subtilis and KlebsiellaPneumoniae species by creating a zone of inhibition of 11 mm. when compared the result with that of streptomycin can see that the activity was merely partial of the activity displayed by std.drug. The investigation result are given below in **Table 3.4.1.**

Table3.4.1 Antibacterial studies of 3a and metal chelates Zn(II),Cu(II) & Ni(II)

Bacteria	Zone of inhibition in mm			
	L_1	$Ni(L_1)_2$	$Zn(L_1)_2$	$Cu(L_1)_2$
E Coli	9.5	19	13	10
Klebsiella	11	15	12	11.5
Bacillus	11	15	11.5	11.5
standard	20	20	20	20
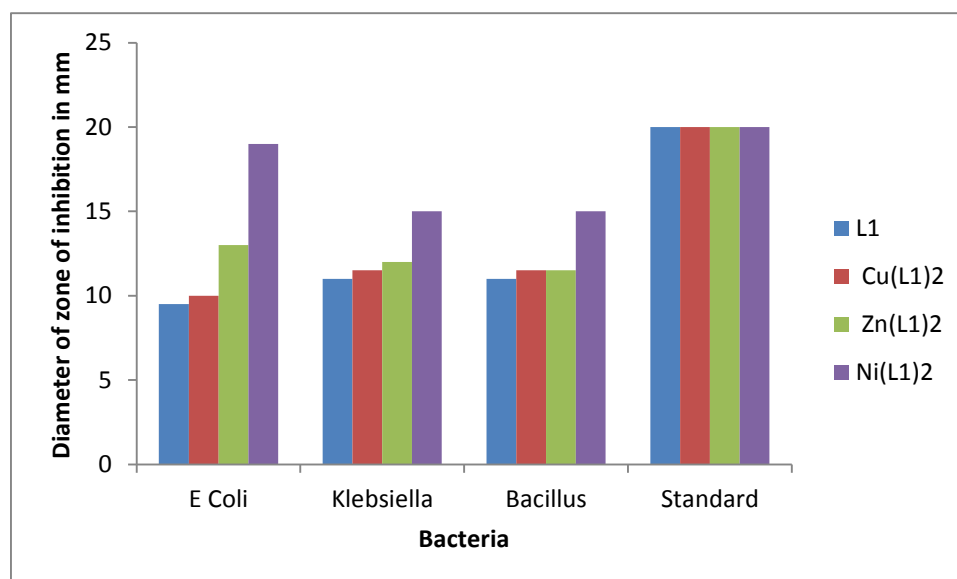
Fig.3.4.1 Antibacterial studies of 3a and metal chelates Zn(II),Cu(II) & Ni(II).

All complexes produced inhibitory actions to all the bacterial creatures and were extra active than the ligand. Towards Ecoli species the ligand **3a** shows a zone of inhibition of 9.5 mm.But its Ni (II) complex showed extreme activity against E.coli species and made a zone of inhibition of 19 mm which is as good as the activity of reference drug, streptomycin.

3.4.2 The antibacterial analysis of 3b and metal chelates Zn(II),Cu(II) & Ni(II)

On comparing the acitivity studies of **3b** it has been found that, methyl substituted thophene ligand displayed more antibacterial activity towards E coli bacterial strains.

Table 3.4.2 Antibacterial studies of 3b and metal chelates Zn(II),Cu(II) & Ni(II)

Bacteria	Zone of inhibition in mm			
	L_2	$Ni(L_2)_2$	$Zn(L_2)_2$	$Cu(L_2)_2$
E Coli	11.5	18.5	14.4	12.3
Klebsiella	10	14.5	12	11.5
Bacillus	9.5	16.1	13.4	11.5
standard	20	20	20	20
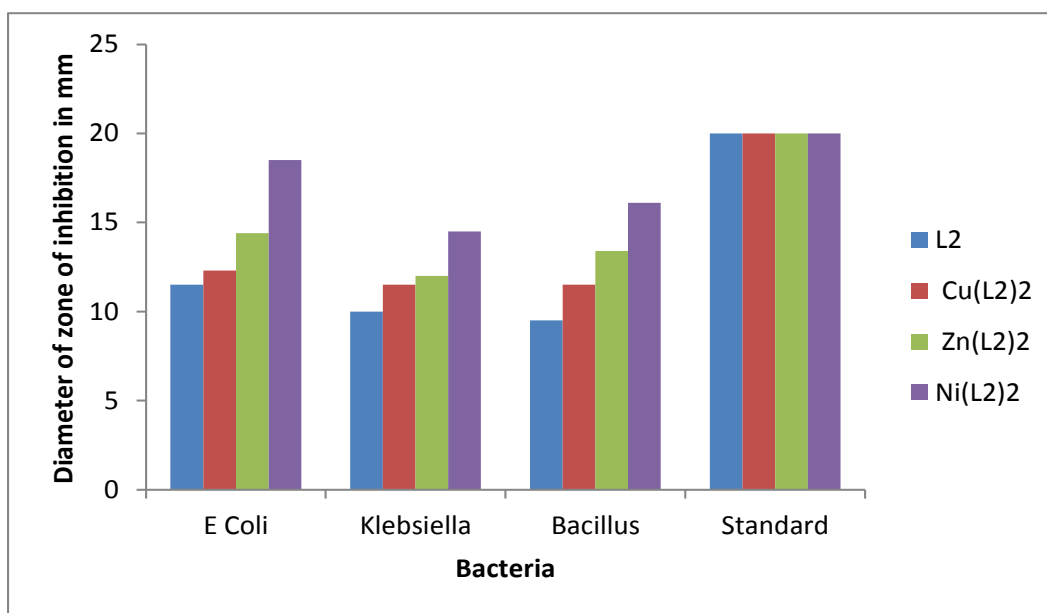
Fig.3.4.2 Antibacterial studies of 3b and metal chelates Zn(II),Cu(II) & Ni(II).

SECTION V

ANTIFUNGAL STUDY OF OF THIOPHENE DERIVATIVE OF CYCLOHEXANE -1,3 DIONE AND THEIR TRANSITION METAL COMPLEXES.

Antifungal study of ligand of (4E,6E) -4,6-bis(aryl)cyclohexane -1,3 diones and their Zn(II) and Ni(II) complexes.

To study the antifungal properties of test compounds three fungal cultures were used and the analysis was done by Kirby Baurer disc plate technique. The synthetic thiophene ligands and their metal complexes were prepared in DMSO with variable concentrations to evaluate their property against Alternaria Alternate, Aspergillius Niger and PencilliumChrysogenum.

3.5.1 Antifungal activity of 3a and their Zn(II) and Ni(II) complexes.

The compound **3a** showed reasonable antifungal activity towards the three fungi at a concentration of 100μg/disc. Metal chelation of ligand **3a** increased its antifungal activity towards a maximum. Among the complexes, Ni(II) possess greatest antifungal activity at all the concentrations. The end result discloses that the test drug has a comparable zone of inhibition with that of reference drug.

Table 3.5.1 Antifungal studies of 3a and their Zn (II) and Ni (II) complexes.

Fungi	Diameter of zone of inhibition in mm								
	L_1			$Zn(L_1)_2$			$Ni(L_1)_2$		
	100 μg	250 μg	500 μg	100 μg	250 μg	500 μg	100 μg	250 μg	500 μg
Aspergilus	9	12	14.5	10.5	14	16	11.5	14.5	19
Pencillium	10.5	12.5	16	12	14.5	17	13	15	18.5
Alternaria	10	13.5	17	11	15	18	12	15.5	19.5
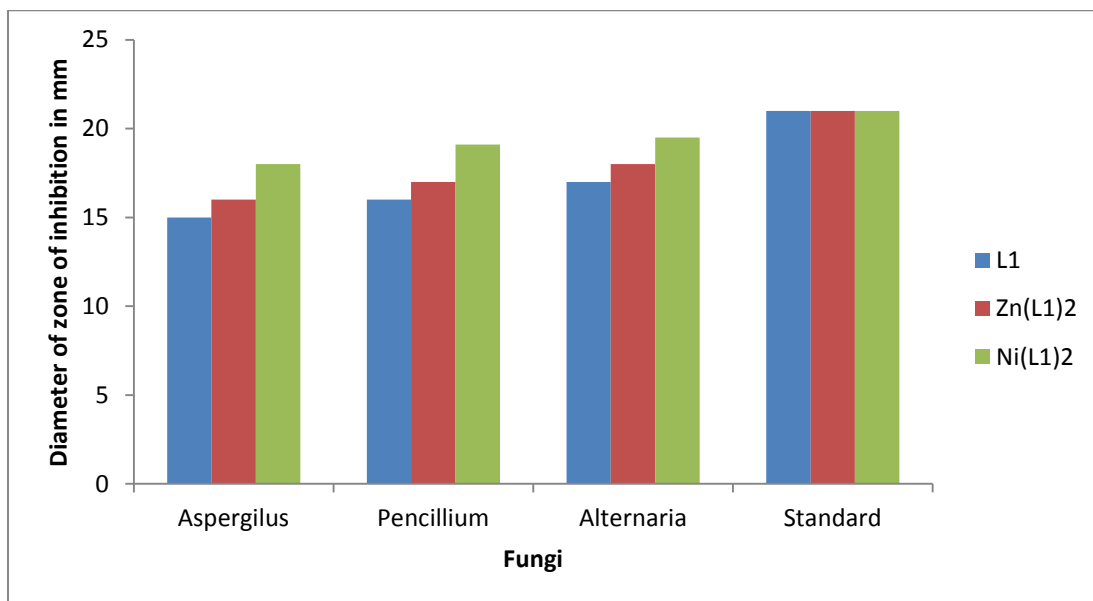
Fig. 3.5.1 Antifungal studies of 3a and their Zn(II) and Ni(II) complexes.

3.5.2The Antifungal studies of 3b and their Zn(II) and Ni(II) complexes.

The peak level of antifungal properties of the methyl derivative of analogues was observed at the concentration of 500μg/ml.From this it is right to conclude that there is a positive response in the antifungal activities against higher concentration of the compounds. Among the synthetic ligands,methyl derivative of ligand was found to be powerful antifungal agent and thiophene derivative was least active compounds.

Table 3.5.2 Antifungal studies of 3b and their Zn(II) and Ni(II) complexes.

Fungi	Diameter of zone of inhibition in mm								
	L_2			$Zn(L_2)_2$			$Ni(L_2)_2$		
	100 µg	250 µg	500 µg	100 µg	250 µg	500 µg	100 µg	250 µg	500 µg
Aspergilus	9.5	12.5	15	10.5	12.7	16	11.2	14.3	18
Pencillium	11	13	16	12	15.2	17	14	18.4	19.1
Alternaria	12	14	17	13	15.2	18	16	18.5	19.5
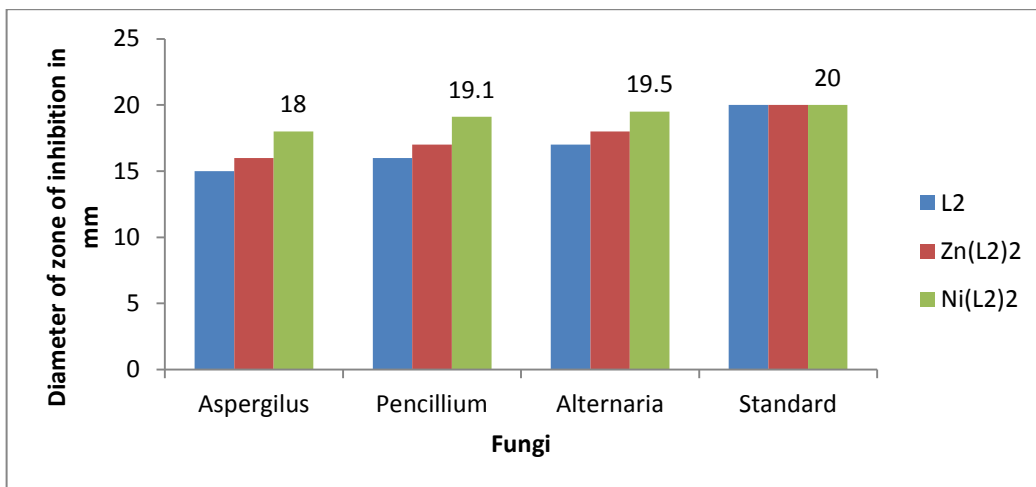
Fig 3.5.2 Antifungal studies of 3b and their Zn(II) and Ni(II) complexes.

CHAPTER IV

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES OF (4E,6E)-4,6-BIS(POLY NUCLEAR) CYCLOHEXANE - 1, 3 –DIONES AND THEIR METAL CHELATES.

INTRODUCTION

This chapter discusses the synthesis and characterization of curcuminoid analogues with naphthyl, substituted naphthyl and anthracenyl rings rather than phenyl rings in natural curcuminoids. In section 1 synthetic details of the ligands,(4E,6E)-4,6-bis(naphthalene-ylmethylidene) cyclohexane -1,3 dione (4a), (4E,6E)-4,6-bis (methoxynaphthalene-ylm ethylidene) cyclohexane -1,3 dione (4b), (4E,6E)-4,6-bis (hydroxynaphthalene-ylm ethylidene) cyclohexane -1,3 dione (4c) &(4E,6E)-4,6-bis (Anthraceneyl-methylidene) cyclohexane -1,3 dione (4d). Section II describes the synthesis and characterization of metal complexes of the previous ligands with transition metal ions Cu (II), Zn (II) and Ni (II) .Section III refers to the invitro and invivo antitumour activity of the couple, ligands and metal complexes. Section IV illustrates the antibacterial activity of the synthesized compounds. Section V describes the antifungal activity of both ligands and metal complexes structures. Addition of polynuclear ring structure in the α, β unsaturated diketone component changes the chemical and biochemical stuffs of the compounds.
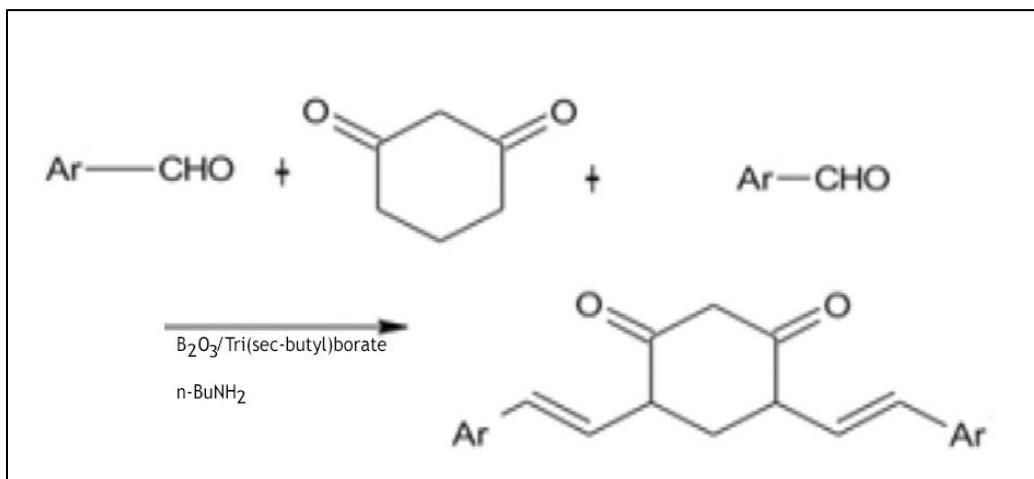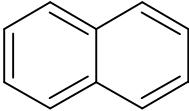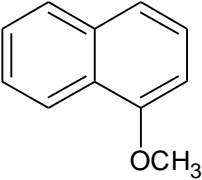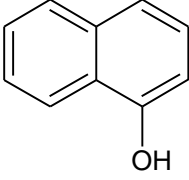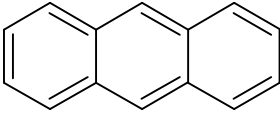
Scheme 4.1.1.

SECTION I

4.1.1. Synthesis of substituted derivatives of (4E, 6E) -4,6- bis (polynuclear) cyclohexane -1,3- diones.

The curcuminoid analogues based on polynuclear ring were prepared by the reaction of aldehydes (1-naphthaldehyde, 4-methoxynaphthaldehyde, 4-hydroxynaphthalehyde & Anthracene-1- carboxaldehyde) with cyclohexane-1,3 dione-boric oxide complex. The solvent used here was ethyl acetate and the condensation is carried out in the presence of tri-butyl borate and n-butyl amine.

Compound	Ar
4a	
4b	OCH$_3$
4c	OH
4d	

The starting aldehyde, structure of synthetic analogues, its IUPAC name & yield has been enclosed in the table given below in **Table 4.1.1**. The synthesized compounds are brown coloured crystalline powder and their %yield was nearly 72. All these three ligands were almost soluble in organic solvents like DMSO,acetone, chloroform ethyl

acetate etc. The analytical and physical data of 4a, 4b, 4c and 4d were shown in **Table 4.1.2.**

Table 4.1.1 synthetic details of(4E,6E)-4,6-bis((aryl) cyclohexane -1,3 diones
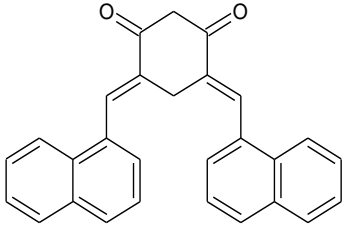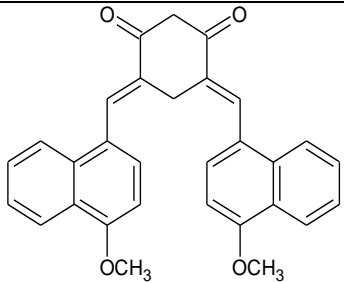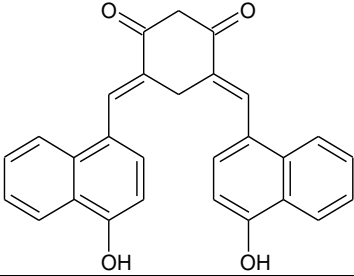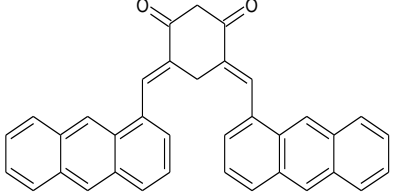
	Starting aldehyde	Structureof synthetic analogue	Analogues systematic name	Yield (%)
4a	1- Naphthaldehyde		(4E,6E)-4,6-bis(naphthalene-ylm ethylidene)cyclohex ane -1,3- dione	67
4b	4-methoxy naphthaldehyde		(4E,6E)-4,6-bis (methoxy naphthalene-ylm ethylidene)cyclohex ane -1,3- dione	72
4c	4-hydroxy naphthaldehyde		(4E,6E)-4,6-bis (hydroxy naphthalene-ylm ethylidene)cyclohex ane -1,3- dione	64
4d	Anthracene-1-carboxaldehyde		(4E,6E)-4,6-bis(Anthraceneyl-methylidene)cyclohe xane -1,3- dione	87

Table 4.1.2 Analytical and physical data of polynuclear analogues.

combinations	MP.(^{0}C)	Elemental analysis		Electronic λ max (nm)
		C	H	
		Found/ (calculated)		
4a	172	85.89 (86.59)	5.07 (5.15)	273 307
4b	184	79.92 (80.35)	4.03 (5.35)	277 309
4c	186	79.48 (80.00)	4.31 (4.76)	293 334
4d	194	87.31 (88.00)	4.08 (4.91)	293 413

4.1.2. Spectral Characterisation of curcuminiod polynuclear analogues.

The curcuminoid analogues **4a**, **4b**, **4c** and **4d** were synthesized and described through several spectral procedures like electronic, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral techniques.

Electronic spectra

The more intense transition takes place at 293 nm and weak transition take place at 413 nm. The n→$\pi*$ absorption of carbonyl chromophore takes place at greater wavelength and the existence of α, β −unsaturation too, raises the wavelength of carbonyl absorption maximum.

IR spectra

IR spectra of **4a**, **4b**, **4c** & **4d** are described by the presence of strong bands at 1634cm^{-1} and 1617 cm^{-1} respectively due to enolised conjugated C=O group. The hydrogen bonding and better conjugation in the compound causes the C=O frequency decreases.Furthher more it shows that there is no other peak in the range of 1600-1800cm^{-1}. This clearly suggests that the compound be existent in the intramolecularly

H- bonded enolic form.The significant IR values and their possible assignments are specified in **Table.4.1.3.**

Table 4.1.3   Infrared spectral details of naphthyl analogues.

Synthetic analogues				Feasible IR specifications
4a	**4b**	**4c**	**4d**	
1614	1657	1634	1639	V(C=O) chelated
1585	1599	1593	1553	V(C=C) phenyl
1537	1532	1537	1532	V(C-C) alkenyl
1501	1515	1514	1501	V_{as}(C-C-C) chelate ring
1439	1434	1475	1414	V_s(C-C-C) chelate ring
1137,1085	1125,1046	1133,1074	1143,1023	β (C-H) chelate ring
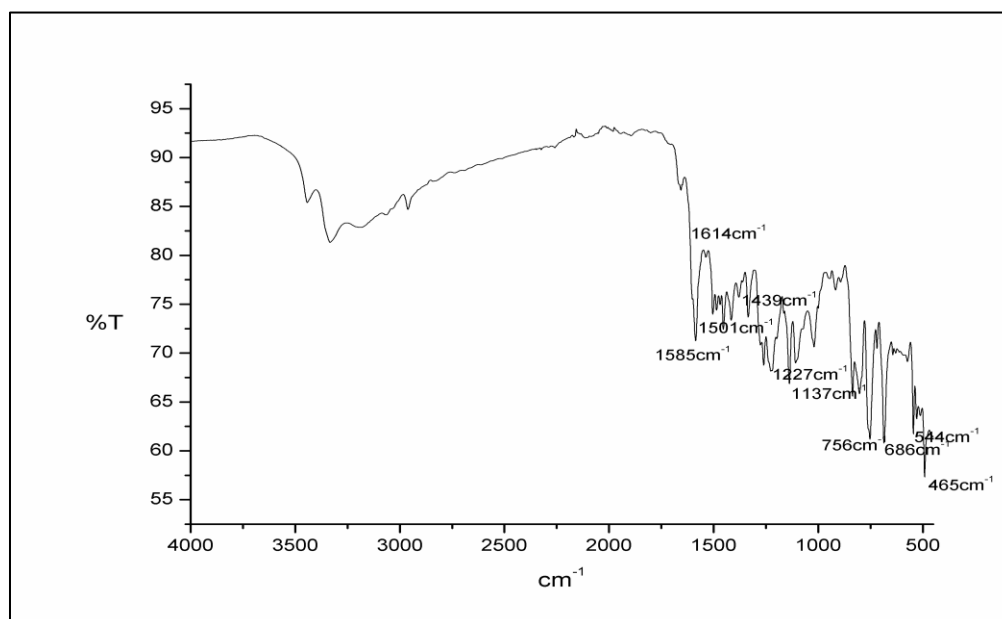
Fig.4.1.1 IR spectrum of 4a.
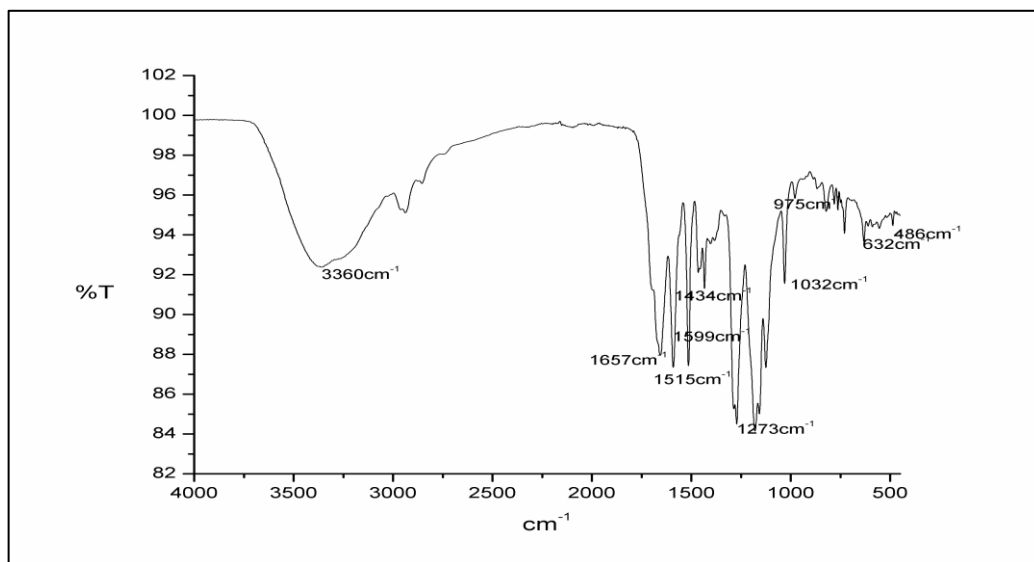
Fig.4.1.2 IR spectrum of 4b.
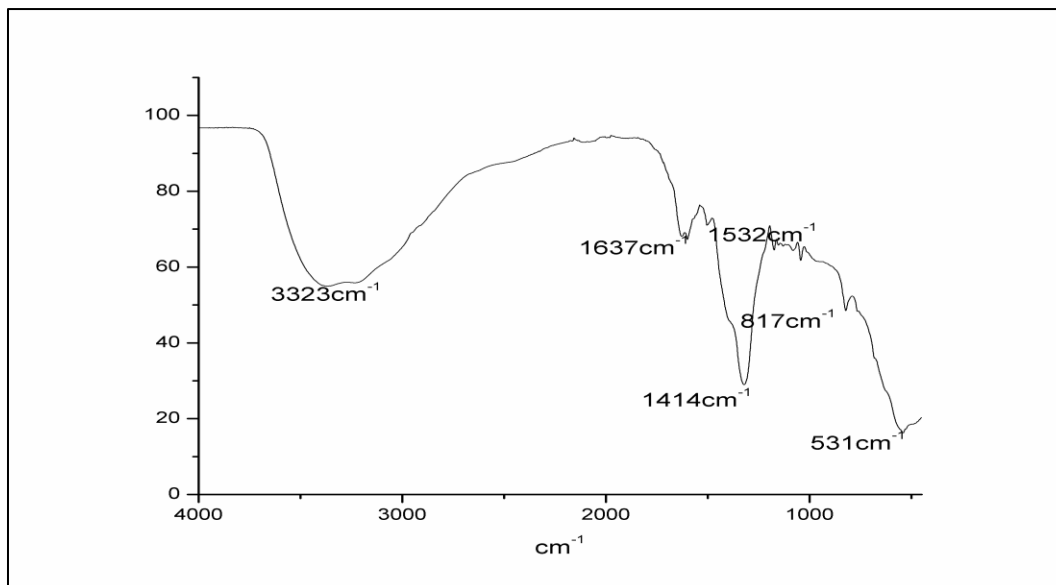
Fig.4.1.3. IR spectrum of 4d.

^{1}H NMR spectra

After the NMR analysis of all synthetic analogues, it should be understood that one enolic proton and one methine proton are present in these compounds. The enolic was proton present at 16ppm and methine proton was present at 5.90 ppm.The ^{1}H NMR spectra of all compounds show specific peaks corresponding to hydroxyl, enolic, phenyl, methine, alkenyl and methoxy groups.The methoxy protons on **4b** and hydroxyl protons on **4c** show a signal at 4.071 ppm & 10.534 ppm. Enolic presence of (4E,6E)-4,6-bis (Anthraceneyl-methylidene) cyclohexane -1,3- dione is reinforced by the information that ^{1}H NMR spectra indicated a one proton singlet at ~ 16.1ppm due to strong intra molecularly hydrogen bonded enolic proton and one more one proton singlet at ~ 6ppm, which is because of methine proton.The peaks due to alkenyl protons are decected to assigned in the range 6.61-8.99 ppm and that due to aryl protons are viewed in the range 7.614-7.760 ppm.The peaks of numerous proton signals detected in the spectra of the compounds are specified in **Table 4.1.4**.The^{1}H NMR spectra of compound **4c** & **4d** is emphasize in **Figure 4.1.4 & 4.1.5.**

Table 4.1.4 ^{1}H NMR spectral details of polynuclear synthetic analogues.

Ligands	Chemical shifts (δ ppm)					
	Enolic proton	Methine proton	Alkenyl protein	Aryl proton	Substituent	
					Methoxy	Hydroxyl
4a	15.89	5.96	6.4-8.346	7.92-8.23	-	-
4b	16.1	5.94	7.31-7.56	7.41-8.04	4.071	-
4c	16.01	5.91	7.142-7.776	7.213-8.130	-	10.534
4d	16.1	5.12	6.6-8.99	7.614-7.760	-	-
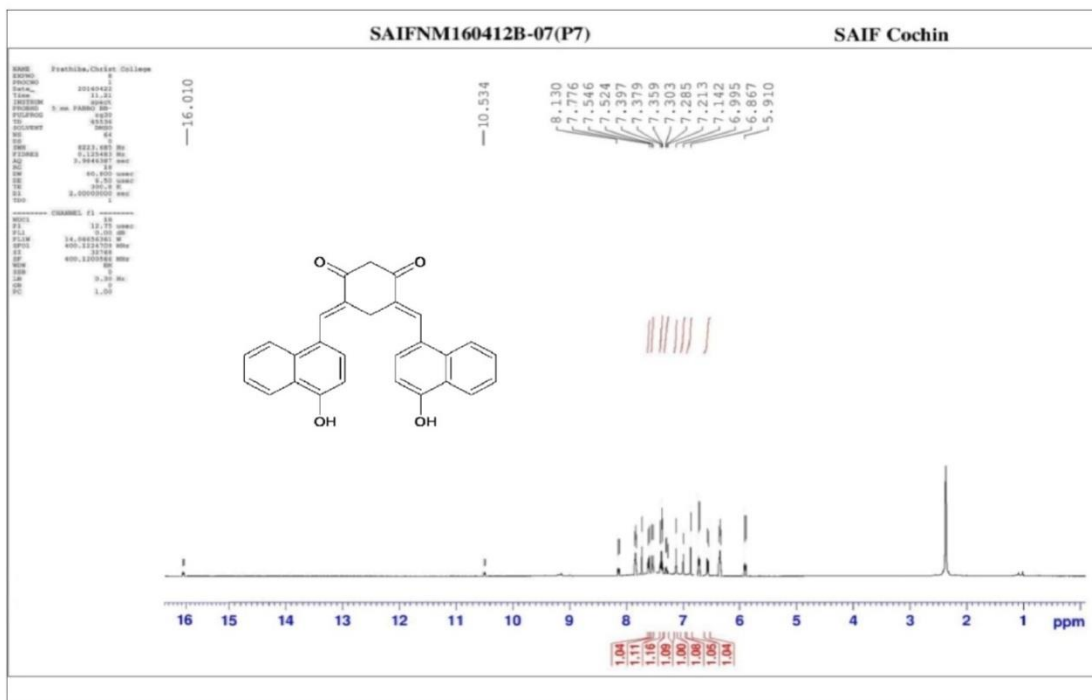
Fig.4.1.4 ^{1}H NMR spectrum of 4c.

^{13}C NMR spectra

The ^{13}C NMR spectral details of all the synthetic analogues were given in **Table 4.1.5-4.1.8.**In **4a**, a peak is present at 103.225 ppm which is due to the CH_2 (methine) carbon . The carbonyl carbon develops a peak   at~ 194.351 ppm ppm while the alkenyl carbon atoms develop signals   at 135.302 & 123 ppm correspondingly. The next carbon atom have two attachment one to the naphthyl ring and other to olefinic carbon providing a peak at 136 ppm.The aromatic carbon atoms are present between 124 – 137 ppm.
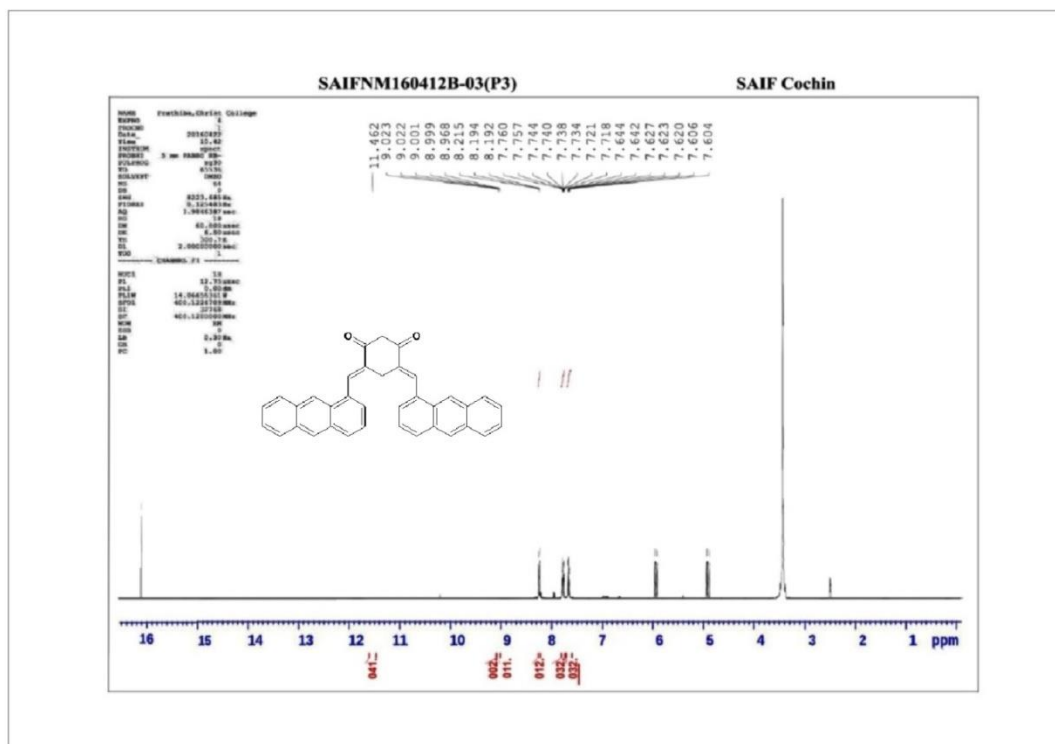
Fig.4.1.5   ¹H NMR spectrum of 4d.

Table 4.1.5 ¹³CNMR spectral data of 4a.

C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'	C6,C6'	C7,C7'
103.225	194	136.623	123	135.302	132.392	122
C8,C8'	C9,C9'	C10,C10'	C11,C11'	C12,C12'	C13,C13'	C14,C14'
131	126.91	127.91	130.14	131.29	134.27	124.61
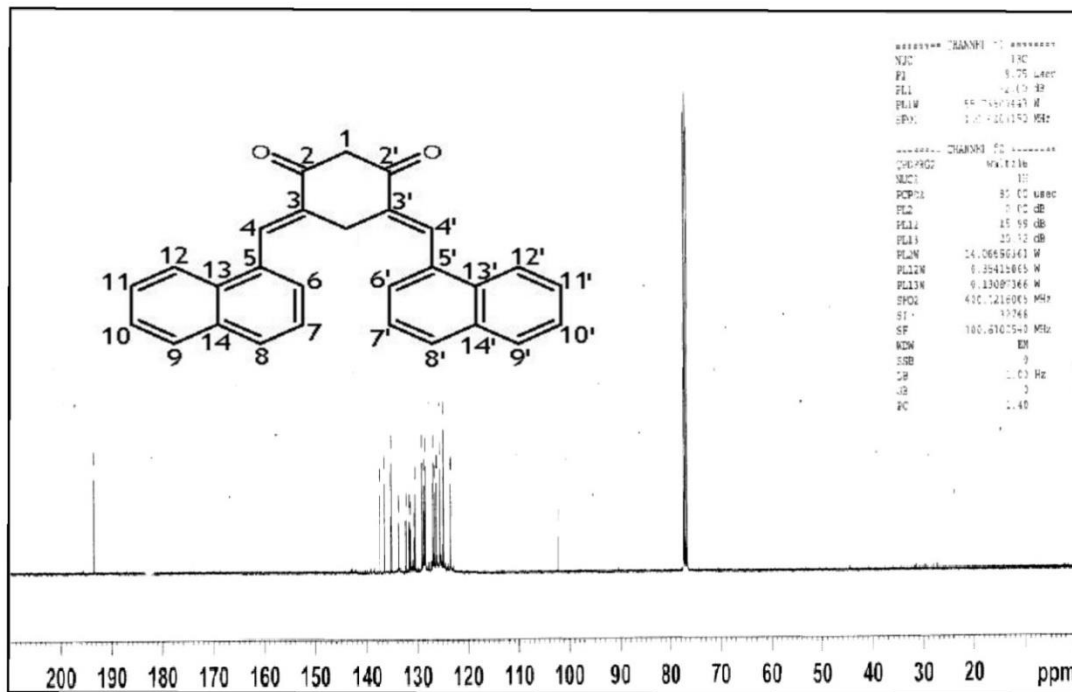
Fig.4.1.6 ^{13}C NMR spectra of 4a.

In the ligand **4b**, a peak is present at 101.38ppmwhich is due to the CH$_2$ (methine) carbon. The carbonyl carbon develops a peak at~ 191.98 ppm while the alkenyl carbon atoms develop signals at 137.291 & 122.442ppm correspondingly. The aromatic ring carbon atoms exhibit a peak in the range of 124-164pm. In **4b** methoxy carbon atom is present at position 57.60ppm. The carbon which is attached to the alkenyl carbon atom (C5) is down shielded and present at a position ~ at 155.25ppm. ^{13}C NMR spectrum of **4b** is described in **Fig.4.1.7** .The spectral analysis is specified below in **Table 4.1.6.**

Table4.1.6 ^{13}C NMR spectral data of 4b.

C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'	C6,C6'	C7,C7'	C8,C8'
101.38	191.98	137.291	113.74	155.25	162.346	132.169	130.51
C9,C9'	C10,C10'	C11,C11'	C12,C12'	C13,C13'	C14,C14'	C15,C15'	
122.44	128.19	128.62	123.58	131.11	128.423	57.60	
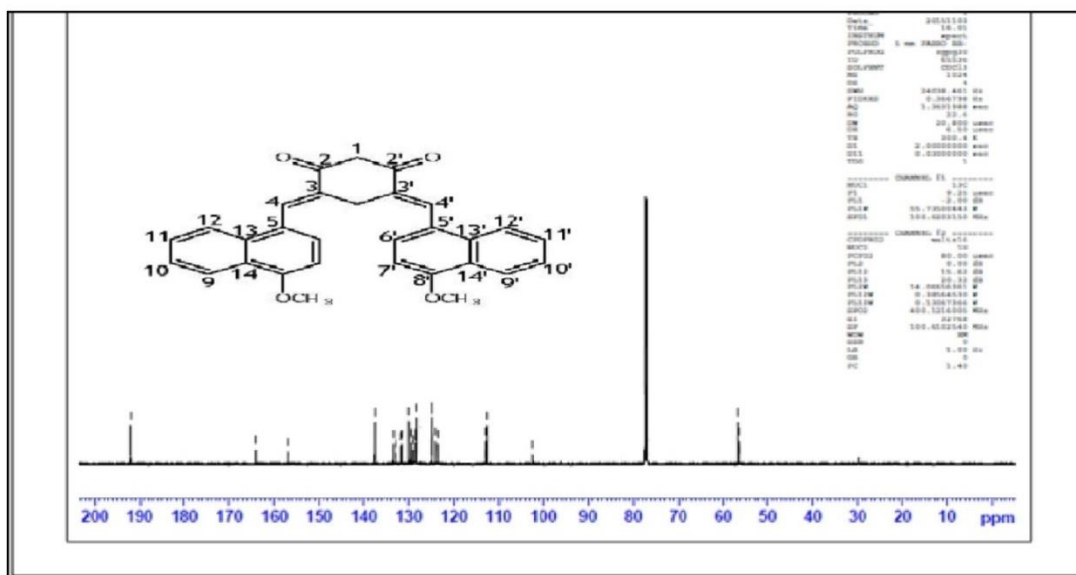
Fig.4.1.7 ^{13}C NMR spectra of 4b

In **4c**, a peak is present at 101.66 ppm which is due to the CH_2 (methine) carbon. The carbonyl carbon develops a peak at~ 193.76 ppm while the alkenyl carbon atoms develop signals at 137.599 & 123.942 ppm correspondingly. The aromatic ring carbon atoms exhibit a peak in the range of 120-156 ppm. The C8 carbon which is linked to the hydroxy group is seen downfield at 156.43 ppm.The downfield position of this signal can be explained due to the electronegativity of oxygen attached to it.

^{13}C NMR spectral analysis of 4c is specified under in **Table 4.1.7.**

Table 4.1.7   ^{13}C NMR spectral data of 4c.

C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'	C6,C6'	C7,C7'
101.66	193.76	138.42	121.01	139.51	132.23	120.65
C8,C8'	C9,C9'	C10,C10'	C11,C11'	C12,C12'	C13,C13'	C14,C14'
156.43	128.32	126.34	125.64	124.43	123.23	122.24

Table 4.1.8 ^{13}CNMR spectral data of 4d.

C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'	C6,C6'
107.22	195.13	140.25	122.11	134	130.10
C7,C7'	C8,C8'	C9,C9'	C10,C10'	C11,C11'	C12,C12'
125.70	128.23	130.31	132	132.18	126.74
C13,C13'	C14,C14'	C15,C15'	C16,C16'	C17,C17'	C18,C18'
124.76	132.15	124.33	121.18	122.55	120.85
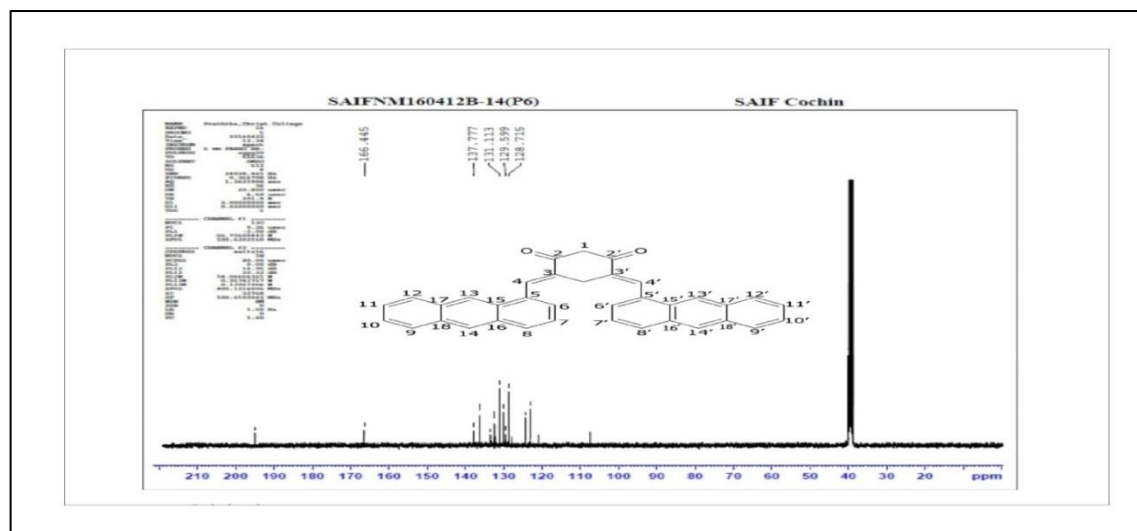
Fig.4.1.8 ^{13}C NMR spectral data of 4d.

Mass spectra

The mass spectra of (4E,6E)-4,6-bis (naphthalene-ylm ethylidene) cyclohexane -1,3-dione (**4a**), displays molecular ion(M+3 ion) peak at 343.The base peak in the spectrum is detected at m/z=143 by the loss of B [Ar-CH=CH-C=O]$^+$ion.The following intense peak is witnessed at m/z=129 which is due to [naphthayl-CH]$^+$ ion,there are also different frangment ions due to the loss of C_2H_2,-CH_2=C=O,-CH_2,-CH=C=O . The m/z values of the fragment ions are depicted in **Table 4.1.9**. and the fragmentation pattern are given in **scheme 4.1.2**.Remaining parts like O, OH, CH_2 etc. are detached from the molecular ion and are displayed in the spectrum.
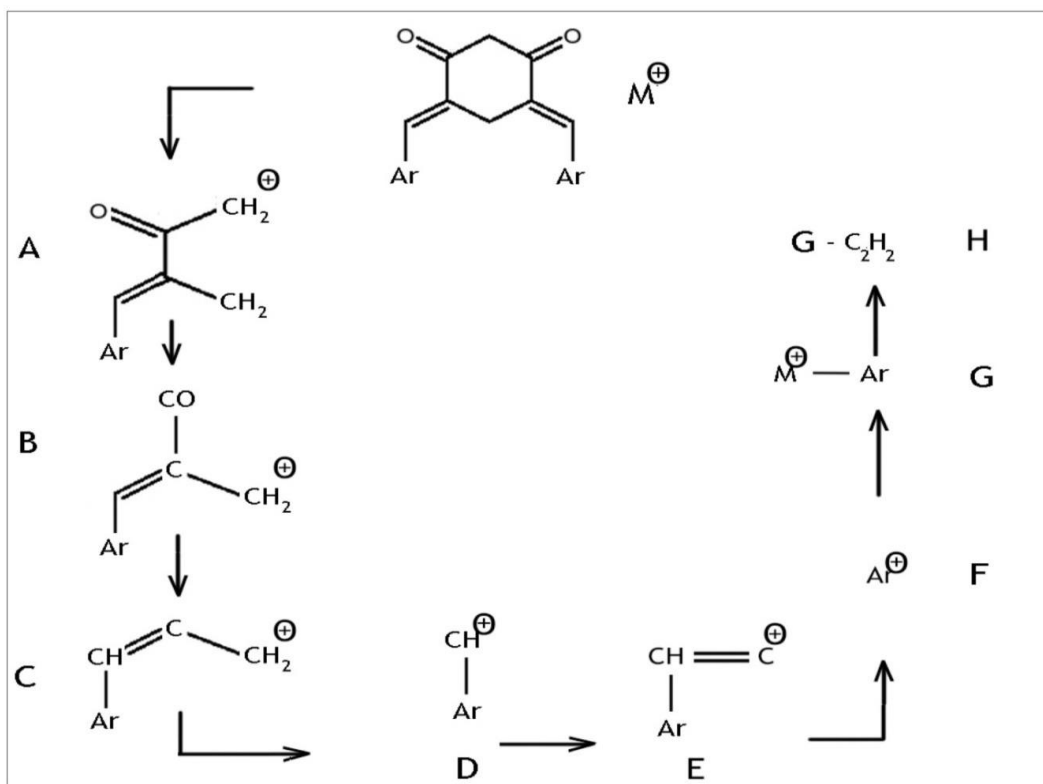
Scheme 4.1.2.

The mass spectrum of **4a** is given in **Fig.4.1.9**. The (M+) ion of **4b** is detected at 448. Further significant points of **4b** are due to fragment ions which are represented in **Table 4.1.10**.The spectrum of **4b** is specified in **Fig.4.1.10**.

Table 4.1.9. The mass spectral peaks of polynuclear analogues.

	Synthetic analogues	Molecular ion peak	I	II	III	IV	V	VI	VII	VIII
Fragments Mass pattern	4a	343	204	199	165	143	150	129	232	220
	4b	448	179	224	196	151	163	137	257	214
	4c	408	241	210	182	157	168	144	263	233
	4d	489	258	214	202	190	177	121	312	258
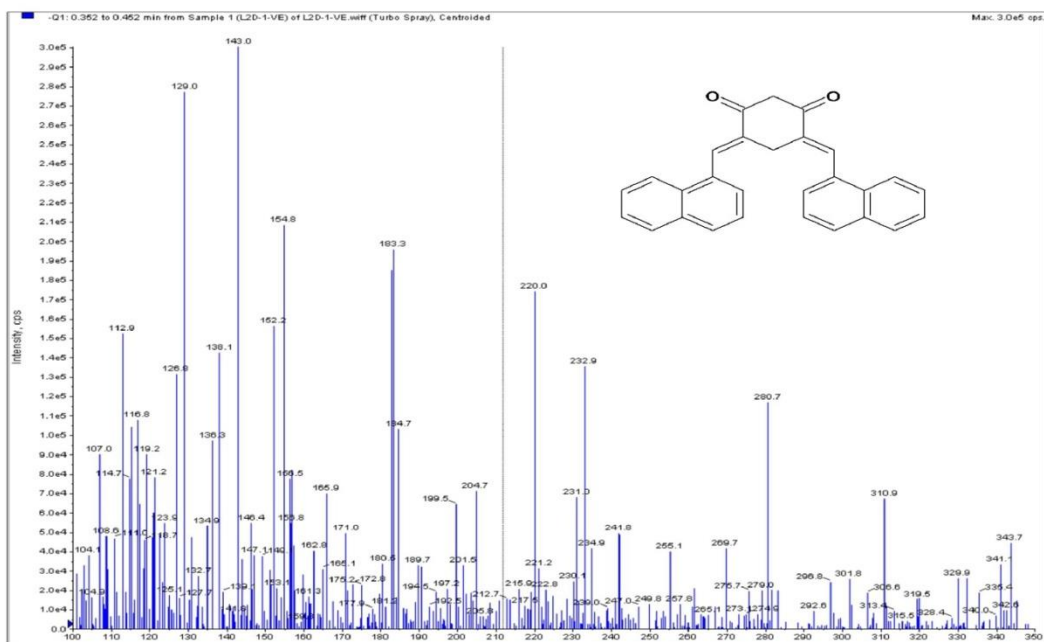
Fig.4.1.9 Mass spectrum of 4a.

Mass spectrum of (4E,6E)-4,6-bis(hydroxynaphthalene-ylmethylidene)cyclohexane -1,3 dione (**4c**) disclosed an powerful molecular ion peak at 408.The succeeding intense peak is observed at m/z is 196 by the loss of $[Ar-CH=CH-C=O]^+$ ion. The peaks at 241,210,263,223,182,157 are due to fragment ions.The development of fragment ions can be described from the fragmentation pattern assumed in scheme below. The mass spectrum of **4c** is given in **Fig.4.1.11**.
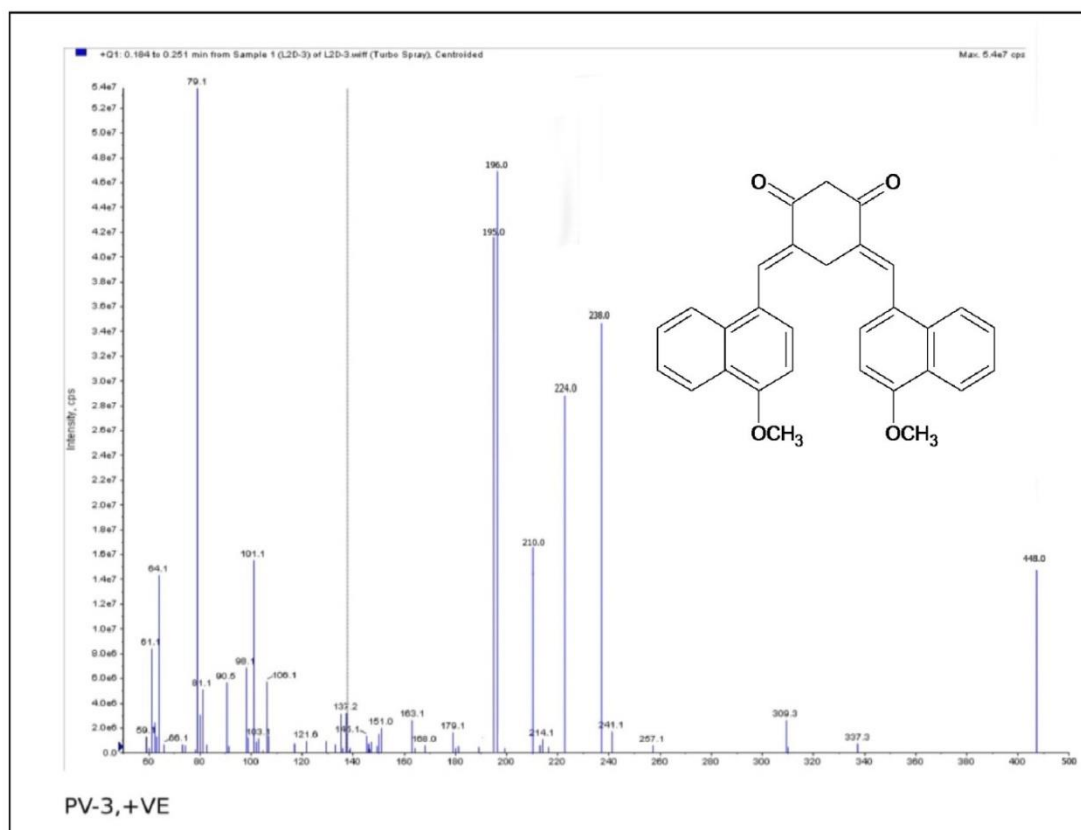
Fig.4.1.10 Mass spectrum of 4b.
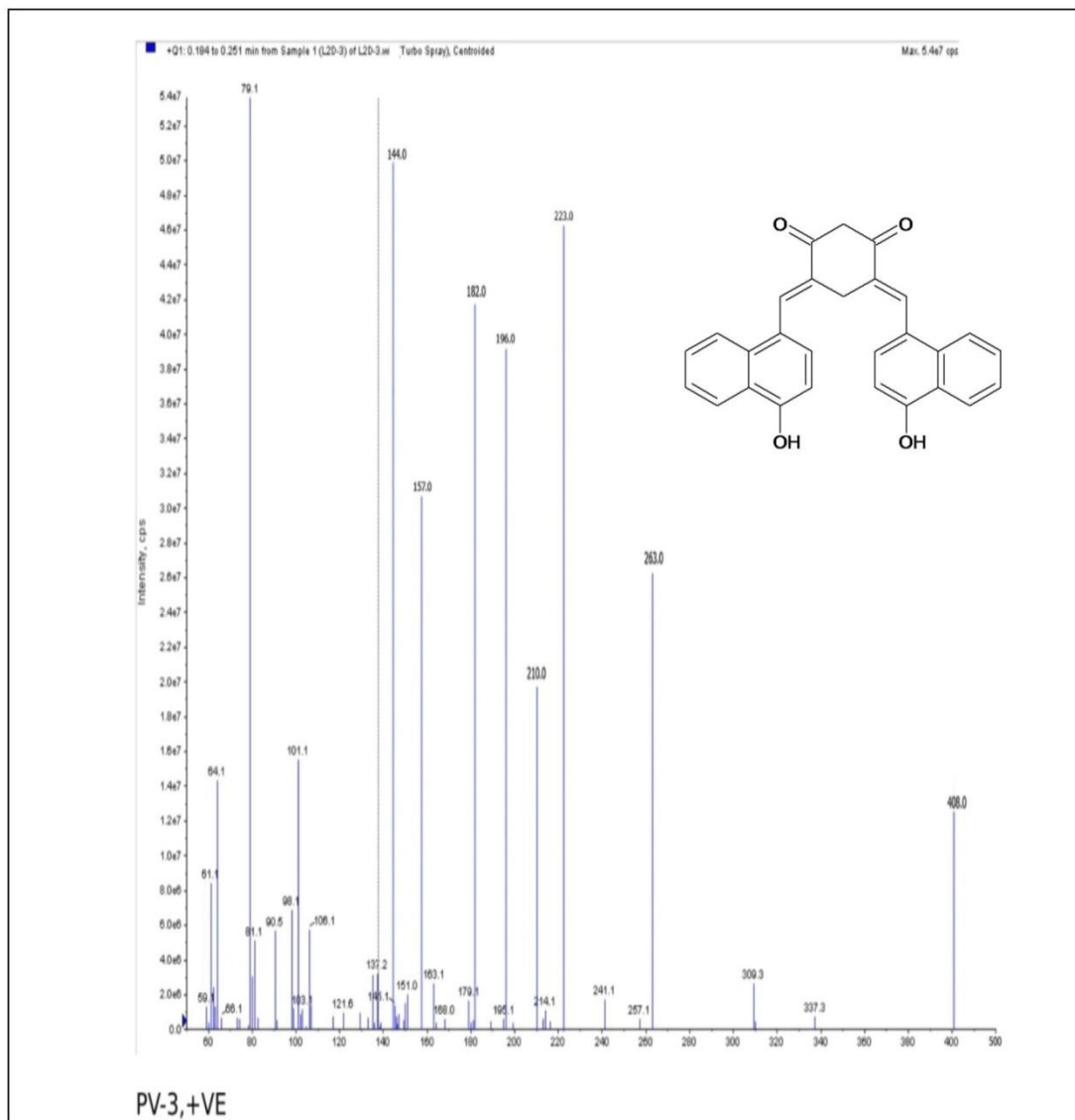
Fig.4.1.11. Mass spectrum of 4c.
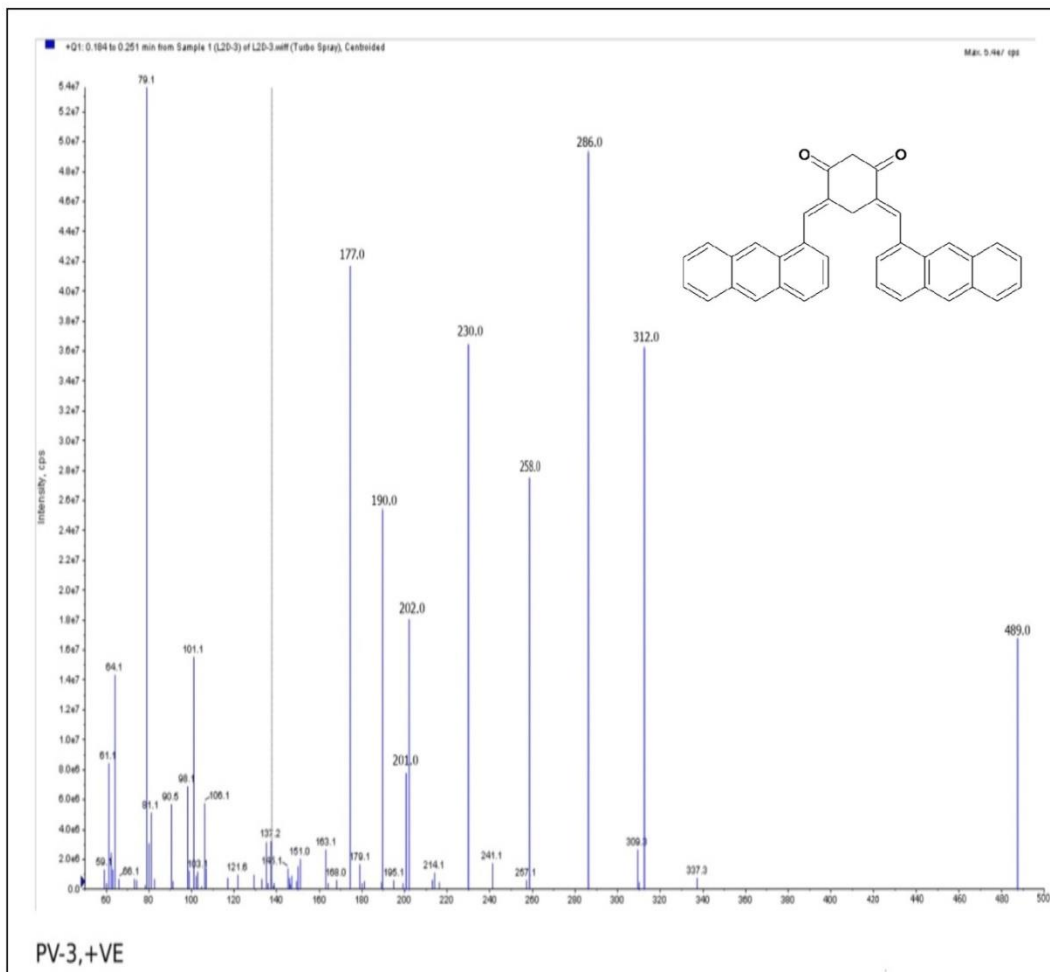
Fig.4.1.12 Mass spectrum of 4d.

THERMOGRAVIMETRIC ANALYSIS OF (4E, 6E)-4,6-BIS(METHOXY NAPHTHALENE-YLM ETHYLIDENE) CYCLOHEXANE -1, 3- DIONE

Thermo gravimetric examination of the synthetic analogues was done in the domain of $39.42^{o}C$ to $721.42^{0}C$.The thermogram achieved displayed a two stage breakdown pattern .The analogue is steady upto 160^{0}.The decay method initiates gradually with a sharpended fall in mass (34.47%) likely about 236.33^{0}.The % mass failure matches to the removal of the aromatic fragment from the ligand.The next stage of decay indicates a mass reduction of 71.932%. This is due to the removal of next aryl part from the synthetic analogues. The top temperatures are witnessed at 236 and $357.99^{0}C$. Thermogram of the ligand is given below in **Fig.4.1.13.**
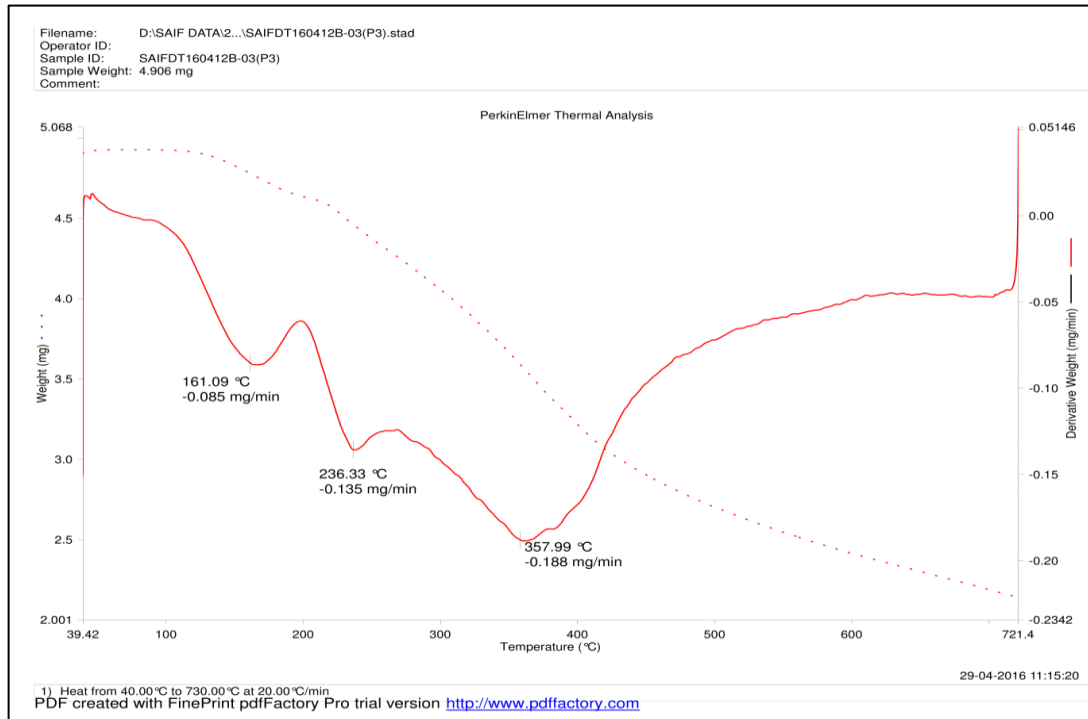
Fig 4.1.13 Thermogram of 4b.

Thus all the spectral evidences such as UV,IR,NMR,mass spectra and TG analysis support the structure of the compounds as in **Figure 4.1.14.**
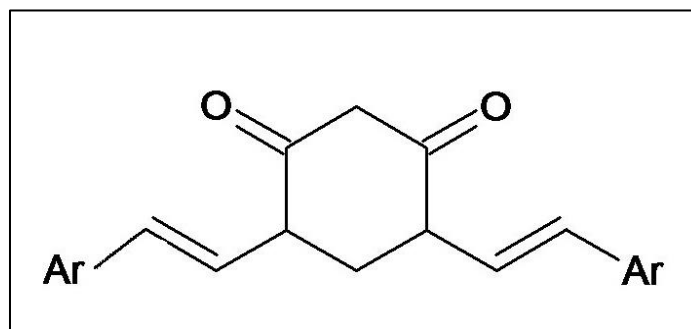
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SECTION-II

SYNTHESIS AND CHARACTERISATION OF TRANSITION METAL CHELATES OF (4E,6E)-4,6-BIS(POLYNUCLEAR)CYCLOHEXANE -1, 3-DIONES

4.2.1 Synthesis of metal complexes of (4E,6E)-4,6-bis (polynuclear)cyclohexane -1,3 diones

The generally adopted procedure is outlined here.To a retreated solution of diketone (0.002mol) in methanol (25ml), an aqueous solution of metal salt (0.001mol) was combined and the reaction mixture was reduced for almost 4 hr and lowered to room temperature. The precipitated complex was filtererd and recrystallized from hot methanol. The metal salts used were copper(II) acetate,Zinc(II) acetate &Nickel(II) acetate for the preparation of Cu(II),Zn(II) & Ni(II) complexes respectively.

4.2.2 Characterization of metal complexes

The characterization data of synthetic analogues of **4a**, **4b**, **4c** & **4d** are given in **Table 4.2.1** - **4.2.5** respectively.

Table 4.2.1   Analytical and spectral data of metal complexes of 4a.

Metal selected	M.P (^{o}C)	Elemental study (%) Found/(calculated)			Electronic λ max (nm)	Distinguishing IR stretchingbands (cm^{-1})		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu	203	79.40 (80.04)	4.33 (4.76)	7.13 (7.56)	267 381	1577	1521	483 416
Zn	201	79.66 (79.86)	4.27 (4.75)	7.59 (7.77)	263 383	1594	1519	481 423
Ni	212	80.38 (80.50)	4.56 (4.79)	7.441 (7.031)	264 391	1593	1527	466 431

Table 4.2.2   Analytical and spectral data of metal complexes of 4b.

Metal chelates	M.P. (°C)	Elemental analysis (%) Found/(calculated)			Electronic λmax nm	Characteristic IR stretching bands (cm⁻¹)		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu	187	74.57 (75.03)	4.83 (5.0023)	6.33 (6.622)	267 366	1608	1521	467 458
Zn	191	74.11 (74.89)	4.71 (4.99)	6.72 (6.800)	267 369	1616	1507	466 413
Ni	193	74.38 (75.41)	5.11 (5.027)	6.02 (6.147)	277 391	1593	1509	486 419

Table 4.2.3   Analytical and spectral data of metal complexes of 4c.

Metal chelates	M.P °C	Elemental analysis (%) Found/(calculated)			Electronic λmax nm	Characteristic IR stretching bands (cm⁻¹)		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu	123	74.39 (74.41)	4.17 (4.42)	6.86 (7.03)	259 392	1588	1527	459 407
Zn	127	73.99 (74.2.2)	4.92 (4.41)	6.93 (7.22)	267 387	1578	1514	452 423
Ni	131	73.28 (74.77)	4.17 (4.45)	6.33 (6.53)	268 394	1587	1524	493 417

Table 4.2.4   Analytical and spectral data of metal complexes of 4d.

Metal chelates	M.P. (°C)	Elemental analysis (%) Found/(calculated)			Electronic λmax nm	Characteristic IR stretching bands (cm⁻¹)		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu	173	82.08 (83.156)	4.18 (4.61)	6.007 (6.11)	295 414	1597	1517	458 428
Zn	187	81.74 (82.99)	3.99 (4.61)	5.888 (6.28)	294, 417	1609	1513	462 432
Ni	192	82.91 (83.55)	4.37 (4.64)	4.05 (4.67)	297, 415	1600	1520	448, 421

Electronic spectra

In particular circumstances there is a small change of absorption maxima to longer wavelength that points out the participation of oxygen in carbonyl group of diketone in metal complexation.

IR spectra

The presence of intramolecular H-bonding in the enolic form of ligand at $1594cm^{-1}$ of the IR spectra of metal chelates has vanished and in its place an intense band designated to stretch the coordinated carbonyl component which is seen at $1600cm^{-1}$. Additional bands appear at ~481 cm^{-1} and ~423 cm^{-1} assignable to ν (M–O) vibration.A significant reduction of broad band in the domain of 2600 -3500 cm^{-1}is observed when the synthetic ligand is transformed into metal complex. This is a clear sign of the substitution of the chelated proton by the metal ion while forming complex. The IR spectrum of Cu(II) of **4b**, Zn(II) complex of**4c** and **4d** are represented in **Fig.4.2.1,4.2.2 & 4.2.3.**
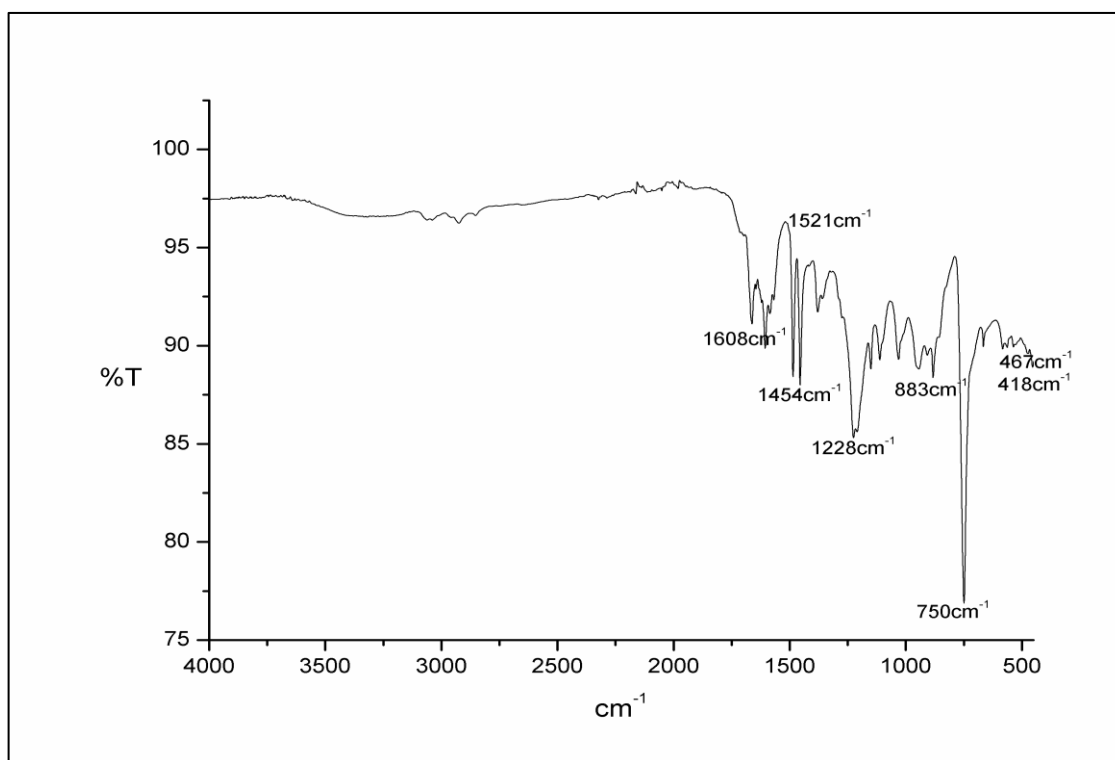
Fig.4.2.1   IR spectrum of Cu(II) complex of 4b.
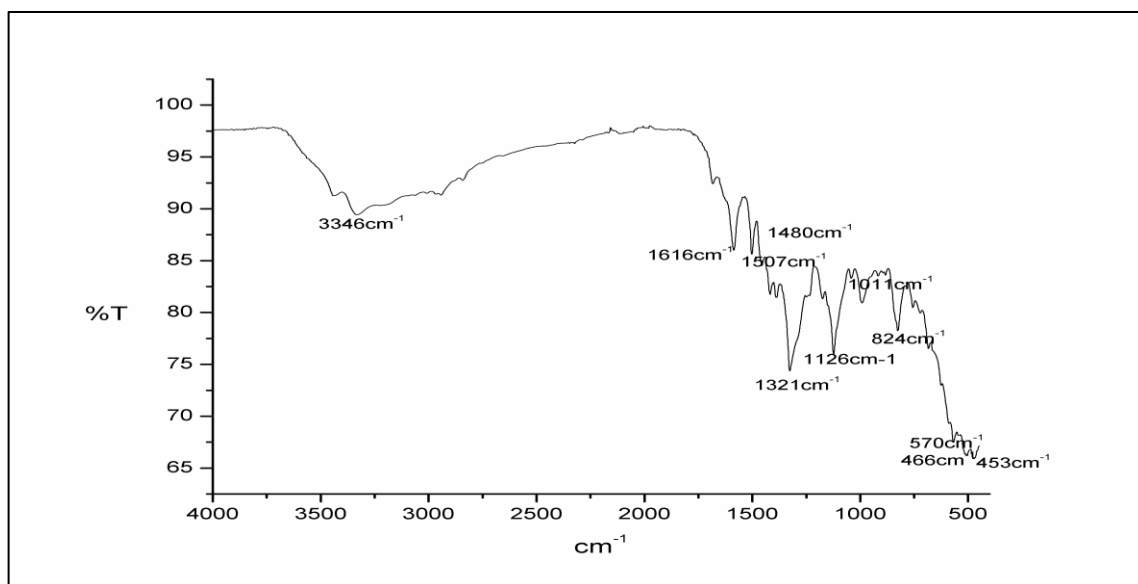
Fig.4.2.2   IR spectrum of Zn (II) complex of 4c.
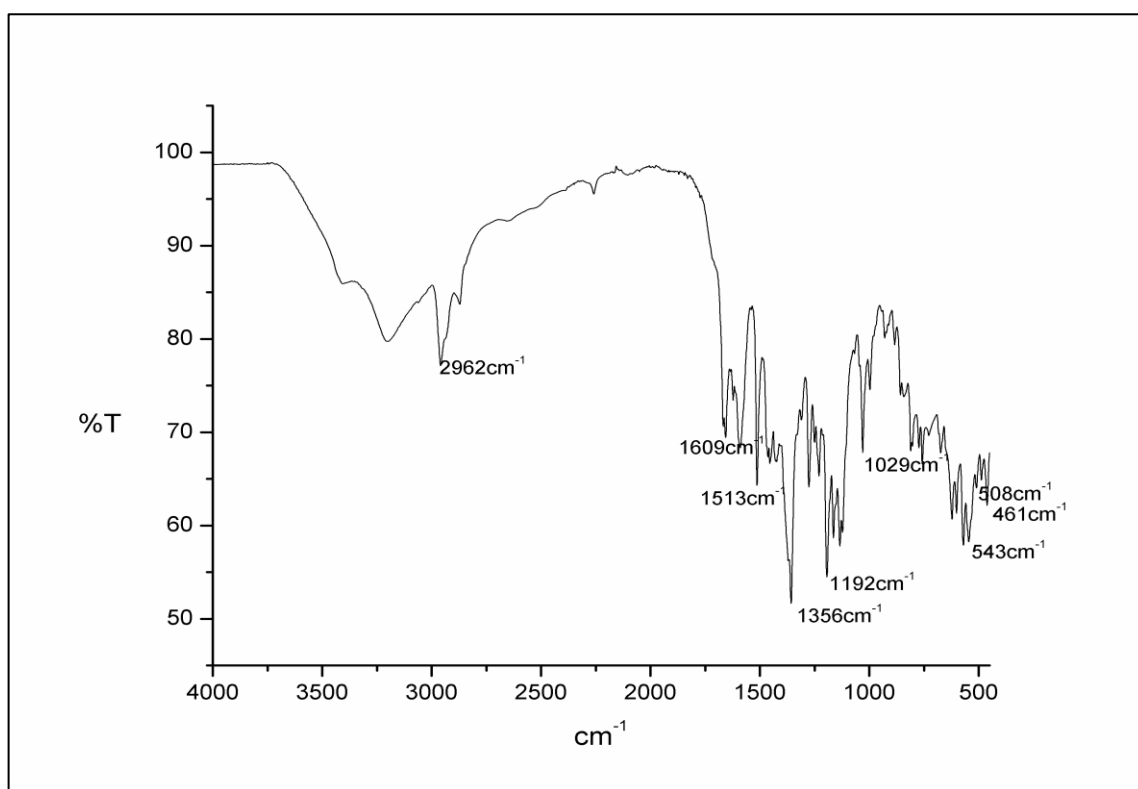
Fig.4.2.3.   IR spectrum of Zn (II) complex of 4d.

¹HNMR spectra

The enolic proton existing in the diketone synthetic analogue is exchanged by copper, zinc and nickel metal atoms while forming complexes.Absence of signal at 16 ppm is a solid evidence for the complexation. The enolic proton has significant role in metal chelation while phenyl and alkenyl protons did not take part in complex formation. The non-altered structure of phenyl and alkenyl protons is a clear indication for this. There is a slight shift of methine signals to the downfield of the spectra. Hence a very close resemblance is seen in the spectra of diketone ligand and metal complex while enolic proton differs. The NMR spectra of Zn(II) complex of **4b** is given in **Fig.4.2.4.**
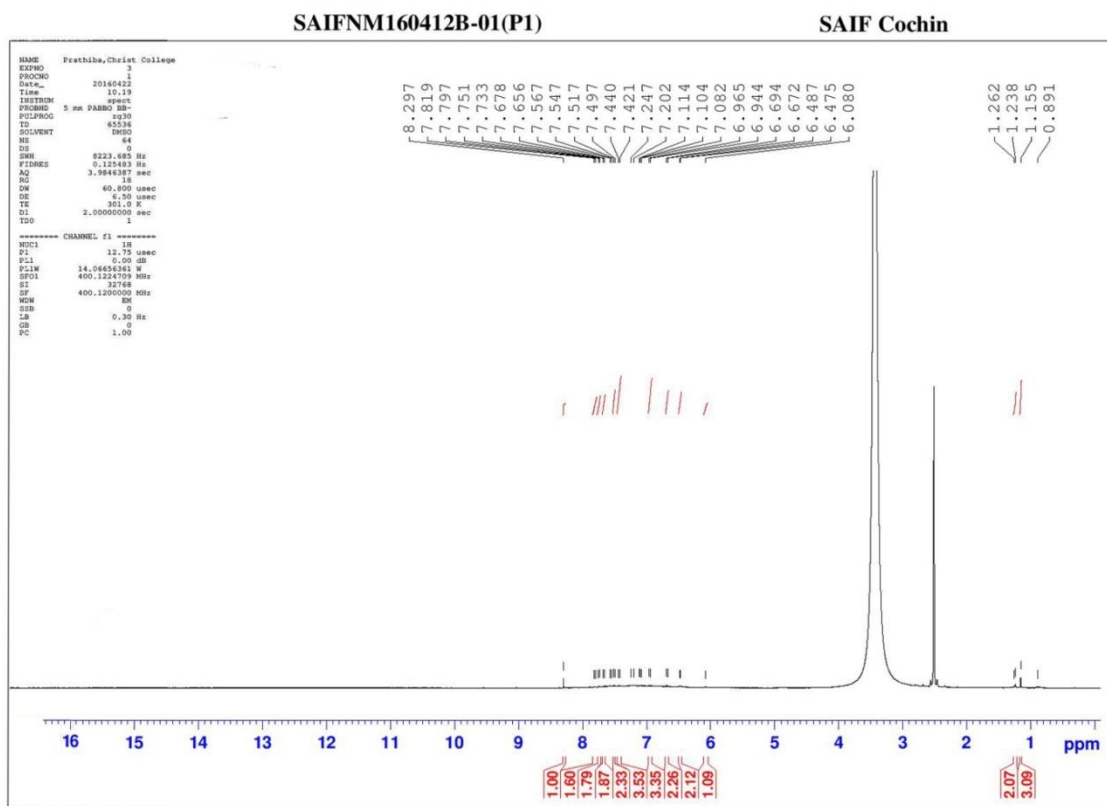
Fig 4.2.4 The NMR spectra of Zn(II) complex of 4b.

Mass spectra

The fragmental arrays of the synthetic analogues of **4a, 4b, 4c & 4d** adopt the **Scheme 4.2.1.** The mass spectrum of Cu (II) complex of **4a** has an intense molecular ion peak at 841.The peak at 638 and 332 is due to the removal of 2 Ar and 4 Ar groups from the molecular ion where Ar is 1-naphthyl .The peak at 453 is due to the [ML]+ fragment where M is metal and L is ligand. The peaks at 181,168,157,138 are all due to fragment ions of the ligand and are observed in the spectrum of ligand. The mass spectrum of Zn(II) complex of **4b** is given below. The molecular ion peak is less intense and observed at 961.The intense peak in the spectrum at 437 is due to the ligand peak. The base peak is observed at 210 which is assigned to $[C_7H_5O_2Zn]+$.The peaks at 647 and 333 are due to the removal of 2Ar and 4 Ar groups respectively from the molecular ion where Ar is 2-methoxy naphthyl.
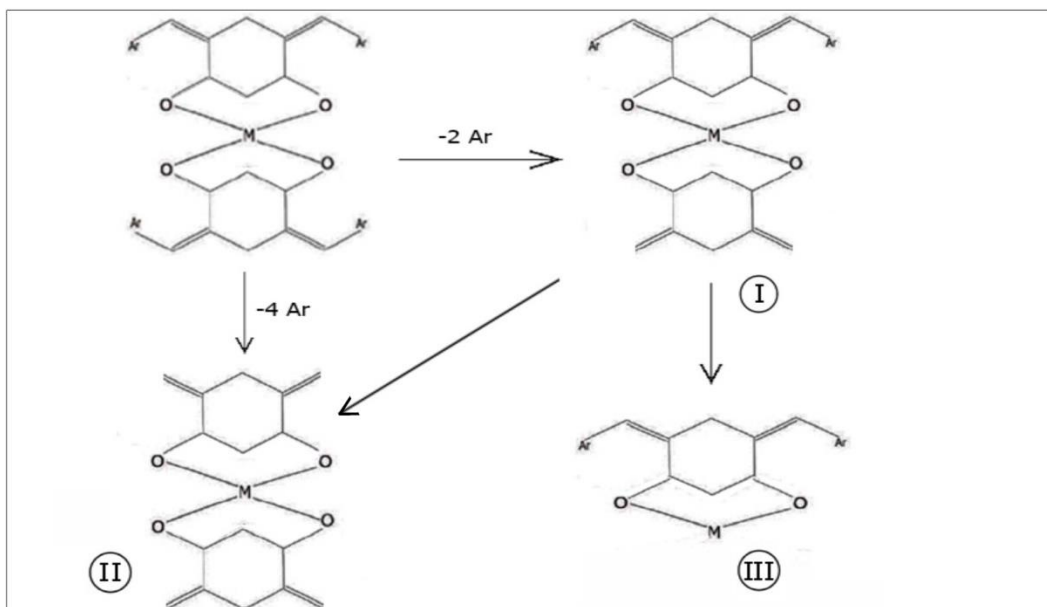
Scheme 4.2.1.
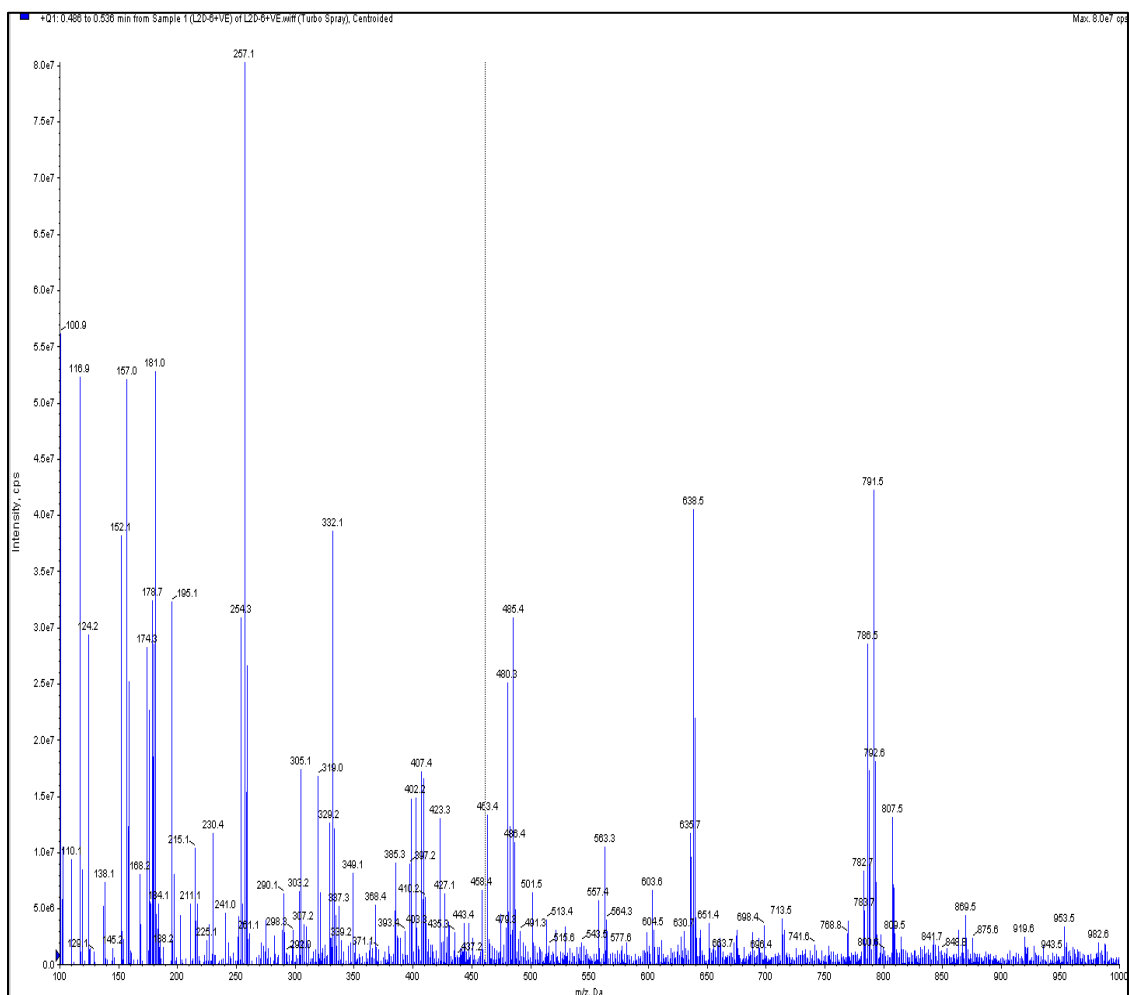
Fig 4.2.5 Mass Spectrum of Cu(II) complex of 4a.
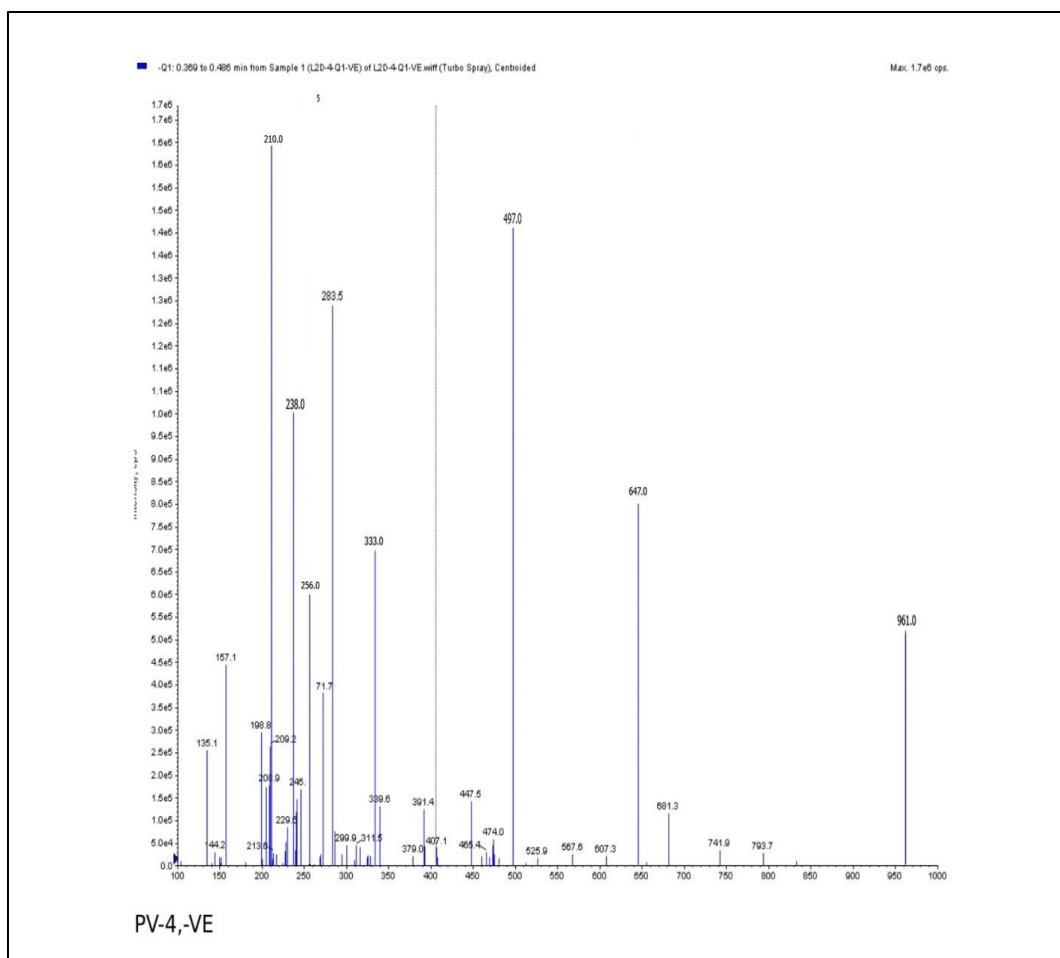
Fig.4.2.6. Mass Spectrum of Zn (II) complex of4b.

In 4d, the peak at 1035 is because of molecular ion peak.There is a removal of two aryl group from the complex, it is indicated by a peak at 681and then the complex undergoes another removal of two aryl pat and it gaves a peak at 327.One of the marked peak at 489 ,it is due to the (L) [+]
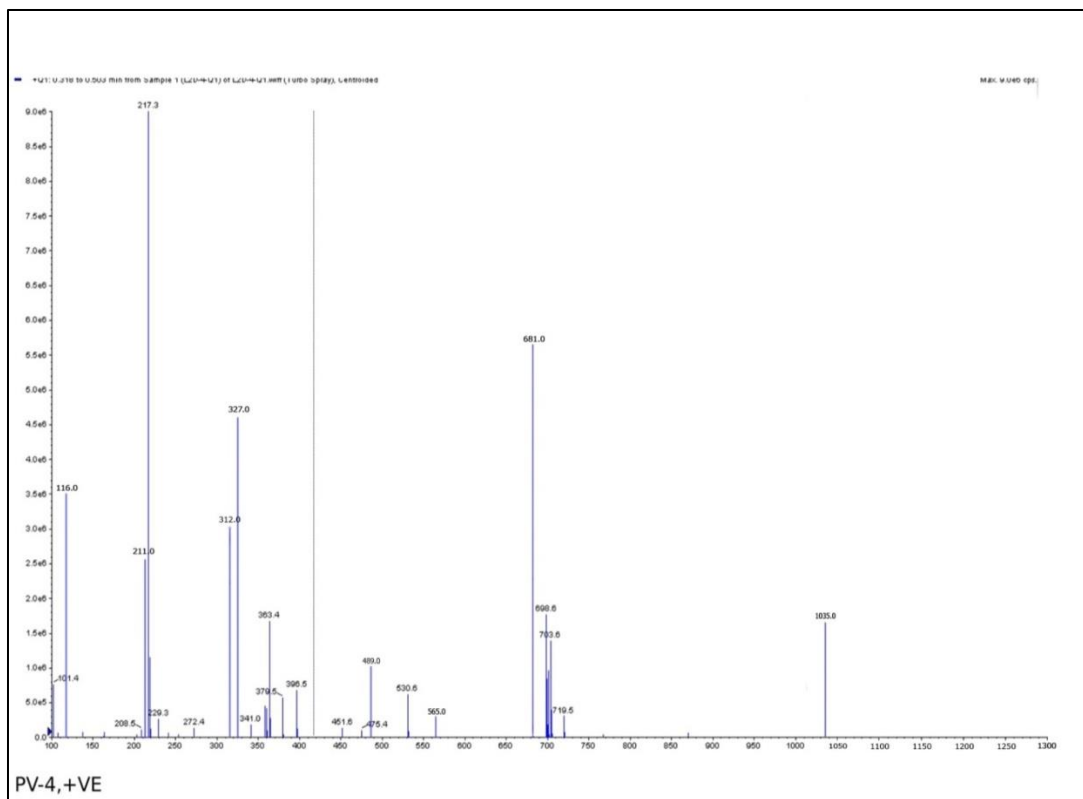
Fig.4.2.7.   Mass Spectrum of Cu (II) complex of 4d.

The observed UV, IR, ^{1}H NMR and Mass spectral data clearly reveals that metal chelates of Cu,Zn & Ni are having ML$_2$ stoichiometry(metal ligand ratio is 1:2).
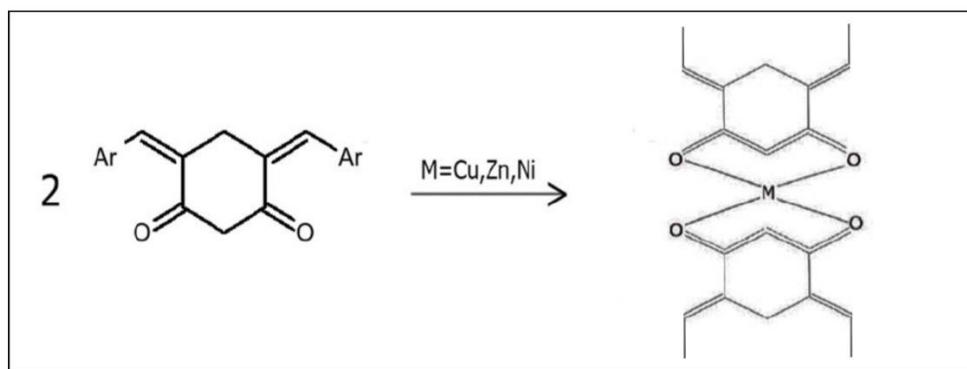
SECTION III

INVITRO CYTOTOXICINVESTIGATION OF (4E, 6E)-4, 6 BIS (ANYHRACENEYL METHYLIDENE) CYCLOHEXANE 1, 3 DIONEAND METAL COMPLEXES

4.3.1. *In vitro* **Cytotoxic studies of (4E,6E)-4,6 bis (anyhraceneyl methylidene)cyclohexane 1,3 dione(4d)&metal complexes Cu(II), Zn(II), Ni(II)**

In vitro cytotoxic activity of **4d** and metal complexeswere studied for short term in vitro cytotoxicity using Daltons's lymphoma ascites cells (DLA) and Ehrlich's Ascites Carcinoma (EAC) cancer cells. The cytotoxic features were deal with, in terms of the percentage cell death brought up by them. The ligands as well as the metal complexes possess appreciable cytotoxic activity in both DLA and EAC cell lines in a dosage dependent aspect. . The result of the present work with EAC and DLA cancer cell lines are specified in **Table 4.3.1**

Table 4.3.1. In vitro studies of (4d) &metal complexes towards EAC& DLA

Conc µg/ml	% Cell death to EAC				% Cell death to DLA			
	L_4	$Cu(L_4)_2$	$Zn(L_4)_2$	$Ni(L_4)_2$	L_4	$Cu(L_4)_2$	$Zn(L_4)_2$	$Ni(L4)_2$
200	67	97	87	81	63	95	86	77
100	48	76	69	65	39	74	67	61
50	31	61	57	52	25	60	53	48
20	19	43	41	38	11	41	39	31
10	4	26	21	19	3	23	20	11
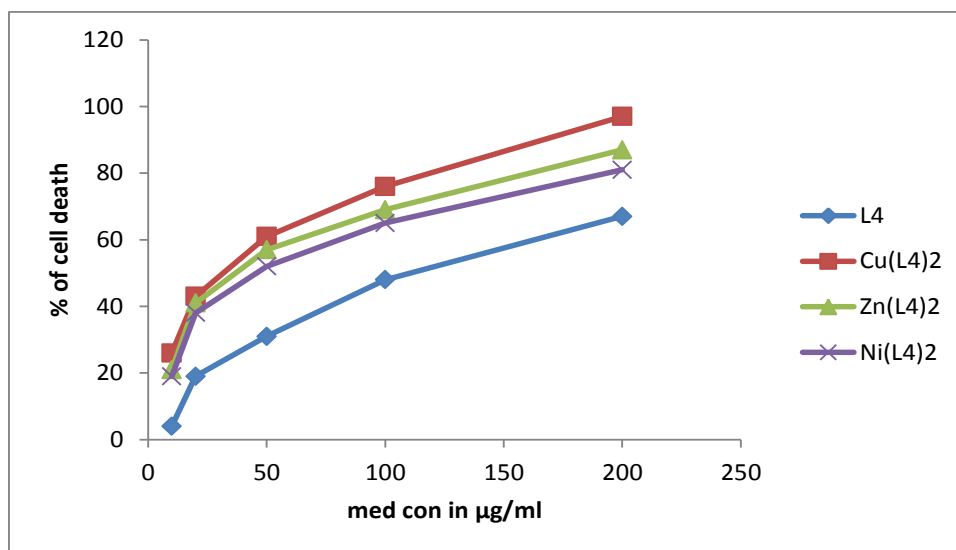
Fig.4.3.1. In vitro Cytotoxic studies of (4d)&metal complexes towards EAC.
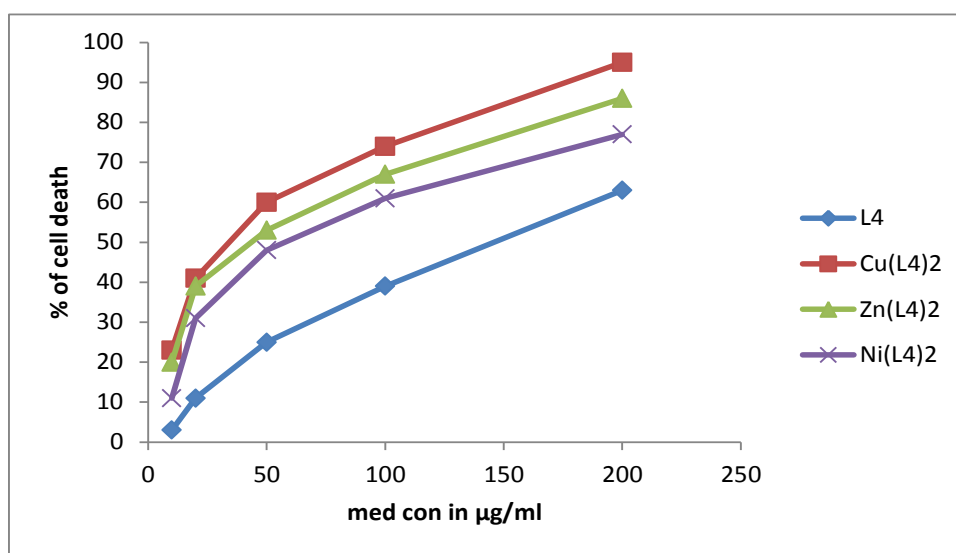
Fig.4.3.2. *In vitro* cytotoxic studies of (4d) & metal complexes towards DLA.

The results disclose that the test compounds offered remarkable cytotoxic property. About 65% cytotoxicity to cancer cells was detected at a dose of 200 µg/ml for the(4E,6E)-4,6 bis (anyhraceneyl methylidene)cyclohexane 1,3 dione and 98 % cytotoxicity was offered by its Cu(II) complex at the equivalent dosage.All the metal complexes gave good results with more than 80 % cell death at higher concentration. Metal complexes showed reasonable activity even their lower concentrations.. The activity of copper complex was nearly four times than that of the ligand. The metal

chelation remarkably increases the cytotoxic character because even at very low concentration namely 50 μg/ml,it offered about 50% cell death. Thus it is working out that the ligand and metal complexes with the polynuclear anthracenyl ring has extreme effect in developing cell death.

In vivo antitumour studies of 4d and metal complexes

In the present study the anticarcinogenic activities of the polynuclear test compound 4d and their Cu & nickel complexes were assessed in vivo because these two complexes gave good result in in vitro studies.The influence of these analogues to boost the life span of ascites tumour carrying animals were considered.Feasible EAC cells were vaccinated into the peritoneal cavity of mice so as to grow tumour in them.medicines (ligand and metal complexes) were directed as ip injection at various concentrations for 10 days after tumour injection.The death rate of animals due to tumour problem was noticed and the % increase in life span determined. The results of the study are given in **Table 4.3.2.**

Table 4.3.2 Effect of 4d their Cu & Ni complexes on ascites tumour reduction

Animal groups	Concentration (μg/ml)	No.of animals with tumour	No.of days survived	%ILS
1. Control		5/5	17.3±1.1	
2.Standard drug		5/5	31.6±3.1	82.65
3.L	20	5/5	29.8±2.5	72.25
4. L	10	5/5	28.5±3.3	64.73
5. L	5	5/5	25.6±2.8	47.97
6.Cu(L)$_2$	20	5/5	31.9±2.4	84.39
7. Cu(L)$_2$	10	5/5	30.6±3.0	76.87
8. Cu(L)$_2$	5	5/5	26.6±2.8	53.75
9.Ni(L)$_2$	20	5/5	28.0±2.2	61.84
10.Ni(L)$_2$	10	5/5	26.1±2.75	50.86
11.Ni(L)$_2$	5	5/5	24.0±2.01	38.72

The examination disclosed that intraperitoneal application of ligand; its Cu (II) and nickel complexes meaningfully elevated the life span of EAC persuaded ascites tumour carrying mice. The(4E,6E)-4,6 bis (anyhraceneyl methylidene)cyclohexane 1,3 dione as well as the copper and nickel metal complexes showed considerable antitumour

activity.The life period of animals was found to be meaningfully increased to 29,31 and 28 days for ligand,Cu(II) and Ni(II) complexes when the control animals survived for an average of 17 days after tumour induction. The % ILS for (4E,6E)-4,6 bis (anyhraceneyl methylidene)cyclohexane 1,3 dioneligand,Cu(II) and nickel complexes with dosage 20 μg/ml was found to be 72.25 ,84.39 and 61.84 % respectively and it was proportionate with the value 82.65 % for the recognized drug cyclophosphamide. The supreme value for the no.of days of existence was detected with Cu(II) complex of(4E,6E)-4,6 bis (anyhraceneyl methylidene)cyclohexane 1,3 dioneie 31.9 days which is larger than 31.6 days for the reference drug .The Cu(II) complex revealed nearly the similar activity as the recognized drug.So the examination discloses that Cu(II) complex is authentic in diminishing tumour progress in mice and boost the life span of the animal. Copper complexes of curcuminiod analogues of the ring system were found to be more active in cytotoxic studies, may be due to extended conjugation.
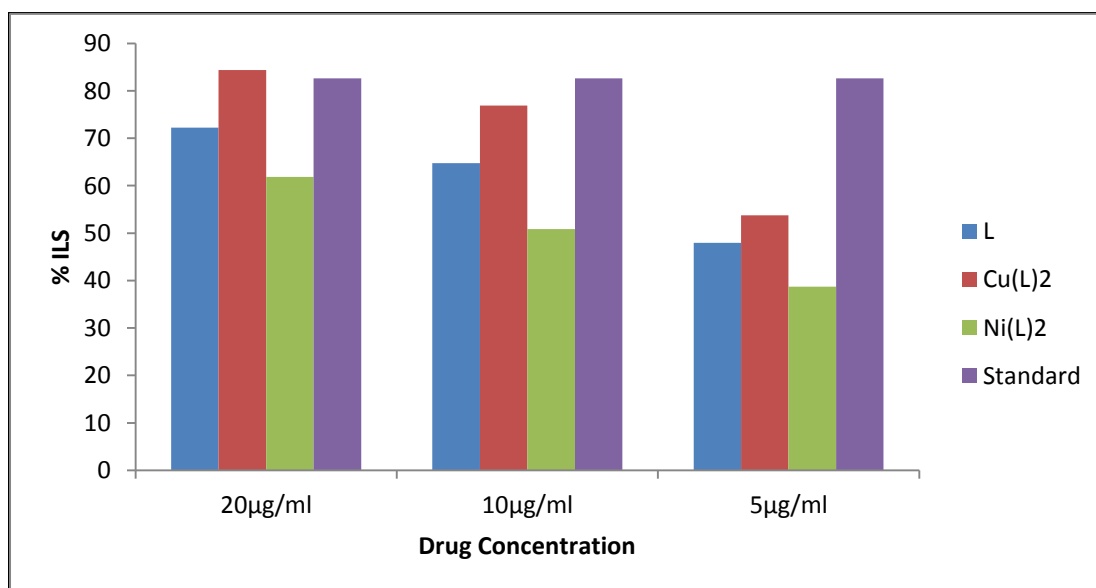
Fig.4.3.3 The % ILS with different conc. of4dand their Cu & nickel complexes.

In vivo cytotoxic on solid tumour development.

Effect of 4d and its Cu (II) complex on solid tumour.

Albino mice (male, 5-6 weeks old) weighing 18-25 kg were used for the study purpose. Five animals form a study group and there were 14 such groups. Ascites tumour was made in these animals by introducing viable DLA cells in 0.1 ml of PBS into the

peritoneal cavity. Out of 14 groups, one group was kept as control group which wasn't treated with any drug. It was labeled as group 1 another group was treated with the standard drug,cyclophosphamide and it was labeled as group 2.Group III and IV were treated with 4dand its Cu(II) complex at a dose of 200 μmol /kg body weight.The medicine were applied by intraperitoneal inoculation from the first day of tumour introduction and extended for next 10 days.The tumour growth on animals in each group was detected by calibrating the diameter of tumour production from seventh day of tumour induction upto 31 rst day.The tumour volume was computed by the formula $V=4/3 \pi r_1^2 r_2^2$ where r_1 is the minor radius and r_2 is major radius.There was substantial decrease of solid tumour volume in mice conducted with 4dand its Cu(II) complex .The computed tumour volume was 6.285 cm^3 for the control group on the 31 ^{rst} day.The reference drug injected mice displayed the reduced tumour volume 2.782 cm^3.The(4E,6E)-4,6 bis (anyhraceneyl methylidene)cyclohexane 1,3 dione used group considerably lessened the tumour volume to3.01 cm^3 . Comparing with that of the control group,the ligand produced a decrease in volume of 3. 275 cm^3 . The tumour volume on day 31 for copper complex of ligand was 1.335 cm^3 .The decrease in tumour volume was 4.95 cm^3 with respect to control group. The decline in tumour volume for ref.drug was 3.503 cm^3 .The Cu(II) complex of 4d had revealed a projecting effect in dropping tumour volume equivalent with the ref.drug may be due to the liphophilic character of copper complex.The results of the study are given below in **Table.4.3.3**

Table 4.3.3   Effect of 4d & Cu complexes on solid tumour development.

Compounds	Tumour volume on 31st day
Control group	6.285 cm^3
4d (L)	3. 01cm^3
Cu (L)$_2$	1.335 cm^3
Std.group	2.782 cm^3

SECTION-IV

ANTIBACTERIAL EXAMINATION OF CURCUMINOID EQUIVALENTS WITH SUBSTITUTED NAPHTHYL RING AND METAL COMPLEXES

4.4.1 Antibacterial studies of (4E, 6E)-4, 6-bis (polynuclear) cyclohexane -1, 3 diones and their metal complexes.

The synthetic diketone analogues and their metal chelates checked for their antibacterial character.The antibacterial inspection were executed on three kinds of bacterial strains i.e.Escherichia Coli, Klebsiella Pneumoniae & Bacillus Subtilis with agar well diffusion method.The experimental compounds exposed variable degree of inhibition in opposition to different bacterial strains. The result of the antibacterial nature of methoxy and hydroxy substituted synthetic analogues and their complexes confirmed that the ligands and their complexes acquired almost equal antibacterial nature to that of recognized drug streptomycin. The activity is indicated in terms of diameter of zone of inhibition in mm. In all circumstances metal complexes acquired improved antibacterial character compared to that of synthetic diketone moiety. From which it can be understood that metal complexation intensify the activity.The results of the antibacterial examination are specified below.

Table 4.4.1 Antibacterial studies of 4a (L_1) and their Cu (II), Zn(II) & Ni(II) complexes.

Bacteria	Zone of inhibition in mm			
	L_1	$Ni(L_1)_2$	$Zn(L_1)_2$	$Cu(L_1)_2$
E Coli	15.7	18.1	17.1	18.9
Klebsiella	13	13.9	13.1	14.8
Bacillus	18.9	8.7	8.3	10.3
standard	20	20	20	20
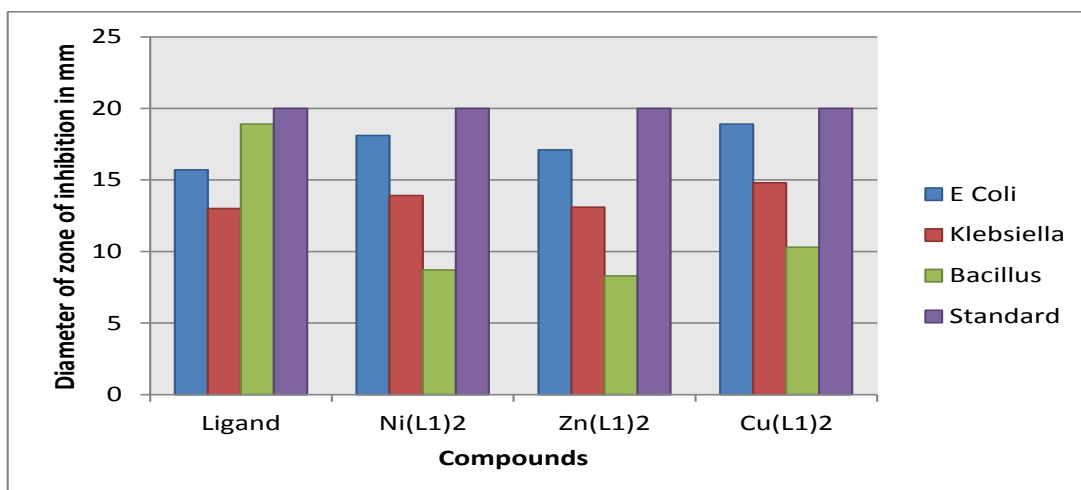
Fig.4.4.1. Antibacterial examination of 4a and their Cu (II), Zn (II) & Ni (II) complexes.

Table 4.4.2 Antibacterial studies of 4b and their Cu(II),Zn(II) & Ni(II) complexes

Bacteria	Zone of inhibition in mm			
	L_2	$Ni(L_2)_2$	$Zn(L_2)_2$	$Cu(L_2)_2$
E Coli	18.2	19.3	18.3	20.7
Klebsiella	16.5	17.7	17.1	18.2
Bacillus	12.1	13.4	12.9	14.6
standard	20	20	20	20
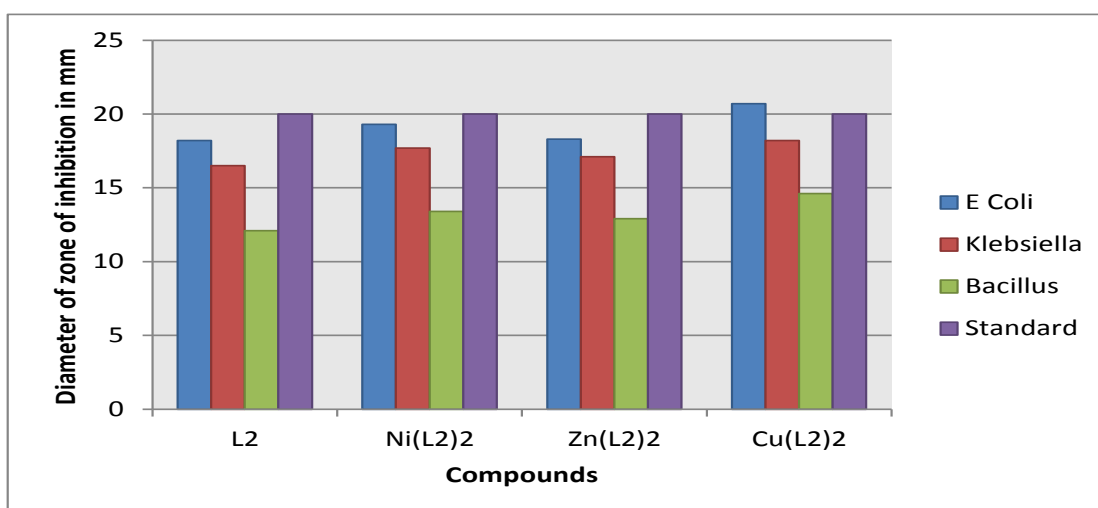
Fig. 4.4.2 Antibacterial studies of 4b   and their Cu(II),Zn(II) & Ni(II) complexes.

Table 4.4.3 Antibacterial studies of 4c and their Cu(II),Zn(II) & Ni(II) complexes

Bacteria	Zone of inhibition in mm			
	L_3	$Ni(L_3)_2$	$Zn(L_3)_2$	$Cu(L_3)_2$
E Coli	16.9	18.7	16.8	20
Klebsiella	14.4	16	14.9	17.2
Bacillus	10.2	12.5	11.7	13.3
standard	20	20	20	20
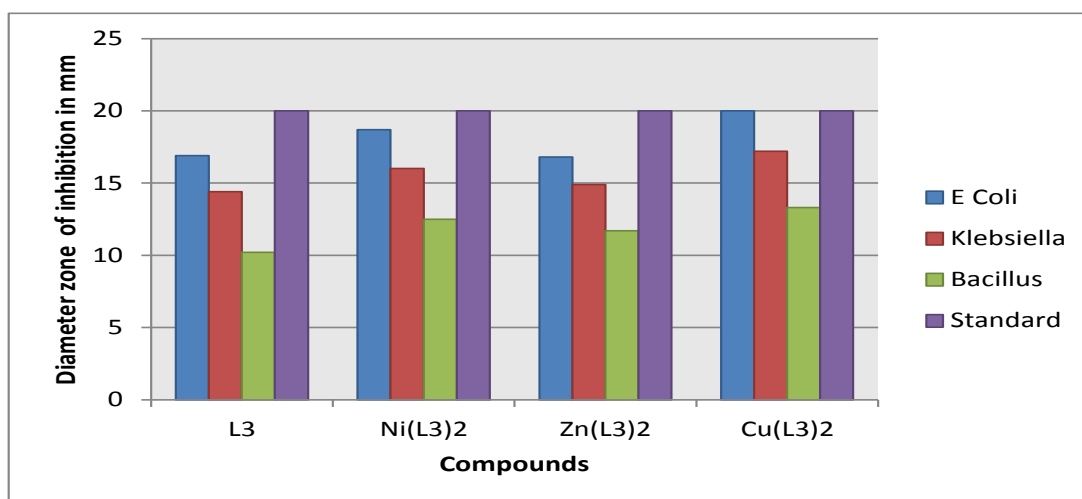
Fig. 4.4.3 Antibacterial studies of 4c and their Cu(II),Zn(II) & Ni(II) complexes.

All the ligands were fairly operative towards E.Coli bacterial strains generating a zone of inhibition of 15.7mm,18.2mm and 16.9 mm correspondingly..The synthetic diketone which doesn't contain any substituent on aromatic ring ie. (4E,6E)-4,6-bis(naphthalene-ylm ethylidene)cyclohexane -1,3 dione(4a),displayed minimum activity.

The activity of synthetic diketones analogues with various bacterial strains monitored the order E.Coli>Klebsiella>Bacillus.For all the three ligands,Cu(II) complexes possessed maximum antibacterial activity. This can be explained on the basis of chelation theory. They were thought to act by favoring the breakdown of permeability barrier of cell wall of microorganisms. Chelation reduces the polarity of metal ion considerably, mainly because of the partial sharing of its positive charge with donor groups and possible electron π delocalization on the whole chelate rings. The lipids and polysaccharides are some important constituent of cell wall and membranes, which are preferred for metalion interaction. In addition to this, cell wall also contains many

amino phosphates carbonyl and and cysteinyl ligands which maintain the integrity of the membrane by acting as a diffusion barrier and also provides suitable sites for binding. Chelation can considerably reduce the polarity of the metal ion, which in turn increases the lipophilic character of the chelate, and the interaction between metal ion and the lipid favoured. This may lead to breakdown of the permeability barrier of the cell, resulting in interference with the normal cell process. They lead to a zone of inhibition in the range 20mm in opposition to E.Coli which is identical that of reference drug. The Cu (II) complex of **4b**, created a zone of inhibition of 20.7mm against E.Coli which is better operative than the recognized drug.The Cu (II) complex of **4a**&**4c** was similarly extremely active towards Klebsiella species.

The Ni (II) complexes further displayed considerable activity next to Cu (II) complexes. The Ni (II) complexes of all synthetic analogues were truly active towards E.Coli bacteria making a zone of inhibition in the range 19.3mm.Out of the metal complexes, Zn (II) possessed minimal activity due to lesser lipophilicity of zinc complex compared to copper and nickel complexes.The metal complexes of (4E,6E)-4,6-bis(methoxy naphthalene-ylm ethylidene) cyclohexane -1,3 dione (**4b**), presented more activity to all the three kind of bacterial strains relating with metal complexes of other two synthetic diketones ie. (4E,6E)-4,6-bis(naphthalene-ylm ethylidene)cyclohexane-1,3-dione(**4a**),and(4E,6E)-4,6-bis(hydroxynaphthalene-ylmethylidene)cyclohexane-1,3-dione (**4c**), may be due to the presence of more electron releasing methoxy moiety in **4b**.

SECTION-V

ANTIFUNGAL EXAMINATIONCURCUMINOID EQUIVALENTS WITH POLY NUCLEAR RING AND METAL COMPLEXES

4.5.1 **Antifungal nature of curcuminoid analogues with polynuclear ring**

In vitro antifungal functioning of 4a,4b,4c and 4d and their metal complexes were checked. The funguses used for the current study were Aspergillus Niger, Penicillium Chrysogenum andAlternaria Alternate. In the present sector antifungal study was done by Kirby Baurer method.A control disc was retained without any application test compounds.The readings of the examination are given in **Table 4.5.1, 4.5.2, 4.5.3 and 4. 5.4.**

Table 4.5.1   Antifungal studies of 4a (L_1) and its Ni (II) complexes.

Fungi	Diameter of zone of inhibition in mm					
	L_1			$Ni(L_1)_2$		
	100μg	250μg	500μg	100μg	250μg	500μg
Aspergillus	14.7	16.8	17.5	15.8	20	21.5
Penicillium	13.2	15	16.9	14.6	18.3	21
Alternaria	12.6	16.8	16.4	14	17.3	20.1

Table 4.5.2 Antifungal studies of 4b (L_2) and its Ni(II) complexes.

Fungi	Diameter of zone of inhibition in mm					
	L_2			$Ni(L_2)_2$		
	100μg	250μg	500μg	100μg	250μg	500μg
Aspergillus	16.2	17.7	19.2	18.8	20.7	24
Penicillium	15.1	16.3	18.4	16.9	19.9	23.7
Alternaria	13.5	15.9	17	15.9	17.6	22

Table 4.5.3 Antifungal studies of 4c (L$_3$)   and its Ni(II) complexes.

Fungi	Diameter of zone of inhibition in mm					
	L$_3$			Ni(L$_3$)$_2$		
	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	13	14.5	17	16.8	18	19.7
Penicillium	12	14.7	15.3	14.2	16.3	18.2
Alternaria	12.5	13	15.9	13	17.1	19

Table 4.5.4.Antifungal readings of 4d (L$_4$) and Ni(II) complexes.

Fungi	Diameter of zone of inhibition in mm					
	L$_4$			Ni(L$_4$)$_2$		
	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	16	17.3	19	18.2	19.7	23
Penicillium	14.7	16.2	18.4	17.2	18.6	21.7
Alternaria	12.8	14.8	18	16.4	17.9	20

Their antifungal activity depends on the groups existing on the aryl part.From these results we can conclude that there is a positive response in the antifungal activities against higher concentration of both the ligand and metal chelates.For the ligand(4E, 6E)-4,6-bis (methoxy naphthalene-ylm ethylidene)cyclohexane -1,3 dione (4b), extreme activity was gotten with Aspergillus species with a zone of inhibition of 19.2mm. Among the synthetic ligands, the one with unsubstituted aryl compound were found to be least antifungal agent compared to methoxy substituted one.The result discloses that the test drug supported by methoxy group was found to be highly active. This is because in this compound the methoxy group is present in para position of aryl ring. Thus it can be stated that the presence of methoxy group in the aryl ring of these type of unsaturated 1, 3-diketones is a structural requirement for their biological activities. The Ni (II)

complexes of **4a**, **4b**, **4c**&**4d** showed extremely significant activity. The nickel complex of **4b** was exactly powerful against Aspergillus fungi generating a zone of inhibition of 24mm which is better than that made by the reference drug fluconazole. The nickel complex of **4d** had also shown noticeable activity to all fungi species. Its functioning is equivalent to that of recognized drug which creates a zone of inhibition of 23mm. A relative study of the antifungal nature of Ligands and their nickel Complexes at 500 μg concentration is given below in **Figure4.5.1**.
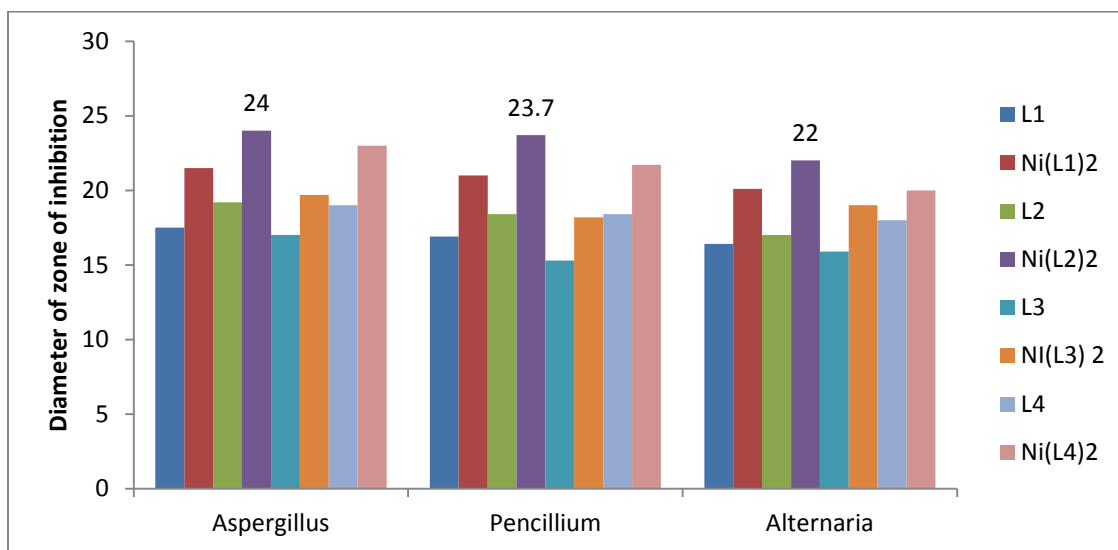
Fig4.5.1 Relative study of 4a, 4b, 4c, 4d and their nickel Complexes at 500μg conc.

PART-V
CONCLUSION

CONCLUSION

In the present work fourteen (4E,6E)-4,6-bis (aryl)cyclohexane -1,3 diones synthesized and they were characterized by using different spectral procedures. Depending on the type of aryl moiety present the ligands were categorized to four different groups. Group 1 with electron releasing moiety are on the phenyl ring, Group 2 with electron withdrawing moiety on phenyl ring, Group 3 heterocyclic thiophenyl ring, Group 4 with polynuclear naphthyl and anthracenyl cyclic rings on the ligands.

The keto-enol tautomeric forms of the (4E,6E)-4,6-bis(aryl)cyclohexane-1,3-diones were confirmed by spectral analysis. The entire unsaturated ligands develop complexes with transition metals keeping a stoichiometry [ML$_2$]. Here the unsaturated diketone moiety serves as excellent chelating operator. In the complex formation process, the oxygen atoms which are present on the diketo moiety are bonded to the metal. This is one of the strong proofs for the formation of metal complex. From the spectral information we can conclude that only the oxygen atoms were involved in complexation whereas the common binding group such as S excluded from this process. The unsaturated cyclic ligands and its respective complexes possess substantial antimicrobial character and they also checked for its *in vitro* and *in vivo* anticancer performance. All the (4E,6E)-4,6-bis(aryl)cyclohexane -1,3 diones and their metal complexes show significant antibacterial, antifungal activity. They also show significant *in vitro* and *in vivo* anticancer activity. The whole data were projected here.

Antibacterial studies

The antibacterial analysis of (4E,6E)-4,6-bis (aryl)cyclohexane -1,3 diones conducted upon three bacterial strains namely Escherichia Coli, Klebsiella Pneumoniae & Bacillus Subtilis.Excellent activity were reported for all the unsaturated ligands and their chelates. For the comparison of result, we checked the data obtained from the reference drug. From the results obtained it is understood that complexation process greatly enhances antibacterial properties of unsaturated cyclic ketones. They display significant antibacterial character upon Escherichia Coli group and reasonable activity upon Klebsiella Pneumoniae & Bacillus Subtilis. Group 3 thiophene based ligands and their metal chelates exhibit minor antibacterial nature than another three groups. A

metaphorical analysis of antibacterial nature of (4E,6E)-4,6-bis(aryl)cyclohexane-1,3-diones of Type 1,2 & 4 upon the creature Escherichia Coli was performed and it was dectected that the extreme activity was acquired with ligand based on anthracene. This is because of electron releasing groups and poly nuclear moiety on the ligand. The ligand,(4E,6E)-4,6-bis(2,4dihydroxybenzylidene)cyclohexane-1,3-dione (**4c**) was also showed noticeable activity towards the bacterial stains. In the compound **4b** an OH group is present in the ortho and para positions of the aryl ring. In the curcuminoids also, it has been reported that their antibacterial and other biological activities depend to a large extend on the presence of groups such as OH in the aryl ring. The results are summarized in **Fig.5.1.**

Out of the three complexes, Copper chelates exhibited significant activity towards all bacterial species than the zinc and nickel complexes. The supreme activity observed for Cu(II)complex of (4E,6E)-4,6-bis (methoxy naphthalene-ylm ethylidene)cyclohexane -1,3 dione (**4b**) with a zone of inhibition of 20.7mm. This can be explained on the basis of chelation theory. They were thought to act by favoring the breakdown of permeability barrier of cell wall of microorganisms. Chelation reduces the polarity of metal ion considerably, due to the partial sharing of its positive charge with donor groups and possible electron π delocalization on the whole chelate rings. The lipids and polysaccharides are some important constituent of cell wall and membranes, which are preferred for metalion interaction. In addition to this, cell wall also contains many amino phosphates carbonyl and and cysteinyl ligands which maintain the integrity of the membrane by acting as a diffusion barrier and also provides suitable sites for binding. Chelation can considerably reduce the polarity of the metal ion, which in turn increases the lipophilic character of the chelate, and the interaction between metal ion and the lipid favoured. This may lead to breakdown of the permeability barrier of the cell, resulting in interference with the normal cell process.

Fig.5.1 Antibacterial study of 1b,2b,3b,4b & its Cu (II) chelates.

Antifungal activity

The (4E,6E)-4,6-bis (aryl) cyclohexane-1,3-dione and its metal chelates is analyzed for its antifungal character upon the fungi creature namely Aspergillus niger, Penicillium chrysogenum andAlternaria alternate. They applied as drugs in different concentrations such as [100, 250, 500μg/ml] and checked the average diameter of zone of inhibition. From the analysis it is found that benzaldehyde derivatives with both electron releasing and electron withdrawing group substituted ones display minimal activity. Thiophene substituted, group 3 ligands exhibited reasonable activity upon the fungi species. Maximum value was obtained with nickel complex of **4b** and **4d**. This may due to the polynuclear nature of the ligand.when rings are added to benzene ring, the value generally increases. With anthracene derivatives maximum results were obtained. A comparison of their activities is given below in **Fig.5.2.**

In general Group 1 & 2 (4E,6E) 4,6bis(aryl)cyclohexane-1,3diones and their chelates exhibits significant activity upon fugal species. from the analysis it is found that group 2 ligands with electron withdrawing substituent on the benzaldehyde display more activity than the group 1 ligands with electron releasing substituents. Zone of inhibition of 18mm was found with the ligand 3c and 19.1 mm with their metal chelates.In the

case of group 4 ligands , 4d exhibited more activity than the others. Its metal chelates exhibited a zone of inhibition of 23mm.
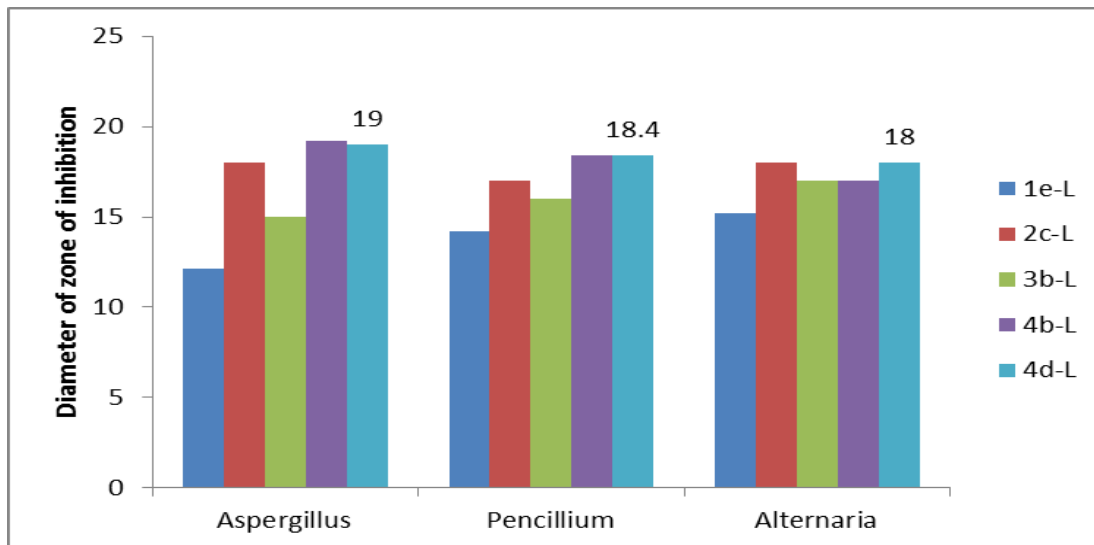
Fig.5.2 AntIfungal study of 1e,2c,3b,4b & 4d ligands.

In vitro cytotoxicity study

In vitro cytotoxic activity of (4E,6E)-4,6 bis (aryl) cyclohexane 1,3 dioneand metal complexes were studied for short term in vitro cytoxicity using DLA and EAC cancer cells. The cytotoxic features was dealt with, in terms of the % cell death brought up by them.At higher level concentration ie at 200 μg/ml they showed maximum activity. In group 1 the ligand **1c** is more active than others. This may be due to the presence of two methoxy group in ligand **1c**.When electron with drawing substituents were present in the ligand its cytotoxic character is minimized shortly. While comparing the result of group 1 & 2 more active one is **1c.** Among the ligands tested for cytotoxic character, ligand, **4d** from group 4 displays more activity than the other group ligands. The ligand possesses an anthracenyl ring in the system and it showed 67% of cell death. Apart from **4d** all other ligands needed a drug concentration more than 200 μg/ml for 50% cell death. The ligand with heterocyclic thiophenyl ring,hydroxyl & methoxy substituted naphthyl ring were too significant in their cytotoxic character. It may be assumed that **3a**&**4d** are more advanced ligands for in vitro cytotoxic analysis against EAC &DLA.The activities of the ligands & complexes are compared and projected in **Fig.5.3**
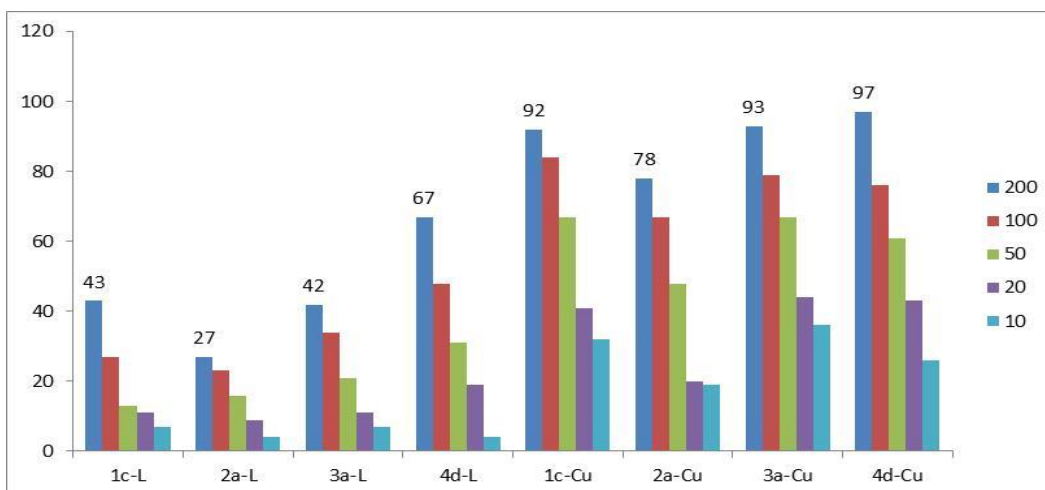
Metal chelates of copper, zinc and nickel also analyzed for cytotoxic activity and found that they are more effective than the unsaturated diketones. When metal complexes are considered it is found that copper chelates exhibited remarkable activity than the other complexes. Supreme activity was for the Cu (II) complex of **4d** having 97% cell death. The thiophene, dimethoxy and dihydroxy based copper chelates also display noticeable activity in the analysis. In short, chelation considerably affects the cytotoxic properties of diketones. Plasma copper concentration increases in neoplastic and autoimmune diseases as an immune mediated physiological response to the disease state. Treatment with copper complexes is a therapeutic support of this increase in plasma copper and the attendant distribution of copper too affected tissues to enable de-novo synthesis of copper dependent enzymes required to bring about remission by reestablishing normal tissue function.

In vivo antitumour studies

Those drugs which display significant activity in invitro analysis selected for in vivo studies and the drug concentration is similar to in vitro analysis. It is observed that period of life span of tumour burden mice increases considerably and it is compared with the result obtained from reference drug. Outstanding results were found at a con. 20μg/ml. among the ligands tested **4d** giving maximum results and % of ILS is 72.25%. The ligand **3a** also exhibited significant extension of life span and it is 50.34 %. Like in in vitro analysis, here also Metal chelation increases the life span of mice especially the copper complex. The metal chelates were introduced as drugs with various

concentrations and the noticeable result was found at a concentration 20 μg/ml. A comparative study of %ILS for ligands and their Copper complexes are given in **Fig.5.4**.
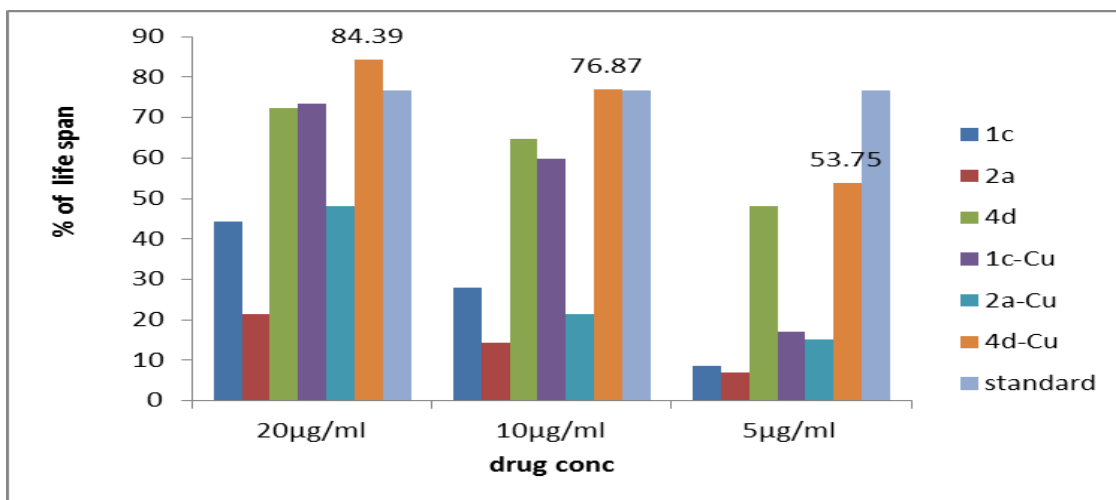
Fig.5.4   %   ILS of tumour bearing mice by the administration of ligands & chelates.

Effect of compounds on solid tumour development

The potency of compounds on solid tumour growth was investigated with ligands **4d** and **3a** and their Cu (II) & Zn (II) chelates .The data are equivalent with the results of the in vivo studies carried out in tumour burden mice. It is observed that the ligand **4d** and its copper complex are found to be extremely efficient in decreasing the solid tumour volume.The application of both ligand and complex as drugs to mice substantially reduced the tumour volume to 3.01 and 1.335 cm^3 .The reference drug treated mice group exhibited the reduced tumour volume 2.782cm^3 while the   control group gave a tumor volume of   6.285 cm^3 .For ligand **3a** treated group the tumour volume was 3.11 cm^3 and for complex treated group the volume was 2.21 cm^3.So the ligand 3a with thiophenyl ring and its Zn(II) complex was also effective in reducing solid tumour volume.

Thus **1c**,**3a**, **4b** and **4d** and their metal chelates displayed significant activity to various biological screenings. More studies have to be conducted in order to get the optimum concentration with maximum result and also to extend the work to human beings.

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