"SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL APPLICATIONS OF TRANSITION METAL CHELATES OF 1,7-DIARYL HEPTANOIDS"

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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CERTIFICATE

This is to certify that the thesis entitled "Synthesis, Characterization and Biological Applications of Transition metal chelates of 1,7-Diarylheptanoids" is an authentic record of the research work carried out by SEENA THOMACHAN, 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. JOHN .V. D

(Supervising Teacher)

DECLARATION

I, SEENA THOMACHAN hereby declare that the thesis, entitled “Synthesis, Characterization and Biological Applications of Transition metal chelates of 1,7-Diarylheptanoids” 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. John. V. D, Associate Professor (Rtd), Department of Chemistry, Christ College, Irinjalakuda, Kerala and further that no part thereof has been presented before for any other Degree.

SEENA THOMACHAN

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DEDICATED

TO

MY FAMILY

PREFACE

The use of plants and their active principles in the prevention and treatment of chronic diseases is based on experience of traditional system of medicine. The potential of medicinal plants as source for new drugs is still largely unexplored. Now a days there is wide spread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable compared to synthetic drugs. One among the few medicinal plants that has been the subject of scientific investigation is the Turmeric (*Curcuma longa* Linn).Turmeric and its active chemical constituents, curcuminoids have been reported to possess a wide spectrum of biological actions such as antinflammatory, antioxidant, anticancer, antidiabetic, antiallergic, antiviral, antiprotozoal, antibacterial and antifungal activities. Curcumin , the bioactive yellow orange pigment is the most important fraction which is responsible for the biological activities of turmeric. The utility of curcumin is limited by its colour, lack of water solubility and relatively low invivo bioavailability. Structurally curcuminoids are 1,7- diaryl heptanoids .They are a group of naturally occurring 1,3 – diketones in which diketo function is directly attached to olefinic groups. It has three chemical entities in its structure, two aromatic ring systems containing o-methoxy phenolic groups connected by a seven carbon linker consisting of an α - β unsaturated β- diketone moiety. They are ideally suited to act as chelating ligands towards a variety of metals and to form complexes similar to diketones. Literature review has revealed the enhanced biochemical activities of synthetic analogues of curcuminoids and their metal complexes especially as cytotoxic, antibacterial and antifungal agents. The present study is mainly on the synthesis and characterization of a series of curcuminoid analogues (1,7-diaryl heptanoids) and their metal complexes with Cu(II) ,Zn(II),Ni(II) and VO(IV).The cytotoxic, antitumour, antibacterial and antifungal activities of these compounds and their metal complexes were also studied. The thesis is divided into four parts.

Part I. Introduction

The medicinal properties of Turmeric have been attributed mainly due to Curcumin, the bioactive yellow orange pigment. Curcumin has already been the subject of several clinical trials and has been reported to possess medicinal properties. Curcumin is one of the most potent and multi targeting phytochemical against a variety of diseases. Synthetic chemical modifications of curcumin have been studied intensively to identify compounds with similar or enhanced properties of curcumin. Fourteen curcuminoid analogues structurally related with natural curcumin were synthesized in the present work. In the synthesized compounds the α,β unsaturated diketo moiety is retained as such without modification and the phenyl ring part in natural curcumin has been modified .The phenyl ring part has been substituted with heterocyclic rings, polynuclear rings, substituted polynuclear rings trisubstituted and disubstituted phenyl rings with substituents different from that of natural curcumin. The structural and spectral properties were studied by UV, IR, ^{1}H NMR,^{13}C NMR,2D-COSY NMR, Mass Spectral techniques etc. Research in the field of coordination chemistry of biologically important ligands and their synthetic analogues has gained considerable momentum in recent years. It has been revealed that the biological significance of these ligands is enhanced by complex formation with metal ions. Curcuminoids are excellent chelating ligands which can bind with metal ions to form stable metal complexes. Metal complexation of these α,β –unsaturated 1,3-diketones has led to effective changes in their biological activities including antitumour ,antibacterial and antifungal activity. In the present investigation transition metal ions namely Cu^{2+},Zn^{2+},Ni^{2+} and VO^{2+}were complexed 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

This part includes the review of literature related with the chemical and biological studies of curcuminoids, its allied derivatives and metal chelates. The biological activities of curcuminoids like antiinflammatory, antioxidant, antiprotozoal, nematocidal, anti-bacterial antiviral, antitumour activity etc are discussed in this part. The importance of synthetic analogues of curcumin and the metal complexes as biologically significant agents have been well established. Studies related with the cytotoxic nature of curcuminoid analogues and their metal chelates have been extensively discussed in this part.The enhanced pharmacological significance of the compounds due to complexation has also been revealed in this part.This part explains the necessity of synthesis of curcumin analogues and their metal chelates and their biological significance.

Part III. Materials, Methods and Experimental techniques

This part is a general description on various chemicals and methods employed, instruments used and various experimental techniques. The methods used for the synthesis of curcuminoid analogues ,their transition metal chelates and purification of compounds are given.Various spectral techniques involved in characterization of the compounds have been explained. The biological studies conducted include Invitro cytotoxic study, Invivo antitumour study, effect of compounds on solid tumour, Antibacterial and Antifungal studies. Materials, cell lines, animals, chemicals, methods etc employed in the studies are given.

Part IV. Synthesis, Characterization and Biochemical activities of 1,7-diaryl heptanoids

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

Chapter I. The chapter deals with the Synthesis, Characterization and Biochemical activities of methyl substituted 1,7-diaryl heptanoids and their Transition metal chelates.

Section I: Synthesis and Characterization of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(1a) and 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione(1b).

Section II: Synthesis and Characterization of Transition metal chelates of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(1a) and 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione(1b) with Cu(II),Zn(II),Ni(II) and VO(IV).ESR Spectrum of Cu(II) complex is also included.

Section III: Antitumour studies of Methyl substituted 1,7-diaryl heptanoids and the transition metal chelates.The studies include Invitro cytotoxic study of ligands and their metal complexes [Cu(II),Zn(II),Ni(II) and VO(IV)]by Trypan blue dye exclusion method. *In vivo* antitumour studies were conducted in mice with the ligand 1,7-bis(2,5-dimethyl phenyl)-1,6-heptadiene-3,5-dione(**1b**) and its Cu(II) & VO(IV) complexes. The ligands 1a and 1b and their copper complexes were used to find the effect on solid tumour development in mice.

Section IV: Antibacterial studies of methyl substituted 1,7-diaryl heptanoids and their Cu(II),Zn(II) and Ni(II) metal complexes.

Section V: Antifungal studies of methyl substituted 1,7-diaryl heptanoids and their Zn(II) and VO(IV) metal complexes.

Chapter II. The chapter deals with the Synthesis, Characterization and Biochemical activities of 1,7-dithiophenyl heptanoids and their Transition metal chelates.

Section I:Synthesis and Characterization of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (**2a**) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (**2b**).

SectionII: Synthesis and Characterization of Transition metal chelates of 2a and 2b with Cu(II), Zn(II), Ni(II) and VO(IV).

Section III: Antitumour studies of 1,7-dithiophenyl heptanoids and the transition metal chelates. The studies include Invitro cytotoxic study of ligands and their metal complexes [Cu(II), Zn(II), Ni(II) and VO(IV)]by Trypan blue dye exclusion method towards DLA and EAC cell lines. *In vivo* antitumour studies were conducted in mice with the ligands 2a and 2b

and their Cu(II) complexes. The effect of ligands 2a and 2b and their copper complexes on solid tumour development in mice were also studied.

Section IV: Antibacterial studies of 1,7-dithiophenyl heptanoids and their Zn(II),Ni(II) and VO(IV) metal complexes.

Section V: Antifungal studies of 1,7-dithiophenyl heptanoids and their Zn(II) and VO(IV) metal complexes.

Chapter III. The chapter deals with the Synthesis, Characterization and Biochemical activities of chloro substituted 1,7-diaryl heptanoids and their Transition metal chelates.

Section I: Synthesis and Characterization of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(**3a**),1,7-bis(2-chloro phenyl)hepta-1,6-diene-3,5-dione(**3b**), and 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione(**3c**).They were characterized by UV, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral techniques.

Section II: Synthesis and Characterization of Transition metal chelates of 3a, 3b and 3c with Cu(II),Zn(II),Ni(II) and VO(IV).

Section III: Cytotoxic and Antitumour studies of chloro substituted 1,7-diphenyl heptanoids and their transition metal chelates.The studies include Invitro cytotoxic study of ligands and their metal complexes [Cu(II),Zn(II),Ni(II) and VO(IV)]by Trypan blue dye exclusion method towards DLA and EAC cell lines. *In vivo* antitumour studies were conducted in mice with the ligand 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(**3a**)and their Cu(II) and Zn(II)complexes.

Section IV: Antibacterial studies of chloro substituted 1,7-diphenyl heptanoids and their Zn(II),Cu(II) and VO(IV) metal complexes.

Section V: Antifungal studies of chloro substituted 1,7-diphenyl heptanoids and their VO(IV) metal complexes.

Chapter IV. The chapter deals with the Synthesis, Characterization and Biochemical activities of 1,7-diaryl heptanoids with di and tri substituted phenyl ring and their Transition metal chelates.

Section I: Synthesis and Characterization of 1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione(**4a**), 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione(**4b**), and 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione(**4c**) .They were characterized by UV, IR, ^{1}H NMR, ^{13}C NMR ,2D COSY and Mass spectral techniques.

Section II: Synthesis and Characterization of Transition metal chelates of 4a ,4b and 4c with Cu(II),Zn(II),Ni(II) and VO(IV).The metal chelates were also characterised by various spectral techniques .

Section III: Cytotoxic and Antitumour studies of curcuminoid analogues with di and tri substituted aryl rings and their transition metal chelates. The studies include Invitro cytotoxic study of ligands and their metal complexes [Cu(II),Zn(II),Ni(II) and VO(IV)]by Trypan blue dye exclusion method towards DLA and EAC cell lines. *In vivo* antitumour studies were conducted in mice with the ligands 1,7-bis(2,4-dihydroxyphenyl)hepta-1,6-diene-3,5-dione(4b), 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione(4c) and their Cu(II) complexes.

Section IV: Antibacterial studies of 1,7-diphenyl heptanoids with di and tri substituted phenyl ring and their Zn(II),Cu(II) and VO(IV) metal complexes.

Section V: Antifungal studies of 1,7-diphenyl heptanoids with di and tri substituted phenyl ring and their VO(IV) metal complexes.

Chapter V. The chapter deals with the Synthesis, Characterization and Biochemical activities of 1,7-dianthracenyl heptanoids and their Transition metal chelates.

Section I: Synthesis and Characterization of 1,7-bis(9-anthracenyl)hepta-1,6-diene-3,5-dione(**5a**) .The ligand was characterized by UV, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral techniques.

Section II: Synthesis and Characterization of Transition metal chelates of 5a with Cu(II),Zn(II),Ni(II) and VO(IV).The metal chelates were also characterised by various spectral techniques .

Section III: Cytotoxic and Antitumour studies of curcuminoid analogues with anthracenyl ring and its transition metal chelates. The studies include Invitro cytotoxic study of ligand and its metal complexes [Cu(II),Zn(II),Ni(II) and VO(IV)]by Trypan blue dye exclusion method towards DLA and EAC cell lines. *In vivo* antitumour studies were conducted in mice with the ligand and its Cu(II) and VO(IV) complexes. Invivo cytotoxic study on solid tumour development was conducted with the ligand and its Cu(II) complex.

Section IV: Antibacterial studies of 1,7-dianthracenyl heptanoid and their Zn(II),Cu(II) and VO(IV) metal complexes.

Section V: Antifungal studies of 1,7-dianthracenyl heptanoids and their VO(IV) metal complexes.

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

Section I: Synthesis and Characterization of 1,7-dinaphthyl-1,6- heptadiene-3,5-dione(**6a**), 1,7-bis(2-methoxynaphthyl)1,6-heptadiene-3,5-dione(**6b**),1,7-bis(2-hydroxynaphthyl)1,6-heptadiene-3,5-dione(**6c**).They were characterized by UV, IR, ^{1}HNMR, ^{13}CNMR ,2D COSY and Mass spectral techniques. Thermogravimetric and magnetic studies were done.

Section II: Synthesis and Characterization of Transition metal chelates of 6a, 6b and 6c with Cu(II), Zn(II), Ni(II) and VO(IV).The metal chelates were also characterised by various spectral techniques .ESR Spectrum of Cu(II) complex is included.

Section III: Cytotoxic and Antitumour studies of curcuminoid analogues with substituted naphthyl rings and their transition metal chelates. The studies include Invitro cytotoxic study of ligands and their metal complexes [Cu(II), Zn(II), Ni(II) and VO(IV)]by Trypan blue dye exclusion method towards DLA and EAC cell lines. *In vivo* antitumour studies were conducted in mice with the ligands 1,7-bis(2-methoxynaphthyl)1,6-heptadiene-3,5-dione(6b), 1,7-bis(2-hydroxynaphthyl)1,6-heptadiene-3,5-dione (6c) and their Cu(II) complexes.

Section IV: Antibacterial studies of 1,7-dinaphthyl heptanoids and their Zn(II),Cu(II) and VO(IV) metal complexes.

Section V: Antifungal studies of 1,7-dinaphthyl heptanoids and their VO(IV) metal complexes.

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CERTIFICATE

The Institutional Animal Ethics Committee has approved the project of Mrs. Seena Thomachan entitled "Synthesis, Characterization and Biological Applications of Transition metal chelates of 1, 7 – Di Aryl Hepatanoids, approved No. ACRC/IAEC/16-01 / (1) under the direction of Dr. Ramadasan Kuttan.

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1. **Seena Thomachan**, S.Sindhu and John V D, Antibacterial and Antifungal Activities of Curcuminoid Analogue with Chloro Substituted Phenyl Ring and the Transition Metal Chelates, *International Journal of Science and Research* ,4(9),2015,1676-1680.
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Paper Communicated

1. **Seena Thomachan**, S.Sindhu and John V D.**S**ynthesis, Characterization, Antibacterial and Cytotoxic activity of Curcuminoid analogues with substituted Naphthyl ring and their Transition metal chelates , *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry.*

ABBREVIATIONS

ROS	—	Reactive Oxygen Species
COX-2	—	Cyclooxygenase 2
LOX	—	Lipoxygenase
NF-κB	—	Nuclear Factor kappa B
cPLA	—	Cytosolic Phospholipase
HBC	—	Hydrazinobenzoylcurcumin
DMC	—	Demethoxy curcumin
BDMC	—	BisDemethoxy curcumin
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

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PART-I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Plants have been used as a source of food and medicine since ancient times. Today the universal movement towards more natural life style has brought about resurgence of interest in medicinal plants as they are capable of bringing the body into harmony and health. The use of plants and their active principles in the prevention and treatment of chronic diseases [1-4] is based on experience of traditional system of medicine. For centuries medicinal plants have been used throughout the world for the treatment of various diseases. The prospective of medicinal plants[5-7]as source for new drugs is still largely unexplored. Now a days there is wide spread interest in drugs derived from plants. This awareness primarily stems from the belief that green medicine is safe and dependable compared to synthetic drugs. One among the few medicinal plants that has been the subject of scientific exploration is the Turmeric (*Curcuma longa* Linn).

Curcuma longa is a medicinal plant[8] that belongs to Zingiberaceae family (Chattopadhyay *et al.*, 2004). Turmeric powder, obtained from the rhizome of *C. longa*, is commonly used as a spice, food preservative, and food-coloring agent.[9-11](Aggarwall *et al.*, 2007; Di Mario *et al.*, 2007; Menon and Sudheer, 2007). It also has a long history of curative use (Chattopadhyay et al., 2004). Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; Diferuloylmethane], a yellow bioactive pigment, is the major component of turmeric [12,13] (Mohammadi *et al.*, 2005;Menon and Sudheer, 2007; Hatcher *et al.*, 2008). Curcumin exhibits a wide range of biological actions such as antinflammatory[14,15](Punithavathi *et al.*,2000;Siddiqui *et al.*, 2006), antioxidant(Mohammadi *et al.*, 2005; Menon and Sudheer, 2007),anticancer [16] (LoTempio et al., 2005), antidiabetic (Aggarwal et al., 2007),antiallergic[17] (Suzuki *et al.*, 2005), antiviral[18] (Si *et al.*, 2007), antiprotozoal [19] (Reddy *et al.*, 2005) and antifungal activities (Chattopadhyay *et al.*, 2004).The anti-bacterial activity of curcumin has also been established[20] (Chattopadhyay *et al.*, 2004; Di Mario *et al.*, 2007;Rai *et al.*, 2008).

The above mentioned activities have been confirmed both in cultured cells and in animal models [21-24], and have paved the way for ongoing human clinical trials [25].

Curcumin, a naturally occurring highly lipophilic molecule has wide spectrum of pharmacological activities[26] .The curative activity of Curcumin is ascribed mainly to its chemical configuration[27-29] and distinctive physical, chemical, and biological properties. It is a diferuloyl methane molecule [1,7-bis (4-hydroxy-3- methoxyphenyl)-1,6-heptadiene-3,5-dione)]with two ferulic acid residues connected by a methylene bridge. It has three principal functionalities: an aromatic o-methoxy phenolic group, α, β-unsaturated β-diketo moiety and a seven carbon linker[30,31]. Extensive studies in the last two decades has given confirmation for the role of these different functional groups in its fundamental biological activities [32,33]. However, its limited aqueous solubility and degradation at alkaline pH restricts its bioavailability [34-39]. Due to the numerous therapeutic activities ascribed to curcumin, however, there is an intense search for a "super curcumin" without these problems. Various approaches are being sought to triumph over these limitations. These include discovery of natural curcumin analogues from turmeric; finding natural curcumin analogues made by Mother Nature;synthesis of "man-made" curcumin analogues; reformulation of curcumin with inhibitors of metabolism(e.g., piperine); preparation of liposomal [40] and nanoparticle formulations[41,42] of curcumin; conjugation of curcumin prodrugs;preparation of micellar formations[43] and connecting curcumin with polyethylene glycol [44].New allieds with enhanced activity[45-49] are being synthesized with modifications on specific functionalities of curcumin. Structural homologues involving modification of all the groups present in curcumin have been prepared. Keeping in view the significant importance of curcumin as antibacterial, antifungal and cytotoxic agents, this research study was conducted to support the possibility of using synthetic curcuminoid analogues and its transition metal complexes as alternatives of curcumin. **Fourteen curcuminoid analogues structurally related with**

natural curcumin were synthesized in the present work.In the synthesized compounds the α,β unsaturated diketo moiety is retained as such without modification and the phenyl ring part in natural curcumin has been modified. The phenyl ring part has been substituted with heterocyclic rings,poly nuclear rings, substituted polynuclear rings,trisubstituted and disubstituted phenyl rings with substituents different from that of natural curcumin. Pabon in 1964 developed a general method[50] for synthesizing curcuminoids and related 1,7-diaryl heptanoids in good yields from aromatic aldehyde, acetyl acetone, B_2O_3 in presence of n-butyl amine and tri(sec-butyl)borate. In this present work fourteen different aromatic aldehydes were condensed with the 1,3-diketone (acetyl acetone) in dry ethyl acetate medium and the suitable temperature range for condensation was 80-100 ^{0} C.

The series of curcumin analogues prepared were characterized both chemically and biologically.The structural and spectral properties[51,52] were studied by UV,IR,^{1}H NMR,^{13}C NMR,2D-COSY NMR,Mass Spectral techniques etc. Spectral analysis established that the 1,7-diaryl heptanoids synthesized exist entirely in the intramolecularly hydrogen bonded enol form.Thermogravimetric studies were also conducted. **Biological activities investigated in the present work include Cytotoxic activity, antitumour nature, antibacterial activity and antifungal activity of curcumin analogues.**

Cancer chemopreventive effects of curcumin and its analogues

Chemoprevention [53]which is defined as the use of non toxic natural or synthetic chemicals to interfere in multistage carcinogenesis has emerged as a promising and realistic medical approach to reduce the risk of cancer. Curcumin is one of the most widely investigated and well defined chemopreventive phytochemical[54].

The anti-carcinogenic effects of curcumin have been investigated in several animal tumour systems[55].Chemopreventive studies are mainly conducted in animal models They provide an effective means of identifying compounds which can be used safely and also provide an

information base for developing intervention trials in humans.Recent studies have found that curcumin has a dose dependent chemopreventive effect in several animal tumour bioassay systems including colon[56], intestinal[57], stomach [58], oesophageal [59] and oral[60] carcinogenesis. It has been shown to reduce tumours induced by benzapyrene and 7, 12 dimethyl benzaanthracene[61,62] (Singh *et al.*, 1998; Deshpande *et al.*, 1997; Azuine and Bhide, 1992),tumour promotion induced by phorbol esters [63] (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 [64](Huang *et al.*,1994). Low incidence of bowel cancer in Indians has been attributed to the use of turmeric in Indian cookery [65] (Mohandas and Desai, 1999).Administration of synthetic curcumin in the diet during the progression stage appreciably inhibited the multiplicity of persistent adenocarcinomas of the colon [66] (Kawamori *et al.*,1999). Curcumin has been demonstrated to induce apoptosis in a variety of cells including prostate cancer cells [67] (Dorai *et al.*, 2001).It has also been shown to affect the activity of a number of enzymes such as cyclooxygenase [68](Zhang *et al.*, 1999), protein kinase C [69] (Liu *et al.*, 1993) and protein tyrosine kinases [70] (Chen and Huang, 1998). Recently, it has been suggested that curcumin affected arachidonic acid metabolism by blocking the phosphorylation of cytosolic phospholipase (cPLA(2)) and decreasing the expression of cyclooxygenase-2 (COX-2). Furthermore, it also inhibited catalytic activities of 5-lipoxygenase (LOX) [71] (Hong *et al.*, 2004). These activities may contribute to the anti-inflammatory and anti-carcinogenic actions of curcumin and its analogues. (Hong *et al.*, 2004).

Curcumin was found as a good anti-angiogenesis agent[72], explaining its chemopreventive effect at the level of tumour promotion. Anticancer effect of curcumin seems to be potentialized in the presence of oestrogen in breast cancer cells and it inhibits genes which are under the influence of the oestrogen receptor [73]. It allows sensitising ovarian cancer cells

to cisplatin, enhancing chemotherapeutic treatment [74]. Numerous studies have reported that curcumin has potential against several cancers including leukemia, lymphoma, melanoma, and sarcoma,as well as gastrointestinal, genitourinary, breast, ovarian, head and neck, lung, and neurological cancers [75](Anand *et al.*, 2008). Curcumin acts at several stages of cancer development. It blocks transformation, tumor initiation, tumor promotion, invasion, angiogenesis, and metastasis.In vitro and animal studies have revealed that curcumin suppresses carcinogenesis [76] and inhibits the proliferation of a wide variety of tumor cells (Aggarwal *et al.*, 2003).

Research has also revealed the enhanced cytotoxic nature of certain curcumin derivatives and analogues. A mono-carbonyl analog of curcumin was synthesized and In vitro assays showed that this curcumin derivative had greater antiproliferative effects on colon cancer cells than curcumin had [77](Zheng *et al.*, 2013). Another derivative, hydrazinobenzoylcurcumin (HBC), exhibits potent inhibitory activities [78] against the proliferation of several tumor cell lines (Shim *et al.*, 2004). The curcumin derivative bis-DeHydroxyCurcumin (bDHC) also has been shown to induce autophagy on human colon cancer cells [79](Basile *et al.*, 2013).So in the present investigation , **the antitumour activity of a series of synthetic analogues of natural curcuminoids have been studied by in vitro and in vivo methods**.

Curcumin and allieds as anti-microbial agents

Curcumin and synthetic allieds inhibits the growth of varieties of pathogenic microbial organisms such as viruses, bacteria and some fungi[80] (Chai *et al* 2005).Anti microbial effects of curcumin has been well established[81] (Negi *et al* 1999). Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants. Synthetic modification of previously described antibacterial agents has been prominent in the development of new

compounds which may possess an enhanced antibacterial activity or new pharmacological properties.

Both curcumin and the oil fraction suppress growth of several bacteria like *Streptococcus*, *Staphylococcus*, *Lactobacillus*[82] etc.The aqueous extract of turmeric rhizomes has antibacterial effects[83]. Curcumin also prevents growth of *Helicobacter pylori* CagA+ strains *in vitro*[84]. Ether and chloroform extracts and oil of *C. longa* have antifungal effects [85-87]. Crude ethanol extract also possesses antifungal activity[88]. Turmeric oil is also active against *Aspergillus flavus*, *A.parasiticus*,*Fusarium moniliforme* and *Penicillium digitatum*[89].

Considering the potentiality of curcumin and analogues as a source of antimicrobial drugs with reference 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 Curcumin and its Analogues

The most interesting feature of curcuminoids from a coordination chemists point of view is that structurally they are 1,3-diketones ,in which the diketo function is directly attached to olefinic groups. The β-diketo group exhibits keto-enol tautomerism.The methine proton in the keto form and hydroxyl proton in the enol form are acidic and their removal generates 1,3-diketonate anion which forms chelates with almost all metal and metalloid ions [90-94]. Complexation refers to the formation of cyclic complex by coordination of a metal ion with a polydentate ligand. The nature of chelation of β-diketone derivatives were first elucidated by Werner[95] and Morgan[96].Numerous metal 1,3-diketonates have been synthesized and their applications in various fields were studied.

Curcuminoids are excellent chelating ligands which can bind with metal ions to form stable metal complexes. The preparation of Boron-Curcumin complex[97] in the determination of trace amounts of Boron dates back to the 1960's.The colour change when turmeric is mixed

with slaked lime is due to the interaction between Ca^{2+} ions and curcuminoids.Recently stable Cu^{2+},Ni^{2+},Co^{2+},Zn^{2+},Pd^{2+} complexes [98]of synthetic curcuminoids were reported.A 2:1 curcumin Hg(II) complex was synthesized and characterized by Angulo (1986).Curcumin is more active when it interacts with copper and iron[99] and this has been proposed by the mechanism of action in Alzheimers disease animal model.Antitumoural effects of a heteroleptic Palladium (II) complex of curcumin on human prostate cancer cells[100] were reported by A.Valentini *et al.*Curcumin –Al(III)complexes[101] were prepared and their effect in Alzheimers disease were studied.Rare earth complexes (Sm,Eu,Dy) of curcumin[102] have been synthesized by Yu Min Song *et al* and the complexes exhibited excellent antibacterial activity than that of curcumin. A mononuclear 1:1 copper complex of curcumin was synthesized[103] and examined for its superoxide dismutase activity.Ruthenium-arene complexes of curcumin[104] were prepared and studied for their cytotoxic activity. Transition metals usually form stable metal complexes with ML_2 stochiometry where M is metal and L is ligand. They can be synthesized by the reaction between stochiometric amounts of curcumin and metal salts in suitable organic solvents and the mixture is refluxed for few hours and the complex precipitates out. This is separated and purified either by column chromatography and repeated crystallization.

Metal complexation of these α,β –unsaturated 1,3-diketones has led to effective changes in their biological activities including antitumour activity,antibacterial and antifungal activity.In the present investigation **transition metal ions namely Cu^{2+},Zn^{2+},Ni^{2+} and VO^{2+} were complexed with synthetic curcuminoid analogues.Their structural characterization were also done by spectral techniques.The in vitro and in vivo anticancer activity, in vitro antibacterial activity and antifungal activities of the synthesized transition metal complexes were also evaluated.** Many metal complexes of curcumin are shown to be

cytotoxic with cancer causing cells. The most widely used complex in chemotherapy is cis-dichlorodiamine platinum(II), commonly known as cisplatin.

Cu(II) based chelates as cytotoxic agents

Substituted [phenylglyoxal bis(4-methyl-3-thiosemicarbazone)] copper(II) chelates show considerable cytotoxic activity.The presence of electron-donating substituents increased cytotoxicity[105]. Anticarcinogenic activity of copper di-Schiff bases (two novel di-Schiff bases coordinated with active center analogs of Cu_2 Zn_2 superoxide dismutase) effectively catalyzes the production 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[106]. Copper(II) complexes of N-1-isonicotinoyl-3-methyl-4- (p-hydroxybenzilidene)-2-pyrazolin-5-one(IMHBP) also exhibited significant antitumor activity[107].

Copper complexes of synthetic curcuminoid analogues gave enhanced antitumor activity[55]. Four synthetic curcuminoids,1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (curcumin-1);1,7-bis (piperonyl)-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(II) complexes were investigated for their cytotoxic and antitumor activities and were found to be very active. In-vivo administration of Copper(II) complexes of N-Salicyloyl-N´-p-hydroxythiobenzohydrazide and N-benzoyl-N´-thiobenzohydrazide complexes had shown an increase of life time of Dalton's Lymphoma cells bearing mice[108]. The exposure of tumor cells to the mixtures of ascorbate and copper chelates, especially Cu^{2+}-o-phenanthroline and Cu^{2+}-2, 9-dimethyl-o-phenanthroline complexes resulted in the destruction of a large fraction of cell populations[109]. Copper(II) complex of N´-[(2-hydroxy phenyl) carbonothioyl] pyridine-2-carbohydrazide inhibits the expression of c-Src, a non-receptor tyrosine kinase, which plays a significant role in the growth-mediated signalling pathway, thus showing cytotoxicity against the colon cancer cell line[110]. Tris-

(hydroxymethyl)phosphine copper(I) complexes with the new bis(1, 2, 4-triazol-1-yl)acetate ligand had exhibited in-vitro antitumor activity comparable with that of cisplatin, the most used metal-based antitumor drug[111].Copper(II) chelate of trans-bis(salicylaldoximato) showed cytotoxicity comparable to that of adriamycin by inducing cell cycle arrest and apoptosis[112].Thus the importance of Cu (II) ion in cytotoxic activity is well established.

Vanadyl –curcumin complex inhibits mouse lymphoma cell proliferation[113] efficiently compared to curcumin according to Katherine *et al* (2004).The medicinal applications of Vanadyl curcumin complex as cytotoxic agents[114] 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[115].The metal complexes also exhibited antibacterial activity against E.coli,S.aureus,B.subtilis and Pseudomonas aeuroginosa and fungicidal activity against Aspergillus flavus and Candida albicans.

Hence the present study was undertaken with the following objectives:

1. Synthesis and structural characterization of a series of curcuminoid analogues.
2. Synthesis and structural characterization of transition metal complexes (Cu^{2+}, Zn^{2+}, Ni^{2+},VO^{2+})of 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 and their synthesized transition metal chelates.
5. To evaluate the antifungal activity of a series of synthetic analogues of natural curcuminoids and their synthesized transition metal chelates.

PART II

REVIEW OF LITERATURE

CURCUMINOIDS, THEIR ANALOGUES & METAL CHELATES-CHEMICAL AND BIOLOGICAL STUDIES-A REVIEW

INTRODUCTION

The importance of medicinal plants in traditional health care practices providing clues to new areas of research is now well recognized . Several plant[116] species are known to exert wide range of physiological effects in addition to aroma and flavour . Even in the modern world nature is still the greatest source of drugs .The number of plant derived compounds known to be pharmacologically active is very large. WHO estimates that approximately 80% of the developing world's population meet their primary health care needs through traditional plant medicine [117](Bannerman 1982). Traditionally many plants and plant parts like leaves, roots, barks, peels, bulb etc have been used for medicinal purposes .There are many medicinal plants recommended [118] for their therapeutic values. Medicines derived from plants have played a pivotal role in the health care of many cultures[119,120] both ancient and modern. [Newman, Cragg (2007); Butler M.S(2004)]. Phytomedicines are beginning to link traditional medicine and modern medicine.Regardless of all the developments in Synthetic chemistry and Biotechnology, plants are still a vital source of medicinal preparations both precautionary and therapeutic. Right from the beginning , the documentation of traditional knowledge especially on the medicinal use of plants, has provided many important drugs of modern day[121]. With the advent of modern techniques it has become possible to isolate and characterize the bioactive chemical compounds present in Medicinal plants. The Indian subcontinent is gifted with rich and varied local health customs which is harmonized with an equally rich and varied plant genetic resources.In this context one of the best known example is Turmeric [26,122,123] (*Curcuma longa* L*inn*).

Foods from plant source have many bioactive chemical constituents called phytochemicals [124-126]. They fit into several classes of organic compounds such as sulphur containing compounds, terpenoids, flavanoids, polyphenols, carbonyl compounds etc. Most of the healing and biological properties of these plants are due to the occurrence of these type of compounds. Some of the naturally occurring carbonyl compounds and their main plant sources are Piperine (Black pepper), Curcuminoids (Turmeric), Cassumunin A (Indonesian medicinal ginger), Nomillin (citrus fruits), Ellagic acid (fruits, nuts), Quercetin (cereal, grains) etc[127].Amongst the diverse naturally occurring carbonyl compounds, Curcuminoids enjoy a variety of remarkable structural features and abundant practical applications.

TURMERIC : THE GOLDEN SPICE

India has a rich history of using plants in medicinal fields.Turmeric (*Curcuma longa* L*inn*) has been an inevitable drug used in traditional medicine as a household remedy for various diseases [128-130] including hepatic disorders, sinusitis, anorexia, cough, wounds,rheumatism, chicken pox etc. *Curcuma longa* L. belongs to the family Zingiberaceae and is a perennial, tropical herb that is cultivated widely in Asia. Its rhizome is used extensively for imparting colour [131-132]and flavour to foods. Turmeric, a powder from the dried rhizomes, is used for medicinal purposes (Srimal 1997). Turmeric is used as a food additive (spice), preservative and colouring agent in Asian countries. Turmeric has been termed the multi antispice in herbal medicine. Over the past few years, numerous studies have been conducted to identify the medicinal properties [133-137] of turmeric. The use of turmeric dates back nearly 4000 years to the vedic culture in India where it was used as a culinary spice and had some religious significance . India produces nearly all of the world's turmeric crop and consumes 80% of it.

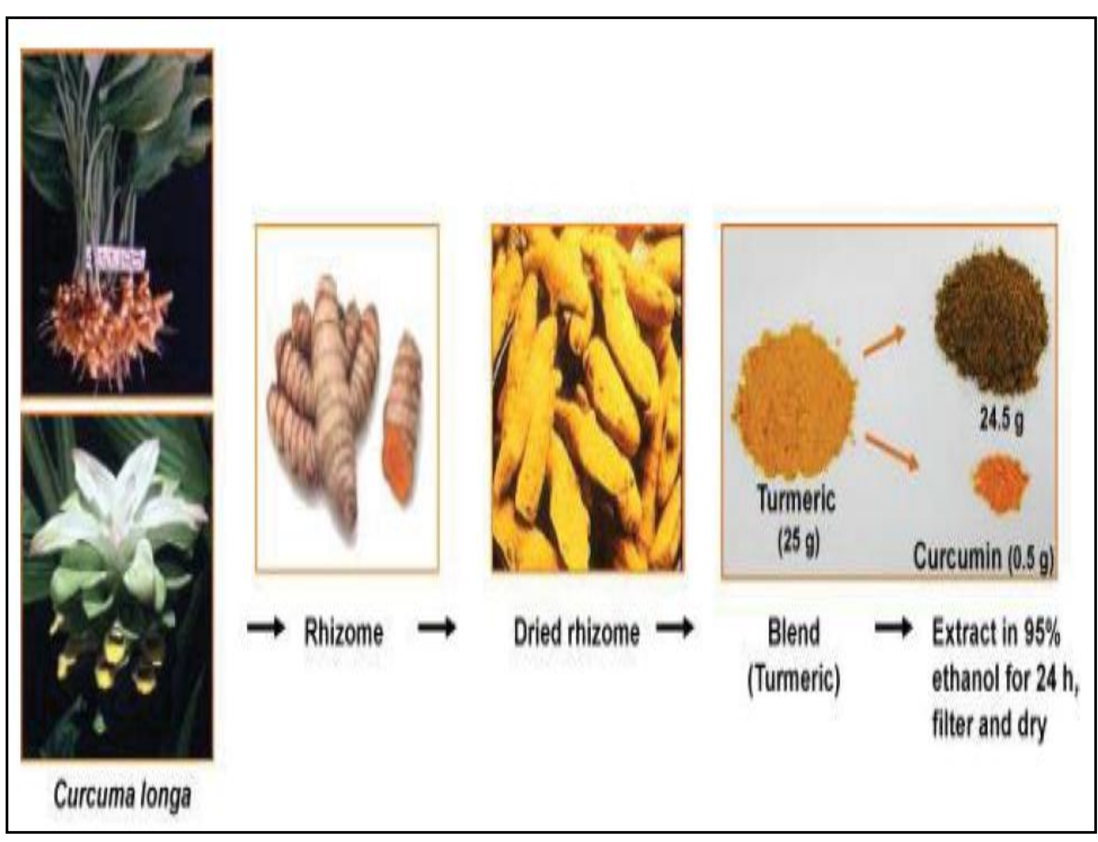
Turmeric contains [138-141] moisture (9%), curcumin (5-6.6%), extraneous matter (<0.5%), volatile oils (<3.5%), sesquiterpenoids, polysaccharides, protein, vitamins, calcium, potassium etc(Balakrishnan 2007).Turmeric is also a good source of ω-3 fatty acids and α- linoleic acid [142](Goud, Polasa and Krishnaswamy 1993).

Curcuminoids is mainly responsible for the yellow colour of turmeric and comprises Curcumin I (94 %),Curcumin II (6%) and Curcumin III (0.3%)[143,144] (Chainani – Wu, 2003).

TURMERIC AND CURCUMINOIDS:

Curcumin, the bioactive [145-150] yellow orange pigment is the most crucial fraction which is accountable for the biological actions of turmeric . Curcumin is the main colouring substance in Curcuma longa and two related compounds demethoxy curcumin (DMC) and bis demethoxy curcumin (BDMC), are altogether known as curcuminoids. Curcumin is the active ingredient of the dietary spice Turmeric. The curcuminoids can be

extracted from Turmeric using Soxhlet extractor [151] and isolation and purification [152-156] was carried out by column chromatography [157](Srinivasan etal 1953). The sighting of curcumin dates back to around two centuries when Vogel and Pelletier [158,159] reported the separation of "yellow colouring matter" from the rhizomes of turmeric and named it curcumin.



In 1910 , Milobedzka and Lampe [30,31] chemically established the structure of curcumin as diferuloyl methane or 1,7 – bis (4- hydroxy -3 - methoxy phenyl)1,6- heptadiene – 3,5 – dione .The structures of Curcumin-I,Curcumin-II and Curcumin-III are given below.

Curcumin
(Curcumin I)

Demethoxycurcumin
(Curcumin II)

Bis-demethoxycurcumin
(Curcumin III)

In 1937 Pabon etal [50] reported its first synthesis from the chloride of carbomethoxy ferulic acid. Later they reported a modified simple procedure for its synthesis. In this method the methylene group of acetyl acetone was blocked with boric anhydride and reacted with Vanillin and the product formation takes place via Knoevenagel condensation. Boron based reagents such as boron oxide, boric acid act as Lewis acids with the β diketone system and reduces the nucleophilicity of the C-3 position. The two terminal methyl groups then undergo dialdol condensation with two Vanillin molecules. Hydrolysis of the intermediate in acid medium results in the formation of Curcumin.

Synthesis of curcumin by the general method proposed by Pabon

1. Substituted benzaldehyde
2. Primary amine
3. Alkyl borate
4. HCl

CURCUMIN-PROPERTIES & BIOAVAILABILITY

Research has identified Curcumin as the agent responsible for most of the biological activity of turmeric like anti inflammatory[160-164], antioxidant[165-170], antiprotozoal[171-173], nematocidal[174], antibacterial[175-178], antiviral[179], antitumour[60,180-184] activity etc.Structurally curcuminoids are 1,7- diaryl heptanoids [33,185]. They are typical 1,3 – diketones in which diketo function is directly attached to olefinic groups. The chemical structure of curcumin shows three chemical functionalities, two phenyl ring systems with methoxy and hydroxy substituents connected by a seven carbon linker which is an α - β unsaturated β- diketone part. Curcumin displays [186] typical keto-enol tautomerism . It is a hydrophobic molecule which is almost insoluble in water and readily soluble in polar solvents like DMSO, ethanol, methanol, ethyl acetate, chloroform etc.

Curcumin (diketone form) ⇌ Curcumin (keto-enol form)

Curcumin has been proved to be safe in investigational studies in humans even at high daily doses of up to 8 gm [187-188] with very less side effects.The main problem faced in clinical trials with curcumin is its poor bioavailability in plasma and tissue .The limited aqueous solubility of curcumin in water, poor absorption, rapid metabolism and systemic elimination have been the main reasons for reducing its bioavailability [34,189]. Bioavailability can be increased by encapsulation of curcumin in the cavities of cyclodextrins [190] or the development of [191,192] nanoparticles and ceramic particles, develop new formulations based on biocompatible organic substances like liposomes[193],micelles [194], glycols, cellulose biopolymers etc.Some methods to increase the bioavailability is described below.

Nanoparticles: With the advent of nanoparticle technology it is possible to develop targeted and triggered drug delivery systems with nano particles.These have been prominent solutions to the bioavailability of therapeutic agents. Nanoparticle-based delivery systems will certainly be suitable for highly hydrophobic agents like curcumin, thus overcoming the limitation of poor aqueous solubility.A recent study by Bisht *et al.* reported the synthesis, physicochemical characterization and cancer related application of a polymer-based

nanoparticle of curcumin namely “nanocurcumin” with less than 100 nm size. Nanocurcumin was found to have similar in vitro activity as that of free curcumin in pancreatic cell lines.

Liposomes are excellent systems used in delivering drugs since they can carry both hydrophilic and hydrophobic molecules. Li *et al.* studied the in vitro and in vivo antitumor activity of liposomal curcumin against human pancreatic carcinoma cells and verified that liposomal curcumin can prevent pancreatic carcinoma growth and, in addition, exhibits antiangiogenic effects. Ruby *et al.* also reported the anticancerous and antioxidant activities of neutral unilamellar liposomal curcuminoids in mice.

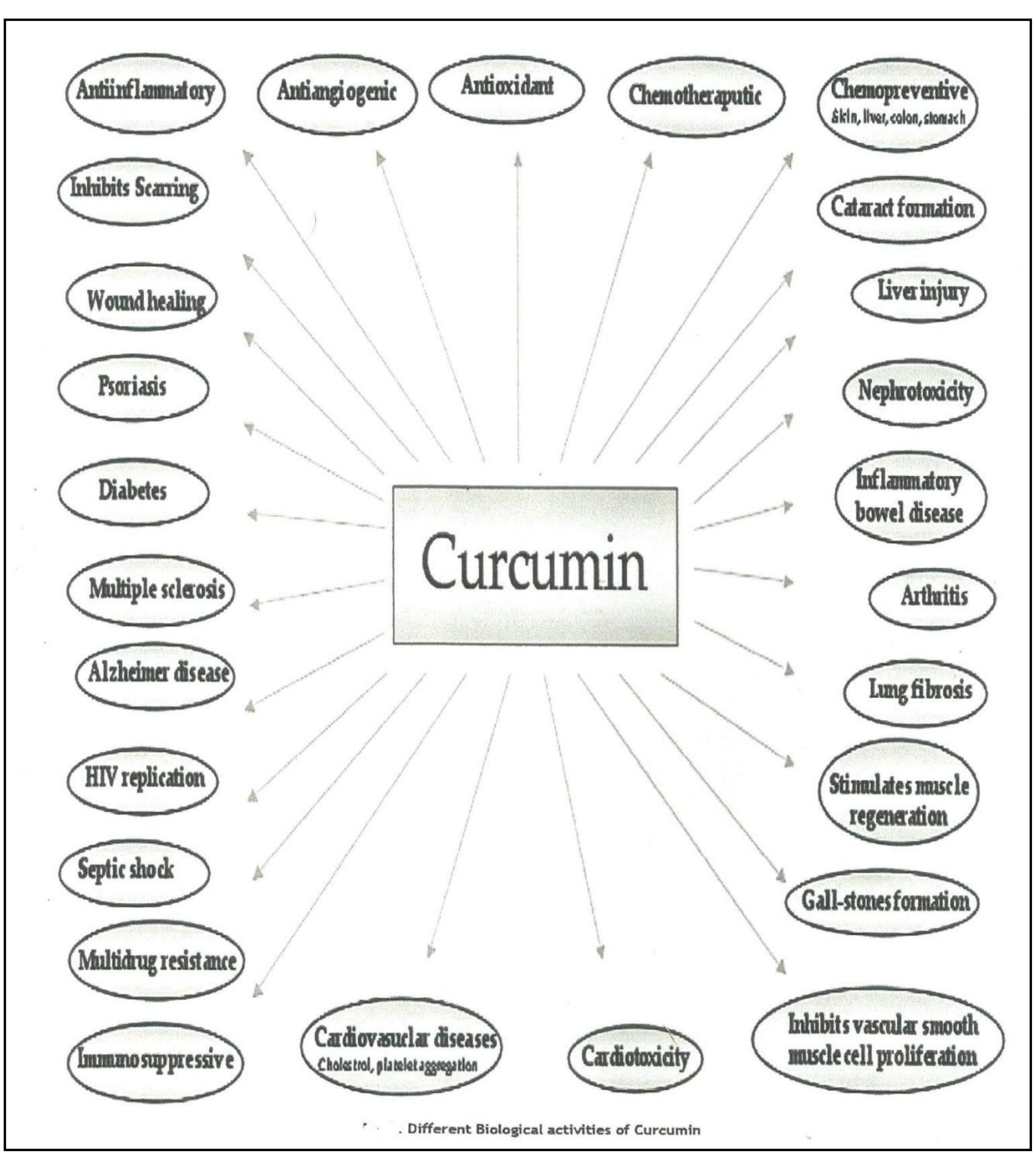
Micelles and phospholipid complexes can improve the gastrointestinal absorption of natural drugs, thereby giving higher plasma levels and lower kinetic elimination resulting in improved bioavailability.The intestinal absorption of curcumin and micellar curcumin formulation with phospholipid was evaluated using an in vitro model consisting of everted rat intestinal sacs.

Researchers hope to achieve improved biological activity of curcumin by structural modifications. Numerous studies dealing with the enhanced biological activity of curcumin derivatives and analogues [195,196] can be found in the literature. A review by Mosley *et al.* systematically describes several studies dealing with the biological activity relationships of curcumin and its derivatives.

Another strategy to increase the biological activity of curcumin is to complex it with metals [113,114] and form coordination compounds.Curcumin molecule becomes an excellent ligand for complexation due to the presence of two phenolic groups and one active methylene group . Several metal chelates of curcumin are reported to possess biological activity over that of free curcumin.

Curcumin : Biological actions and medicinal applications

For the last few years, extensive work has been done to establish the biological behavior and pharmacological actions of curcumin. Curcumin, the yellow bio active component of turmeric has been shown to have a wide variety of biological actions [85]. These comprise its anti inflammatory, antidiabetic, antibacterial, antifungal, antioxidant antiprotozoal, antiviral, antiulcer, hypotensive and hypocholesteremic activities.



. Different Biological activities of Curcumin

Curcumin is a dietary phytochemical with low toxicity . Numerous therapeutic activities,its pharmacological safety and its colour qualifies curcumin as Indian solid gold. Some important biological activities of Curcumin are listed below.

Pharmacological actions of curcumin as an antinflammatory agent [47] have been examined by Srimal and Dhawan (1973) . They reported that " Curcumin was effective in acute as well as chronic models of inflammation. The potency of curcumin is equal to a standard drug , phenylbutazone in the carrageenin – induced edema test in mice". Mukophadhyay etal (1982) demonstrated the activity of curcumin and sodium curcuminate in cotton pellet granuloma models of inflammation in rats [197] and has found that the compounds possessed anti inflammatory activity. Ghatak and Basu (1972) showed the action of sodium curcuminate [198]as an anti inflammatory agent. According to Huang etal (1992),"Curcumin showed inhibitory effects on the proliferation of blood mononuclear cells and vascular smooth muscle cells"[199].Ammon *et al* (1992) demonstrated curcumin as an "inhibitor of leucotriene formation in rat peritoneal polymorphonuclear nuetrophils" [200]. Curcumin offers anti-inflammatory effect through inhibition of Nuclear Factor Kappa B[201] (NF-κB), a molecule that travels into the nuclei of cells and turn on genes related to inflammation. The anti inflammatory role of curcumin is also mediated through down regulation of cycloxygenase -2 (COX-2) and inducible nitric oxide synthetase (iNOS) through suppression of NFκB activation[202].

The antioxidant nature of curcumin was studied as early as 1975 (Sharma O.P).The active antioxidant principle in *Curcuma longa* has been identified as Curcumin [203,204] (Choiu *et al* (1983), Moken *et al* (1984). Curcumin happens to be a potent anti oxidant that can nuetralise free radicals due to its chemical structure.According to Pulla Reddy and Lokesh (1992) "Curcumin can lower lipid peroxidation [205] by preserving the action of antioxidant enzymes like superoxide dismutase , catalase and glutathione peroxide".They also

observed that curcumin is capable of scavenging oxygen free radicals such as superoxide anions and hydroxyl radicals which initiate lipid peroxidation.According to Joe and Lokesh 1994,"Curcumin can hinder the generation of reactive oxygen species (ROS), H_2O_2 and nitrite radical generation [206] which has a significant role in inflammation."Since ROS have been concerned in the advance of various pathological conditions curcumin has the prospective to control these diseases[207,208] through its potent antioxidant activity. Joe B *et al* reported that "The phenolic and methoxy group on the phenyl ring and the 1,3 – diketone system in curcumin seems to be an important structural feature [209] which contribute to its antioxidant activity." Sharma *et al* observed that the phenolic hydroxyl groups are essential for antioxidant activity and an increase in the number of this functional group confers better activity than curcumin.The role of β-diketone moiety for the antioxidant activity was suggested by Sugiyama *et al*[210].

The continuous development of bacterial resistance to presently available antibiotics has necessitated the search for novel and effectual antimicrobial compounds, especially of plant origin. Curcuma oil was found to inhibit the growth of bacterial strains like Staphylococcus albus and S. aureus [211](Chopra *et al* 1941). Bhavani Shankar and Murthi [82] (1979) investigated the activity of curcumin against intestinal bacteria in vitro and observed inhibition of growth of Lactobacilli. Mahady *et al* reported [84] that "Curcumin also prevents growth of Helicobacter Pyloricag A^+ strains in vitro."The studies conducted by Dipti Rai *et al* (2008) has reported [212] that "Curcumin, a dietary polyphenolic compound, has been shown to have a potent antibacterial activity against a number of pathogenic bacteria including Staphylococcus aureus, Staphylococcus epidermidis and Enterococcus. Curcumin inhibits FtsZ assembly[213]: an attractive mechanism for its antibacterial activity. Curcumin inhibited the assembly of FtsZ protofilaments and also increased the GTPase activity of FtsZ. Curcumin reduced the bundling of FtsZ protofilaments in vitro. The results indicate that the

perturbation of the GTPase activity of FtsZ assembly is lethal to bacteria and suggest that curcumin inhibits bacterial cell proliferation by inhibiting the assembly dynamics of FtsZ in the Z-ring". (FtsZ, a prokaryotic homologue of eukaryotic cytoskeletal protein tubulin, polymerizes to form a Z-ring at the mid cell that orchestrates bacterial cell division).

Curcumin exhibits antiprotozoan activity. Curcumin and synthetic derivatives has anti- Leishmania and anti L- amazonensis effect [214,215]. Curcumin has been shown to exhibit activity against Plasmodium falciparum,which is a protozoan. Curcumin exhibits antiviral activity. Mazumber *et al* (1995) demonstrated [216] that "Curcumin being a HIV-I integrase inhibitor may be developed as anti- Aids drug". Eigner and Scholz (1999) reported [133] that curcumin had anti HIV - 1 and HIV-2 activities.

Curcumin had appreciable inhibitory activity against fungal contaminations[217]. Curcumin possessed activity [218] against fungi species namely R.Solani, Pu.recondita and P.infestans.Curcumin exhibited antifungal effect against two phytophagous fungi, namely Fusarium solani and Helmintho sporium oryzae[219]. Shadomy *et al* 1985 used diffusion disc method to test the susceptibility of fungi to curcumin and has found to be very effective against fungal cultures. An in vivo study conducted in guinea pigs[85] infected with T.rubrum established that dermal application of turmeric oil caused the lesions to vanish in 6-7 days. The study of curcumin with fourteen different strains of Candida species showed that curcumin exhibit significant fungicidal activity against them even at a concentration of 250μg/ml. The possible mechanism for the antifungal effect was found to be the down regulation of ERG3 leading to significant reduction in ergosterol of fungal cell.When there is a reduction in formation of ergosterol this will result in accumulation of biosynthetic precursors of ergosterol which causes cell death by generation of ROS[220]. Reduction in proteinase secretion and alteration of membrane- associated properties of ATP ase activity are other possible critical factors for antifungal activity of curcumin[221].

Curcumin acts as an effective anti carcinogenic[70]compound .There are various mechanisms for its activity where induction of apoptosis has a significant role. R.S.Ramsewak etal 2000,showed the activity of curcuminoid compounds[222]against leukemia, colon,melanoma,renal and breast cancer cell lines .His studies revealed that as polarity increased from Curcumin I to III ,the cytotoxic activity decreased and so the methoxy groups are very important for cytotoxicity exhibited by curcumins. According to Chen *et al* 1998, "curcumin induces apoptosis and inhibits cell -cycle progression[70], both are influencial in preventing cancerous cell growth in rat aortic smooth muscle cells". The apoptotic effect may be mediated through inhibition of protein tyrosine Kinase, protein Kinase C and bcl-2 m RNA expression. Curcumin produces apoptotic cell death by causing damage to DNA in human cancer cell lines [223].According to Duvoix etal [224] 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 of GSTP-1 at transcription level. Colon carcinoma is prevented by curcumin through arrest of cell – cycle progression independent of inhibition of prostaglandin synthesis[225]. Curcumin also suppresses tumour growth. Nitric oxide(NO) and its derivatives play a major role in tumour promotion . Curcumin inhibits iNOS and COX – 2 production [226] by suppression of NFκB activation.

Curcuminoids and allied derivatives as chelating ligands

Curcuminoids are rare examples of naturally occurring β - diketone ligands.Similar to other 1,3-diketones curcumin act as complexing ligands with different metals [227-229] and form complexes.Researchers have shown increased interest in preparation, characterization and biological studies of metal curcumin complexes.Coordination compounds with curcumin and its analogues as ligands have been reported for most metallic elements in the periodic table [48,230-231].

The α , β - unsaturated β - diketo moiety of curcumin is an excellent chelating agent. Curcumin is a monobasic bidentate ligand and forms stable complexes with metals (M^{2+}) forming stable structures with 2:1 (ligand : metal) stochiometry [232,233]. Metal coordination of curcumin occurs through the enolic group, where the enolic proton is replaced by the metal ions.

Chemical studies on metal chelates of naturally happening ligand systems and their significance in biology and medicine comprise one of the most vibrant area of modern inorganic chemistry.Biochemical activity of many plant chemicals are known to be allied with their ability to form chelates with metal ion.

Metal complexes of curcumin and allied derivatives : Biological Activities

The potential medicinal applications of curcuminoid–metal complexes are as diverse as those of curcuminoids itself. Metal- curcumin complexes have been reported to exhibit significant antiarthritic activity[234], antimicrobial[235], antifungal, antiviral[236], anti HIV, anticancer[100], antioxidative [27] effects, anti – Alzheimer's disease[99] activity etc.

Curcuminoids act as good chelating ligands and form a large number of complexes with various main group metals[237], d- transition metals and f- elements [102,115] Several Boron, Aluminium [238,239], Gallium [240,241], Indium complexes with curcuminoid analogue ligands have been prepared . Among transition metals Fe, Cu, Mn, Ni, Zn, V, Ru, Re, etc forms a series of curcumin analogue complexes [242-244]. Curcumin complexes have been prepared for lanthanide elements (Sm, Eu, Dy, La) as well as Thorium and Uranium [102,230,245].

The most exciting aspect of metal-curcuminoid analogue complexes is that many of them exhibit very diverse and highly potential health effects as given below.Experimental studies on anticancer nature of metal-curcumin complexes date back to the year 1998.Numerous transition metal complexes of curcuminoid ligands have been effectively tested *in vitro* and *in vivo* for anticancerous effects [246,247]. Among a series of VO(IV),Co(II),

Ni(II), Cu(II), Zn(II) complexes, the copper complexes turned out to exhibit highest selective cytotoxicity and also showed considerable reduction in solid tumour volume in ascites tumour burden mice[55] VO(Curcu)$_2$ exhibited antioxidant,anti rheumatic activity, inhibited angiogenesis in smooth muscle cells and shows significant anticancer activity in mouse lymphoma cells [113,114]. VO(Curcu)$_2$ proved to be harmless and showed no negative symptoms when used as drug at doses upto 2 mmol/kg/day/. Zn^{2+}-curcumin complexes showed anti- cancer , gastro protective and anti-depressant effects in rats [248]. Studies conducted with metal curcuminoid complexes of group 13 elements, Al, Ga and In, the first row transition metals V, Fe, Zn, Cu, the second row transition metals Ru, Pd as well as some rare earth metals revealed their antitumour activity.Copper complexes showed enhanced antitumour activity against three Human cancer cell lines namely ASPC-1 (Pancreatic Carcinoma), MCF-T(breast cancer) and HeLa(Cervical Cancer)[247].

It has been observed that Metal – curcuminoid complexes which can act as potential agents to fight Alzheimer's disease contain the metals Al, Ga, Cu and Zn. In Alzheimer's disease β-amyloid plaques are formed around neurons. Metal ions which can act as significant risk cofactors in AD [249] include, Al^{3+}, Mn^{2+}, Fe^{3+} , Cu^{2+} and Zn^{2+}. Metal-curcumin complexes are inhibitors of metal induced nuerotoxicity. Gallium, Technetium and Rhenium complexes of curcumin and some of its derivatives were successfully tested for selective staining of β - amyloid plaques of AD[250]. It has been proved that Al- curcumin complexes are very active in preventing proteins from the formation of amyloid fibrils and even removing the preformed amyloid deposits.

Antioxidant activities have been presented by curcumin complexes of gallium, indium , vanadyl, manganese and copper . Curcumin complexes of Cu and Mn have been studied for their SOD(super oxide dismutase, which control the formation of super oxide radical anion) activity, free- radical scavenging ability and antioxidant potential.According

to Barik A *et al* "the biological activities of metal curcumin complex changes through ligand variations, Cu(Cur)2 is a less powerful antioxidant [251] than Cu[cur] [OAc][OH], (OAc – acetate)". In the case of copper(II) complexes, the distorted orthorhombic 1 : 1 complex Cu(Curc)(OAc)(OH) (OAc = acetate) and the square-planar homoleptic 1 : 2 complex $Cu(Curc)_2$ have been investigated.These two complexes display different antioxidant potentials due to different geometry of complexes. The 1 : 1 Cu(II)–curcumin complex Cu(Curc)(OAc)(OH) with the larger distortion from the square-planar structure showed a nearly ten times higher SOD activity than the 1 : 2 complex $Cu(Curc)_2$. From this study it was concluded that the 1 : 1 complex would be able to undergo and sustain the distortion from square planar geometry to the distorted tetrahedral one during its reaction with superoxide radical. This allows for the compound to undergo many redox cycles and hence work as a very competent antioxidant.The homoleptic 1 : 2 complex $Cu(Curc)_2$ is planar but rigid and hence cannot undergo the distortions and therefore is a less powerful antioxidant. Singh *et al* prepared gold–curcumin conjugates and reported to exhibit antioxidant activity by DPPH assay.

Antiarthritic activity have been reported for vanadyl and gold complex of curcumin. The activity of the five coordinated curcumin-gold(I) complex were assessed in adjuvant – induced rat polyarthritis model and a reduction in paw swelling was seen after 3 weeks of treatment [234].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 done with boron and copper complexes of curcumin and they were found to be active. A Cu- curcumin complex was found to be effective and was used in the preparation of a vaginal gel against viral infections. Z Sui *et al* have found "Curcumin – Boron complex [252] is a modest inhibitor of HIV- 1 and HIV -2 proteases". Potential anti osteoporotic activity [253] was recently reported by Y.Mawani and C.Orvig, 2014

for three homoleptic rare-earth metal curcumin complexes.Homoleptic compounds Ln(Curc)$_3$ (Ln = Eu, Gd, Lu) were prepared and the toxicity of these complexes was investigated in MG-63 cell lines, an osteoblast-like cell line derived from a human osteosarcoma, for potential activity as antiosteoporotic agents. It was established that the three lanthanide curcumin complexes possessed cytotoxicity toward MG-63 cells similar to cis-platin (*cis*-diammine-dichloroplatinum(II)).

According to M.A.Subhan *et al* "Eu,Ce,La,Y,Cr and Pd complexes of curcumin had shown antibacterial activity [230] against Klebsiella Pneumonia and Escherichia Coli".Complexes had DNA cleaving ability.Curcumin nanoconjugates of cobalt and silver nanoparticles have been shown to display antimicrobial activity [254] by R Sakey and S Hatamie,2012 .Curcumin-Ag (I)complex is quite effective against bacterial strains viz S.aureus,E.coli,B.Subtilis and Bacillus cereus [255]. Mixed ligand-curcumin complexes with rare earth metals like Samarium,Europium and Dysprosium in the +3 state showed antibacterial activity.Saeed Tajbaksh *et al* suggested that In (cur)$_3$ was found to have more antibacterial effect [256] than curcumin.

Curcuminoids are bioactive compounds with powerful medicinal properties present in Turmeric.The present investigation is an endeavour to synthesize synthetic analogues of the dynamic chemical constituent namely Curcuminoids seen in the herbaceous Indian medicinal plant Turmeric.Coordination chemistry of biochemically important plant products have gained significant momentum in recent years.This is evident from the numerous reports on medicinal and other aspects of metal complexes of Curcuminoids and allied derivatives.The main aim of this study is to synthesize,characterize and study the biochemical activities of transition metal complexes of a series of synthetic curcuminoid analogues.

PART-III

MATERIALS, METHODS AND INSTRUMENTAL TECHNIQUES

MATERIALS, METHODS AND INSTRUMENTAL TECHNIQUES

3.1 Materials for synthesis of curcuminoid analogues and metal complexes:

All the chemicals used in this study for synthesis and analytical purposes were of analar grade and purchased from Sigma Aldrich,USA. Fourteen different aldehydes namely 2-methylbenzaldehyde, 2,5-dimethylbenzaldehyde, Thiophene-2-aldehyde, 3-methyl-Thiophene-2-aldehyde, 2-chlorobenzaldehyde, 4-chlorobenzaldehyde, 3,4-dichlorobenzaldehyde, 3,4,5-trimethoxybenzaldehyde,3-ethoxy-4-hydroxybenzaldehyde, 2,4-dihydroxybenzaldehyde, 1-naphthaldehyde,2-methoxy naphthaldehyde, 2-hydroxy naphthaldehyde and Anthracene-9-carboxaldehyde were used along with the 1,3-diketone,acetyl acetone.Metal salts used for the synthesis of metal complexes include copper (II) acetate monohydrate, nickel (II) acetate tetrahydrate,zinc(II) acetate and vanadium(IV) oxide sulphate.Commercial solvents were distilled and used for synthesis.

3.2 Methods for synthesis of curcuminoid analogues and their metal complexes Cu(II),Zn(II),Ni(II) &VO(IV).

3.2.1 Synthesis of different curcuminoid analogues

Acetyl acetone (0.005mol, 0.5g) was stirred for ~ 1 hour with boric oxide (0.0035mol, 0.25g) to obtain acetyl acetone-boron complex. To this reaction mixture, the aldehyde (0.01mol) dissolved in dry ethyl acetate (7.5ml) containing tri(sec-butyl) borate (0.02mol,5.4 ml) were added and the temperature was kept above 80°C. The reaction mixture was stirred and while stirring n-butyl amine (0.1ml dissolved in 1ml dry ethyl acetate) was added drop wise during 40min. Stirring was continued for an additional period of ~ 4h and the solution was set aside overnight. Hot (~60°C) HCl (0.4M, 7.5ml) was added and the mixture again stirred for ~ 1h. Two layers were separated and the organic layer was extracted with 5ml ethyl acetate. The combined extracts were evaporated and the residual pasty material was

stirred with HCl for ~1h. The solid product separated was collected, washed with water and dried in vacuum. The product obtained was a mixture of 1,7-diaryl heptanoid and 6-aryl hexanoid. The products obtained were quantitatively separated by column chromatography using silica gel (60 – 120 mesh) as detailed below.

3.2.2 Separation and purification of 1,7-diaryl heptanoids

The solid product obtained on acidification was dissolved in minimum quantity of ethyl acetate and placed over the column densely packed with silica gel. The eluting solvent used was 1:5 (v/v) acetone:chloroform mixture. As the elution proceeds, two bands were developed in the column. The elution was then repeated by using a 1:2 (v/v) mixture of acetone and chloroform and collected in aliquots, checked by tlc and the combined extracts on removing the solvent in vacuum yield 1,7-diaryl heptanoid in 60 – 70% yield. The isolated 1,7-diaryl heptanoids were recrystallised twice from hot benzene to get chromatographically pure compound.

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

The Cu(II) complexes were prepared by adding a methanolic solution of copper(II) acetate (25 ml, 0.001mol) to a solution of curcuminoid analogue (25 ml, 0.002 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature. The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol. The Cu(II) complexes were synthesized and characterized.

The Zn(II) complexes were prepared by adding a methanolic solution of zinc(II)acetate(25ml,.001mol)to a solution of curcuminoid analogue(25ml,.002mol)in methanol and refluxed gently for 2h. The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol.

The Oxovanadium(IV) complexes were prepared by adding a methanolic solution

of vanadium (IV) oxide sulphate (25ml,.001mol) to a solution of curcuminoid analogue (25ml,.002mol) in methanol and refluxed gently for 2h. The precipitated complex was filtered, washed with 1:1, methanol: water mixture and recrystallised from hot methanol.

The Ni(II) complexes were prepared by adding a methanolic solution of nickel acetate(25ml,.001mol)to a solution of curcuminoid analogue(25ml,.002mol)in methanol and refluxed gently for 2h. The precipitated complex was filtered, washed with 1:1, methanol: water mixture and recrystallised from hot methanol.

3.4 Characterisation of the ligands and metal complexes:

The ligands and metal complexes were characterized by various spectral and analytical techniques.The spectral techniques involve UV, IR, ^{1}H NMR,^{13}C NMR,2D COSY NMR,Mass spectra and ESR spectra for Cu(II) complexes.Elemental analysis of compounds were done to find C,H,S and metal percentage in them by Vario EL III analyzer.The analysis was carried out at STIC,Cusat.

UV-Visible Spectra

The absorption of radiation in the UV-Vis region of the spectrum is dependent on the electronic structure of the absorbing species. UV spectra are characterized by two parameters, the position of the maximum of absorption band at λ max and intensity of band. Only $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions can be achieved by energies in the 200-800nm region. Only those molecules are likely to absorb light in UV-Vis region which contains π and nonbonding electrons. The important transitions observed in the spectrum of analyzed compounds are $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. Absorbance spectroscopy was performed on a Schimadzu UV-VIS -1601 spectrophotometer.

IR spectra

IR spectra is the absorption spectra of compounds that are a unique reflection of their molecular structure.IR spectroscopy is often used to identify structures because functional groups give rise to characteristic bands both in terms of intensity and position (frequency). Molecules experience a wide variety of vibrational motions, characteristic of their component atoms Compounds absorb IR radiations that corresponds in energy to these vibrations. The IR spectra of synthesized compounds are characterized by bands corresponding to C=O, C=C phenyl, C=C alkenyl ,CH=CH trans groups, C-H vibrations etc. The observed position and intensity of the bands in the spectra helps us to identify the presence of hydrogen bonding, conjugation etc in the molecule. It helps to detect the tautomeric structure of the compound. The position and intensity of the carbonyl stretching band depends on the molecular structure in its immediate vicinity and is useful in characterizing the type of carbonyl function.IR spectra (KBr pellets) of compounds were recorded on 8101 Schimadzu FTIR spectrophotometer.

^{1}H NMR spectra

NMR spectroscopy is a research technique that exploits the magnetic properties of certain nuclei. It relies on the phenomenon of nuclear magnetic resonance and provide detailed information about the structure and chemical environment of molecules. Different functional groups are obviously distinguishable and identical functional groups with different neighboring substituents give distinguishable signals. The position and nature of splitting of the signals depends on the mode of the coordination, nature of substituents and extent of delocalization in the chelate ring. The position of functional groups like methine, enol, alkenyl and aryl protons can be identified in the spectrum [257-259].The ^{1}H NMR spectra were recorded on a Varian 300 NMR spectrophotometer.

^{13}C NMR spectra

The Carbon atoms of a molecule could be probed by NMR in the same fashion as hydrogen atoms. The ^{13}C NMR spectrum of a compound displays a single sharp signal for each structurally distinct carbon atom in a molecule. The dispersion of ^{13}C chemical shifts is nearly 20 times greater than that for protons, and this together with the lack of signal splitting makes it more likely that every structurally and magnetically distinct carbon gives only a single peak so that they can be easily identified. In ^{13}C NMR, chemical shifts appear over a range 0 to 220ppm .Saturated carbon atoms, carbonyl carbon,alkenyl and aromatic carbon atoms can be identified from their positions in the spectra. The carbonyl carbon usually appears at the lowest field values and have the largest chemical shifts due to the electronegative oxygen attached to it which produces deshielding. Anisotropy is responsible for the large chemical shifts of carbon in aromatic rings. The ^{13}C NMR spectra were recorded on a Bruker, AV 400 – AVANCE III NMR spectrophotometer.

2D COSY NMR

Correlation spectroscopy is one type of two dimensional NMR spectroscopy which is best known by its acronym,COSY.Two dimensional NMR spectra provide more information about a molecule than one dimensional NMR spectra.In two dimensional experiments,there are two coordinate axes representing ranges of chemical shifts.The signal is presented as a function of each of these chemical shift ranges.The data are plotted as a grid, with two axes representing chemical shift ranges and third dimension constitutes the intensity of the observed signal.The result is a form of contour plot in which contour lines correspond to signal intensity[260].The two dimensional experiments include H-H correlation spectroscopy, COSY and heteronuclear correlation spectroscopy ,HETCOR. COSY NMR spectra were recorded using Bruker, AV 400-AVANCE III FT-NMR spectrophotometer.

Mass Spectroscopy

Mass spectrometry has been an important analytical tool for many years.This works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass to charge ratio.The x axis of mass spectrum is the m/z ratio(mass to charge) and y axis,the ion abundance.The value of m/z at which the molecular ion appears on the mass spectrum gives the molecular weight of the molecule.Fragmentation of the molecular ion produces fragment ions at m/z values corresponding to their individual masses.This technique gives idea about the molecular weight of a molecule and its fragmentation pattern .The potential of mass spectroscopy in the structural elucidation of coordination compounds [261,262] has been demonstrated by Chaston,Reid et al.Metal complexes of substituted β-diketones have been studied extensively by electron impact mass spectrometry. The FAB mass spectra were recorded on a Joel SX–102 mass spectrophotometer from CDRI, Lucknow, India.

C, H, N analysis

C, H, N analysis is very much important in studying the structure of organic parts in metal complexes. A CHN Analyzer is a scientific instrument which can determine the elemental composition of a sample. The analyzer uses a combustion process to break down substances into simple compounds which are then quantified, usually by infrared spectroscopy. C, H, N analysis was done using Vario EL III analyzer. Metal percentage was also calculated using standard methods.

ESR spectroscopy

Electron spin resonance (ESR) spectroscopy is a technique for studying materials with unpaired electrons. The basic concepts of EPR are analogous to those of NMR, but it is electron spins that are excited instead of the spins of atomic nuclei. EPR spectroscopy is particularly useful for studying metal complexes. An unpaired electron can move between the

two energy levels by either absorbing or emitting a photon. EPR spectra can be generated by either varying the photon frequency incident on a sample while holding the magnetic field constant or doing the reverse. In practice, it is usually the frequency that is kept fixed. The unpaired electrons are excited to a high energy state under the magnetic field by the absorption of microwave. The excited electron changes its direction of spin and relaxes into the ground state by emitting phonons. Microwave absorption is measured as a function of the magnetic field by ESR spectroscopy.1,3 diketone copper complexes are popular for ESR studies.

3.5 Thermogravimetric studies

Thermogravimetry (TG) is the branch of thermal analysis which examines the mass change of a sample as a function of temperature in the scanning mode.TG is used to characterize the decomposition and thermal stability of materials under a variety of conditions and to examine the kinetics of the physicochemical processes occurring in the sample. Thermogravimetric curves are characteristic for a given compound because of the unique sequence of the physiochemical reaction that occurs over specific temperature ranges and are function of the molecular structure. TG curves are normally plotted with the mass change (Δm) expressed as a percentage on the vertical axis and temperature (T) or time (t) on the horizontal axis. 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. The thermogram illustrates the temperature at which the compound begins to decompose, decrease in weight loss, formation of intermediate compound,temperature of final decomposition etc.TG studies were conducted using Perkin Elmer Diamond Thermal Analyser System.

3.6 Magnetic studies

The magnetic moment is a useful quantity because it is related to the number of unpaired electrons in a sample. An experimental magnetic moment can be calculated from the following equation.

χ_g =C x L (R-Ro)/W x10^9

[C = calibration factor, L = length of sample, R = sample reading , Ro = empty tube reading and W = weight of sample]

$$\chi_m = \chi_g \text{ x mol.wt}$$

$$\mu = 2.808 (\chi_m \text{ x } T)^{1/2}$$

Therefore by comparing actual to experimental values it is possible to determine the number of unpaired electrons in a sample.The magnetic moments were measured using Gouy type magnetic balance.

3.7 Biological studies of Curcuminoid analogues and their Transition metal chelates

The biological studies conducted include Invitrocytotoxic study, Invivoantitumour study,effect of compounds on solid tumour, Antibacterial and Antifungal studies. Materials, cell lines, animals, chemicals etc employed in the studies are given.

3.7.1 Invitrocytotoxicity study

Materials

All reagents and chemicals used in the study are of analar grade.

Cell lines: Daltons Lymphoma Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cell lines were obtained from the Adayar Cancer Research Institute, Chennai, India. The cells were maintained in mice by intraperitoneal inoculation.The cells were aspired from the peritoneal cavity of the mouse from the 15th day of induction of tumour cells.

Preparation of Reagents:

Normal Saline: This was prepared by dissolving A.R.NaCl (0.85g) in 100 ml distilled water.Normal Saline is essential for preparing cell suspension, as the osmotic pressure due to it is isotonic with the fluids inside the cells and this will not cause death of the cell.

Phosphated Buffer Saline(PBS):It is used for maintaining the pH and isotonicity of the cells, failing which the cells may rapture during experiments. It is prepared 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: Cell viability was determined using this dye which penetrates into dead cells and makes the identification of dead cells easier. It is prepared by dissolving 1 g trypan blue in 100 ml distilled water.

Method

In vitro cytotoxicity studies were carried out using the diketone & metal complexes which are dissolved in minimum quantity of DMSO. The tumour cells (DLA & EAC), aspirated from the peritoneal cavity of tumour bearing mice after 15 days of inoculation were washed with PBS (Phosphate buffered saline) and centrifuged at 1000 rpm for 5 min. Cell viability was determined by trypan blue exclusion method. Viable cells (1×10^6 cells in 0.1 ml) were added to tubes containing various concentrations of the test compounds (200, 100, 50, 20 & 10 μg/ml) and the volume was made up to 1 ml using PBS. Control tube contains only cell suspension. These mixtures were incubated for 3 h at 37°C. 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 number of stained and unstained cells were counted and percentage cytotoxicity (cell death) was evaluated by Trypan blue dye exclusion method .

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

3.7.2 Invivo antitumour studies

Cell lines:EAC and DLA cell lines were maintained as ascites tumours in Swiss Albino mice.The cells were aspirated ,washed thrice with PBS.The cells were suspended in saline so as to get a cell suspension of 1 million cells/ml.One ml of the cell suspension was injected into the peritoneal cavity of fresh Swiss albino mice.The test animals were given normal diet and within 10-14 days ascites fluid that contain cancer cells were accumulated in the abdomen.The animals grow with this tumour and die within 18-20 days.These cells are propagated regularly by transferring it to other normal mice as mentioned above and thus the cell lines were maintained.

Animals: Swiss albino mice(7-8 weeks old weighing 25-30 g,male) were obtained from the Small Animal Breeding Station (SABS), Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala. They were kept in well ventilated cages under standard conditions of temperature and humidity in animal house of Amala Cancer Research Centre. The animals were provided with standard mouse chow (Sai Durga Feeds,Bangalore,India) and water *at libitum*. All animal experiments in this study were carried out with the prior approval of the Institutional Animal Ethics Committee (IAEC) and were conducted strictly according 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

For animal studies, drug suspension was prepared by using carboxy methyl cellulose (CMC), ie, by dissolving 0.05 gm carboxy methyl cellulose in 10 ml distilled water. For preparing the drug, 0.01 gm 1,7-diaryl heptanoids and their metal complexes were dissolved in 5ml of ethanol. Mix both solutions and keep the mixture in a water bath for evaporation of ethanol to get the drug in the slurry form.

The standard drug cyclophosphamide was prepared by dissolving 25mg/kg body weight of mice in phosphate buffered saline (PBS) solution. 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

Groups of Swiss Albino mice (5nos/group) were injected intraperitoneally with EAC cells($1x10^6$cells/animal).One group was kept as control: oral administration of 0.1ml of distilled water /animal without drug treatment. Another group was kept as standard : cyclophosphamide 25mg/kg body weight. All other groups of mice were simultaneously injected (ip) with the test compounds in different concentrations (20µg/ml,10µg/ml,5µg/ml) from the second day of tumour induction upto 10 days.The animals 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.]

Normal Swiss Albino mice

Tumour bearing mice

Determination of the effect of compounds on solid tumour development

Solid tumours were induced in groups of Swiss Albino mice (5nos/gp) by subcutaneous injection of DLA cells on the right hind limbs.One group was kept as control that is not treated with any drug, another group as standard ie given std. drug. Other groups were simultaneously injected (ip) with the test compounds (200 μmol/kg body weight) and continued for 10 days.Tumor diameter was measured every third day for 1month and the tumor volume was calculated using the formula,$V=4/3\pi r_1^2 r_2^2$,where r_1 and r_2 are the minor and major radii respectively.

3.8 Antibacterial assay (Agar well diffusion method)

Agar plates were prepared using sterile Muller -Hinton (MH) agar medium. Bacterial strains of *Escherichia Coli, Klebsiella Pneumoniae* and *Bacillus Subtilis* of 24 h culture were evenly spread into the surface of the agar plates using sterile swab sticks. Wells were cut into agar plates with sterile gel puncture. The curcuminoid analogues and their metal chelates in the concentration 5 mg/ml in DMSO were added in the wells. The pure solvent DMSO act as negative control and streptomycin (5mg/ml) served as positive control. The plates were incubated at 37°C for 24 h and observed for zones of inhibition. 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

Antifungal test was carried out by disc diffusion method. The fungal cultures were maintained in Sabouraud's Dextrose broth. Each culture was uniformly distributed on SDA plates using 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. 2 % DMSO was used to dissolve the drug, which was found to have no adverse effect on the fungal cultures. Drugs of different concentrations [100,250,500μg/ml] were added on each disc with a micropipette. Disc with DMSO but, without drug was used as control. Then the plates were incubated at room temperature for 2-3days. After incubation, zone diameter in mm was measured.

Media used and their composition

Muller Hinton Agar Medium (1 L) The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm 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.

CHAPTER-I

SYNTHESIS, CHARACTERISATION AND BIOCHEMICAL ACTIVITIES OF METHYL SUBSTITUTED 1,7-DIARYLHEPTA-1,6-DIENE-3,5-DIONES AND THEIR TRANSITION METAL CHELATES WITH CU(II), Zn(II),Ni(II) & OXOVANADIUM(IV)

SECTION I

SYNTHESIS AND CHARACTERIZATION OF 1,7-BIS(2-METHYL PHENYL)1,6-HEPTADIENE-3,5-DIONE AND 1,7-BIS(2,5-DIMETHYL)1,6-HEPTADIENE-3,5-DIONE

1.1.1 Synthesis of methyl substituted 1,7–diaryl heptanoids

The 1,7- diaryl heptanoids with monomethyl and dimethyl substituted phenyl rings were synthesized by the condensation of aldehydes, (2-methyl benzaldehyde and 2,5 dimethyl benzaldehyde) with acetylacetone-boron complex (prepared by stirring acetyl acetone and B_2O_3 for 1 hr)in ethyl acetate medium in presence of tri(sec-butyl) borate and n-butyl amine as condensing agent.The reaction leads to the formation of a 1,7-diarylheptanoid which is formed by the condensation of two equivalents of aldehyde with one equivalent of acetyl acetone.It is represented in a schematic way in **Scheme 1.1.1.**

1a – $R_1 = CH_3$, $R_2 = H$

1b – $R_1 = CH_3$, $R_2 = CH_3$

t-butyl borate/ n-butyl amine

Scheme 1.1.1

The products 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(1a) and 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione(1b)were purified by column chromatography over silica gel (60 – 120 mesh) using 4:1 (v/v) chloroform:acetone mixture as the eluent and recrystallised twice from hot benzene to get pure crystalline material. The aldehyde used for synthesis, structures of the compounds prepared,its systematic name, and yield are given in **Table1.1.1.**

Table 1.1.1 Synthetic details of methyl substituted 1,7-diphenyl heptanoids

Compound	Aldehyde used for Synthesis	Structure of Ligands	Systematic name	Yield %
1a	**2-methyl benzaldehye**		1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione	**62**
1b	**2,5-dimethyl benzaldehyde**		1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione	**75**

Both the compounds 1a & 1b are coloured compounds,orange and yellowish respectively, crystalline in nature and show sharp melting points.They were obtained in good yields nearly 62 and 75% respectively. They are readily soluble in organic solvents like acetone, ethanol, methanol, chloroform. The observed C, H percentage and molecular weight

determination (Table1.1.2) together with mass spectral data of the compounds clearly suggest the formation of bis-condensation product.

Table 1.1.2 Analytical & UV spectral data of 1a & 1b

Compounds	MP.(°C)	Elemental analysis (%)		Molecular weight	UV λmax (nm)
		C	H		
		Found/(Calculated)			
1a	123	81.91(82.89)	6.19(6.57)	302(304)	276, 387
1b	159	82.45(83.13)	6.95(7.22)	331(332)	281, 393

1.1.2. Characterisation of methyl substituted 1,7–diaryl heptanoids

The compounds 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(1a) and 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione(1b) synthesized were characterized using various spectral techniques like UV, IR, ^{1}H NMR, ^{13}C NMR,2D COSY NMR and Mass spectra.The spectral data of synthesized ligands are discussed below.

UV spectra

The UV spectra of the compound 1a in methanol show two absorption maxima at 387nm &276nm respectively due to the $n\rightarrow\pi*$ and $\pi\rightarrow\pi*$ transitions (Table1.1.2). The value at 270-300nm are due to $\pi\rightarrow\pi*$ transition and at 360-460nm are due to $n\rightarrow\pi*$ transitions based on earlier reports.The presence of α,β unsaturation increases the wavelength of carbonyl absorption maxima.The high energy band at 276nm is due to $\pi\rightarrow\pi*$ transition of the fully conjugated system.The UV spectra of the compound 1b in methanol show two absorption maxima at 393nm &281nm respectively due to the $n\rightarrow\pi*$ and $\pi\rightarrow\pi*$ transitions (Table1.1.2).The UV spectra of 1b is given below (Fig.1.1.1).

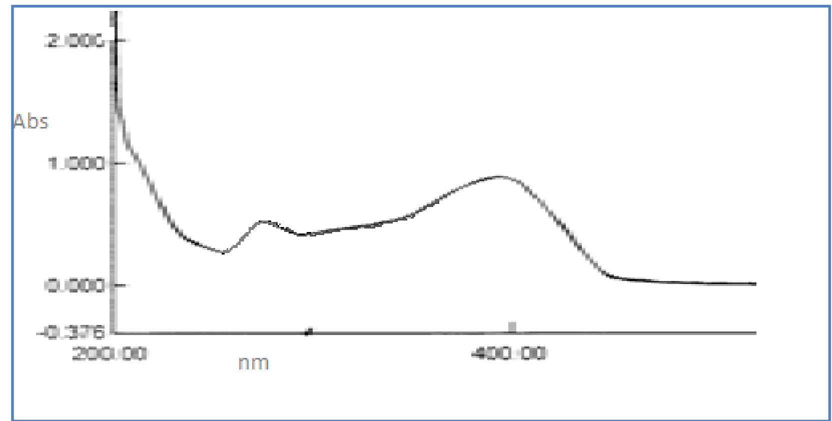


Fig.1.1.1 UV spectra of 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione

IR spectra

In compounds1a & 1b, the position and intensity of the carbonyl stretching frequency depends on the molecular structure in its immediate vicinity and is useful in characterizing the type of carbonyl function. Presence of α,β – unsaturated 1,3 - diketo moiety present in these molecules can be very well established using IR spectroscopy (Table 1.1.3)

Table 1.1.3 IR spectral data of methyl substituted 1,7-diaryl heptanoids

Compounds		Probable IR assignments
1a	1b	
1625	1622	V(C=O) chelated
1598,1571	1564,1554	V(C=C) phenyl
1545	1517	V(C-C) alkenyl
1516	1496	V_{as}(C-C-C) chelate ring
1452	1450	V_s(C-C-C) chelate ring
1134,1041	1132,1067	β(C-H) chelate ring
970	968	V(CH=CH) trans

IR spectra of 1a and 1b are characterized by the presence of strong bands at 1625 cm^{-1} and 1622 cm^{-1} respectively due to the enolised conjugated C=O group. The stretching frequency of acetyl carbonyl group and carbonyl stretching frequency of aroyl group are at ~ 1710 cm^{-1} and at ~ 1660 cm^{-1} respectively. The C=O frequency decreases due to hydrogen bonding and increased conjugation. There is no other band in the region 1600-1800 cm^{-1} which is assignable due to free or bound C=O group.This shows that the compound exist in the intramolecularly hydrogen bonded enolic form.

In the spectra, the intramolecular hydrogen bonded enolic group shows a broad band in the region 2550-3600 cm^{-1}. There are a number of medium intensity vibrations observed in the region 1050-1450 cm^{-1} due to various stretching vibrations of the phenyl group, alkenyl & chelate ring.The band in the region 970 and 968 is assigned to the trans CH=CH vibrations. The IR spectrum of compound 1a is given below in Fig.1.1.2.

IR spectrum of compound 1b is depicted in Fig.1.1.3.

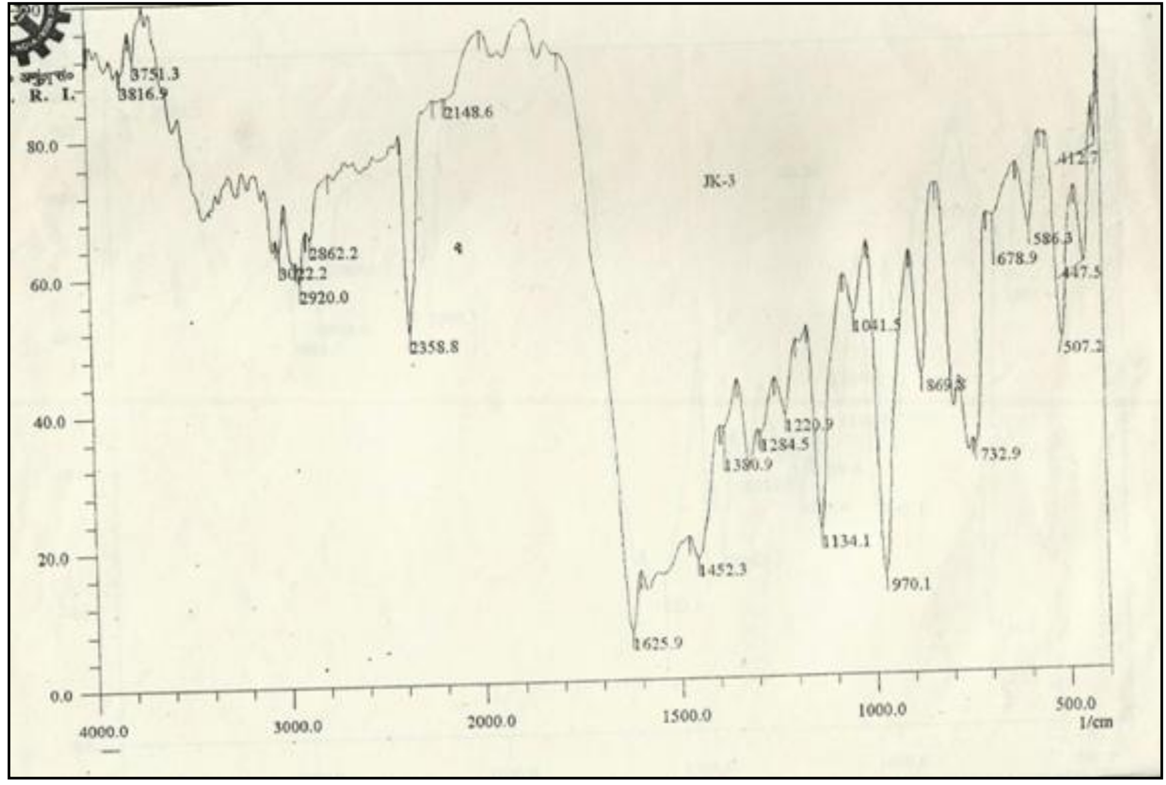


Fig.1.1.2 IR Spectrum of 1,7-bis(2-methyl phenyl) hepta-1,6-diene-3,5-dione

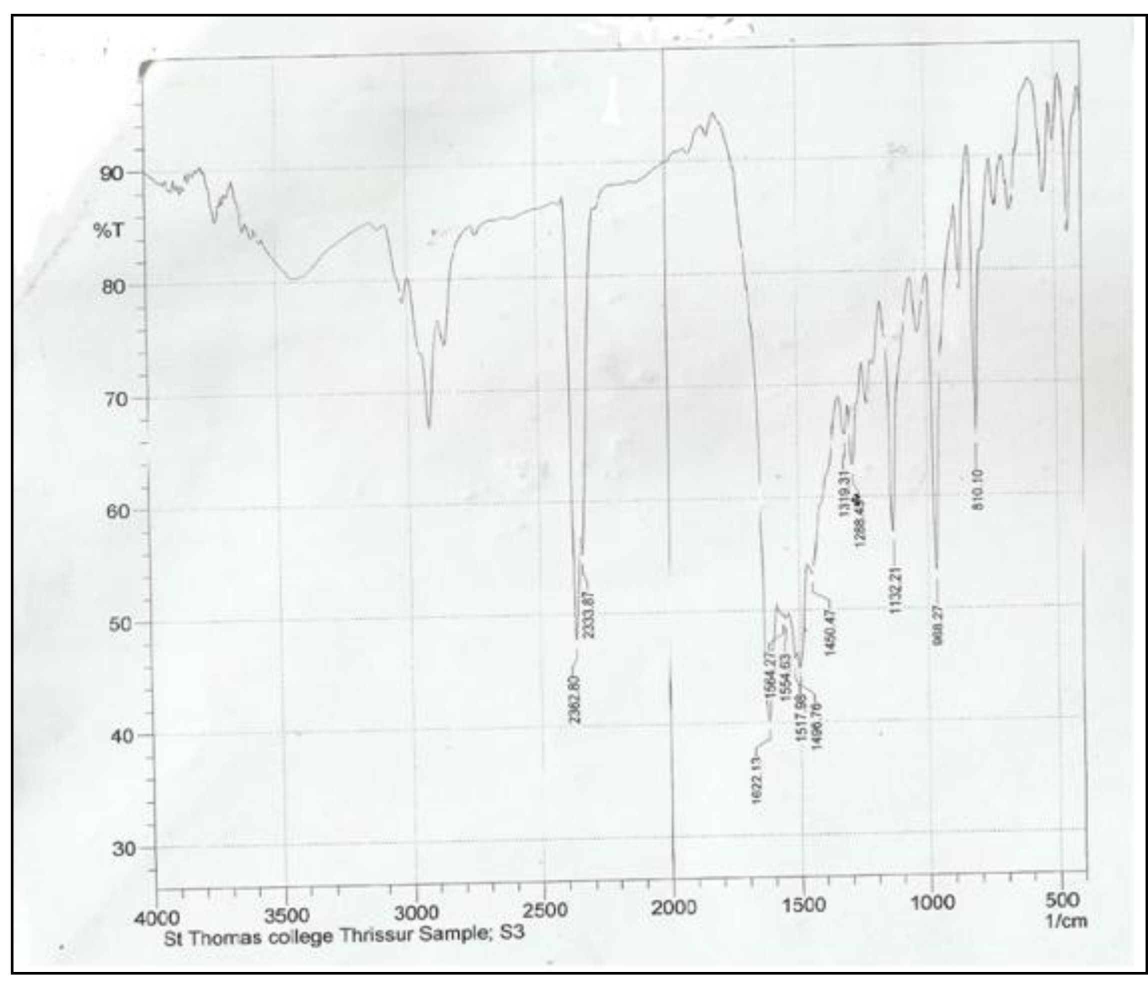


Fig 1.1.3 IR Spectrum of 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione

^{1}H NMR spectra

The ^{1}H NMR spectra of methyl substituted 1,7-diaryl heptanoids also supports the enolic structure of the compound. The peaks corresponding to enolic, methine, alkenyl, methyl and phenyl groups can be observed in the spectrum(**Table 1.1.4).** Ligands **1a & 1b** displayed a one proton singlet at ~ 16ppm assignable to strong intramolecularly hydrogen bonded enolic proton. Another one proton singlet at ~ 5.8ppm can be assigned to the strong intramolecularly hydrogen bonded methine proton. The various proton signals observed in the spectra are given in Table 1.1.4

Table 1.1.4 ^{1}H NMR spectral data of methyl substituted 1,7-diaryl heptanoids

Compounds	Chemical shifts (δ ppm)				
	Enolic	Methine	Alkenyl	Phenyl	Methyl
1a	16.025	5.838	6.529-7.954	7.071-7.415	2.463
1b	15.941	5.835	6.536-7.977	7.199-7.269	2.345 2.417

The aryl protons show signals in the region 7.1 – 7.5ppm and the alkenyl protons show signals in the region of 6.5 – 8.0ppm. The methyl group on aryl ring in 1a showed a signal at 2.463 where the two methyl groups on aryl ring of 1b are present at ~ 2.3,2.4 ppm. The ^{1}H NMR spectra of 1a and 1b are brought out in **Fig.1.1.4 & Fig.1.1.5** respectively.

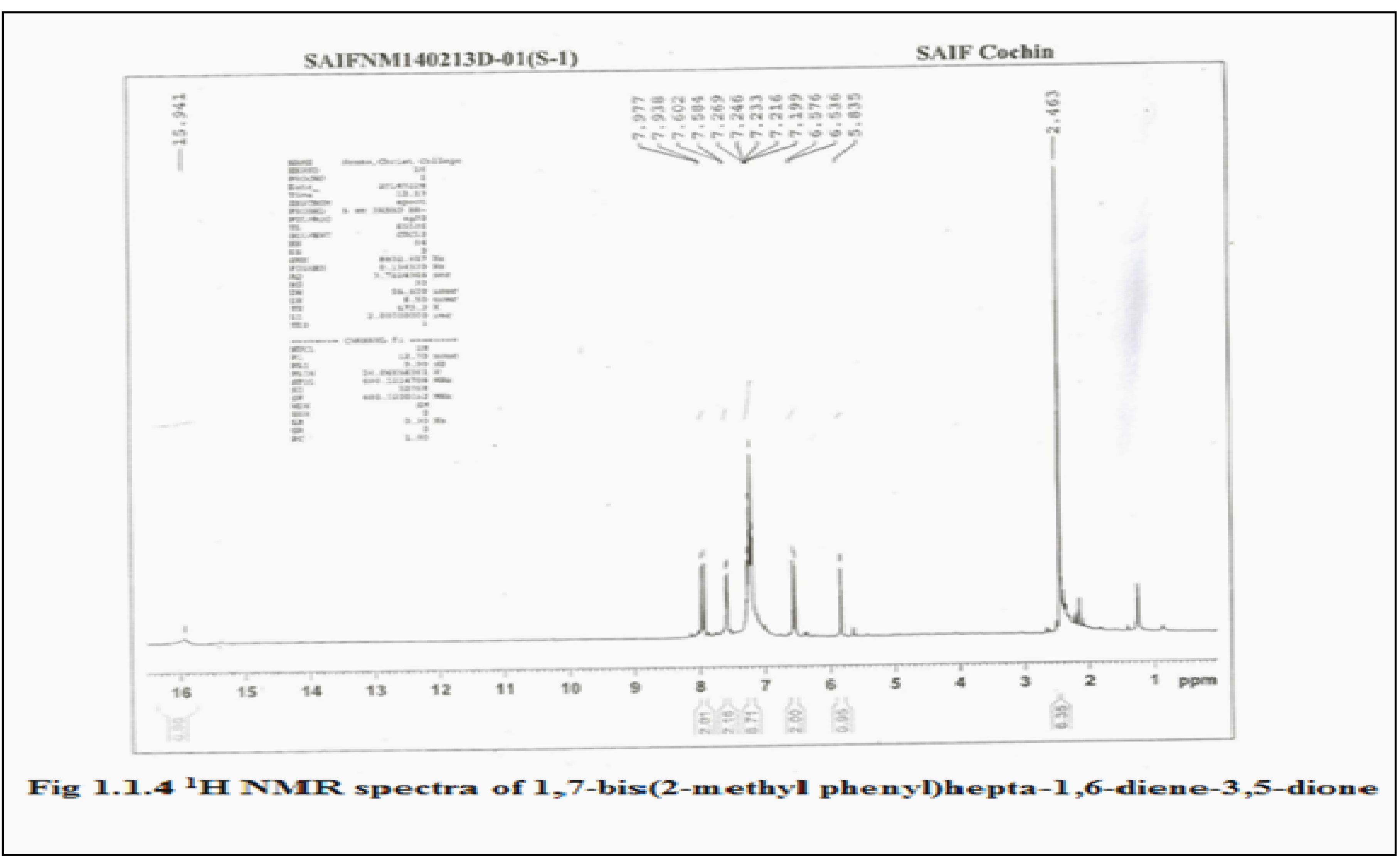


Fig 1.1.4 ^{1}H NMR spectra of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione

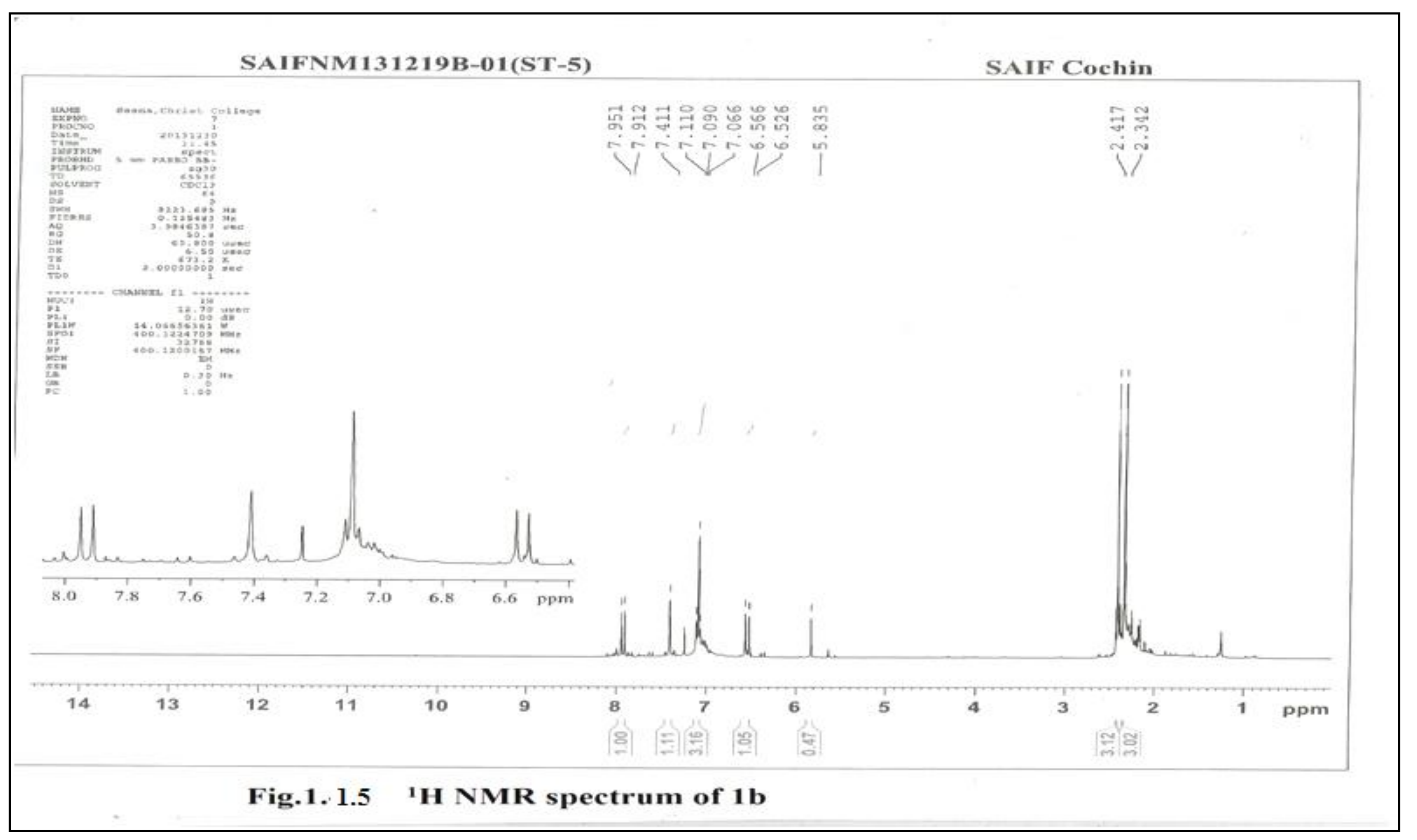


Fig.1.1.5 ^{1}H NMR spectrum of 1b

^{13}C NMR spectra

The ^{13}C NMR spectral details of 1a & 1b are given in Table 1.1.5 & 1.1.6 respectively. The peak corresponding to methine (C1) carbon is present at positions 106.58 & 101.82ppm respectively. Here also there is a possibility of keto-enol tautomerism which makes the shift of C1 carbon to ~ at 100ppm. C2 carbon of carbonyl appears at a position at~ 190ppm. The alkenyl carbon are present at a position nearer to the phenyl ring system. Out of the two alkenyl carbon C3 is downshielded and is present ~ at 139ppm. The aromatic carbon atoms are present between 125 – 140 ppm. **^{13}C NMR spectra of 1a & 1b** are reproduced in Fig.1.1.6 & 1.1.7.

Structure representing different carbon atoms in 1a.

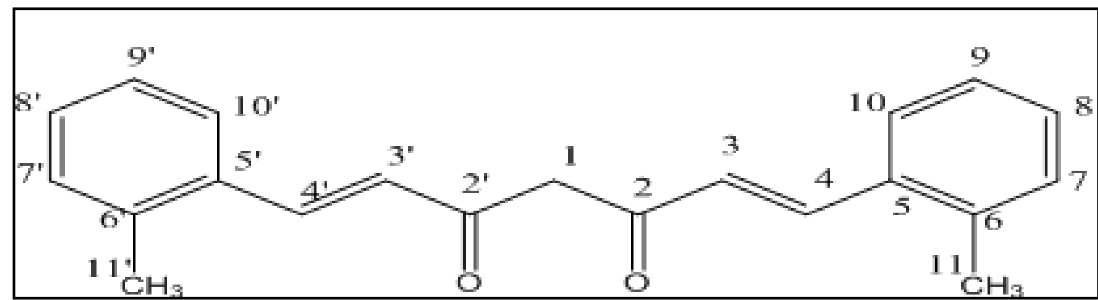


Table 1.1.5 ^{13}C NMR spectral data of 1a (chemical shift in ppm)

C1	**C2,C2'**	**C3,C3'**	**C4,C4'**	**C5,C5'**	**C6,C6'**
106.58	196.67	139.3,138.7	124.4,124.1	133.1,134.4	140.34
C7,C7'	**C8,C8'**	**C9,C9'**	**C10,C10'**	**C11,C11'**	
131.7,130.1	128.8,128.4	126.88,125.9	132.1,132.6	29.29,31.12	

In 1a & 1b 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.

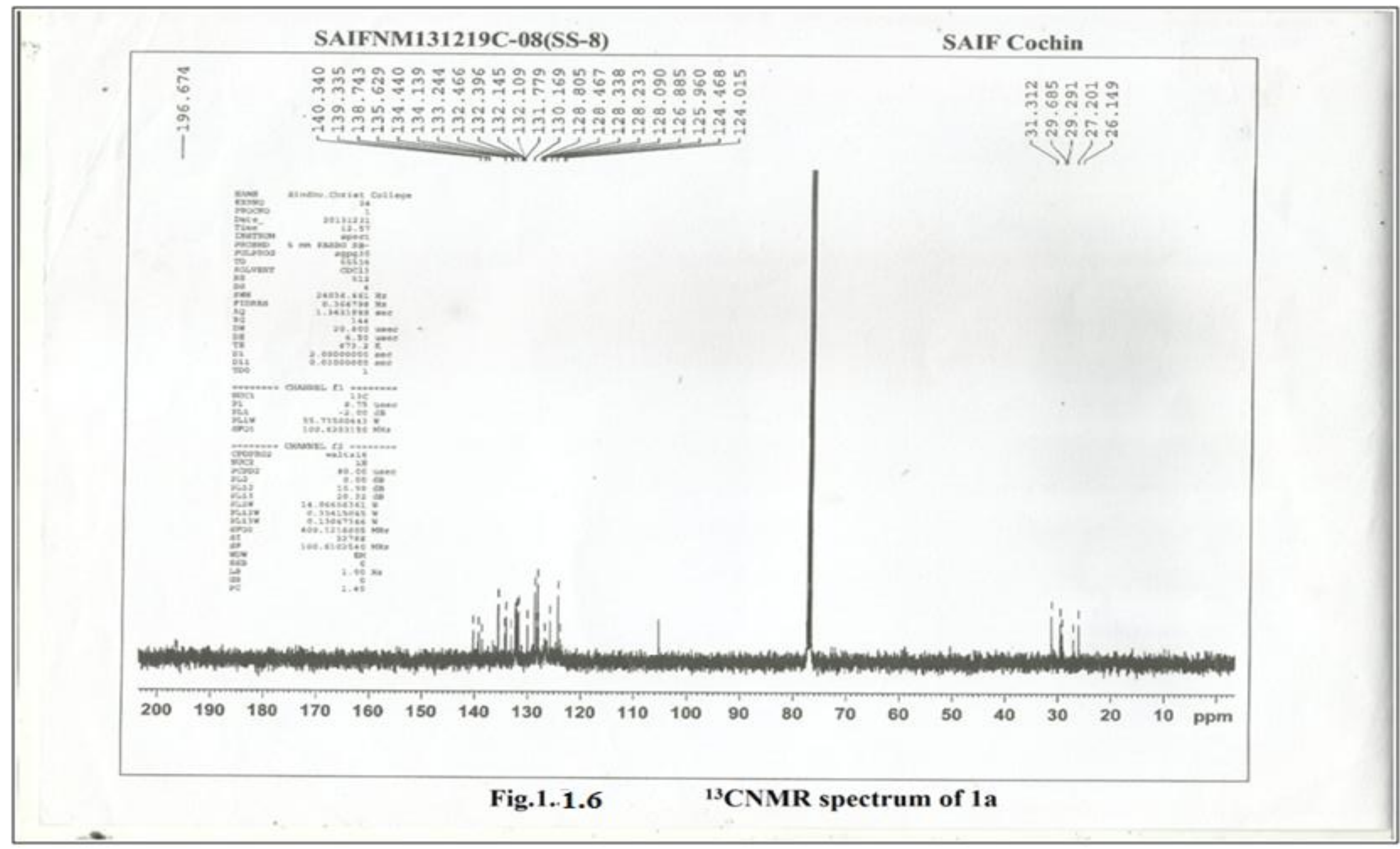


Fig.1..1.6 ^{13}CNMR spectrum of 1a

Structure representing different carbon atoms in 1b.

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

C1	**C2,C2'**	**C3,C3'**	**C4,C4'**	**C5,C5'**	**C6,C6'**
101.82	183.48	138.33	126.76	133.92	135.72
C7,C7'	**C8,C8'**	**C9,C9'**	**C10,C10'**	**C11,C11'**	**C12,C12'**
130.81	130.77	134.92	124.82	20.98	19.33

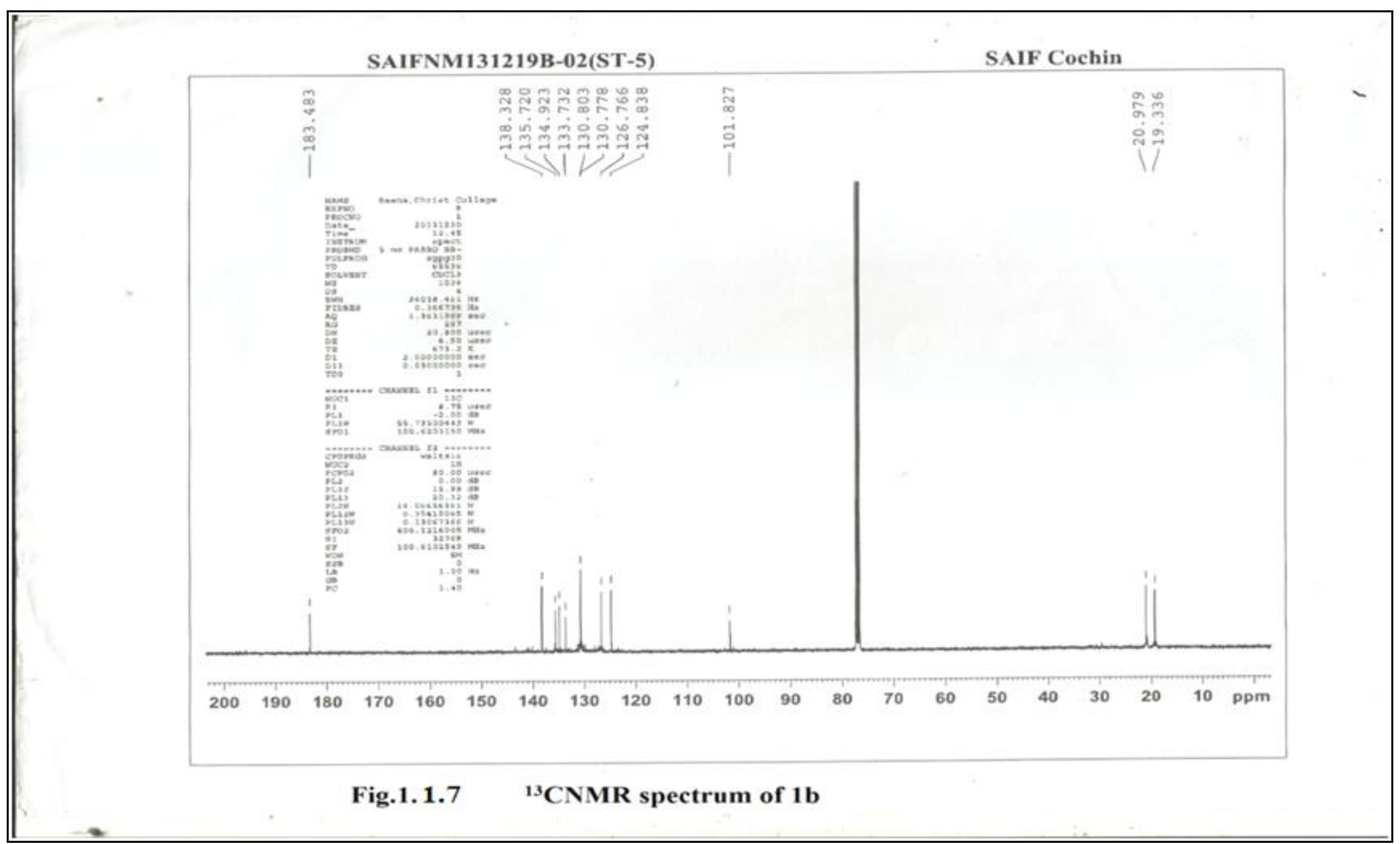


Fig.1.1.7 ^{13}CNMR spectrum of 1b

2D COSY NMR

2D H' – H' COSY NMR spectrum of 1b (Fig.1.1.8) was taken as an additional information to the ^{1}H NMR spectrum. On comparing the spectrum with that of Fig.1.1.5, it was found that methine, alkenyl, phenyl and methyl protons are exactly at the same position as mentioned earlier.

2D C' – H' COSY NMR spectrum of 1b was also taken and is given in Fig.1.1.9 & 1.1.10. On comparing the ^{13}C NMR spectrum of 1b , the values of the methine and alkenyl carbon atoms were ascertained. The methine carbon atom has a value 101.82ppm, apart from the usual value of ~ 50ppm. This is because the carbon atom is flanked between two carbonyl groups. Out of the two alkenyl carbon atoms the C3 carbon has a value of 138.33ppm and C4 has a value of 124ppm. This is very clearly established by the COSY NMR.

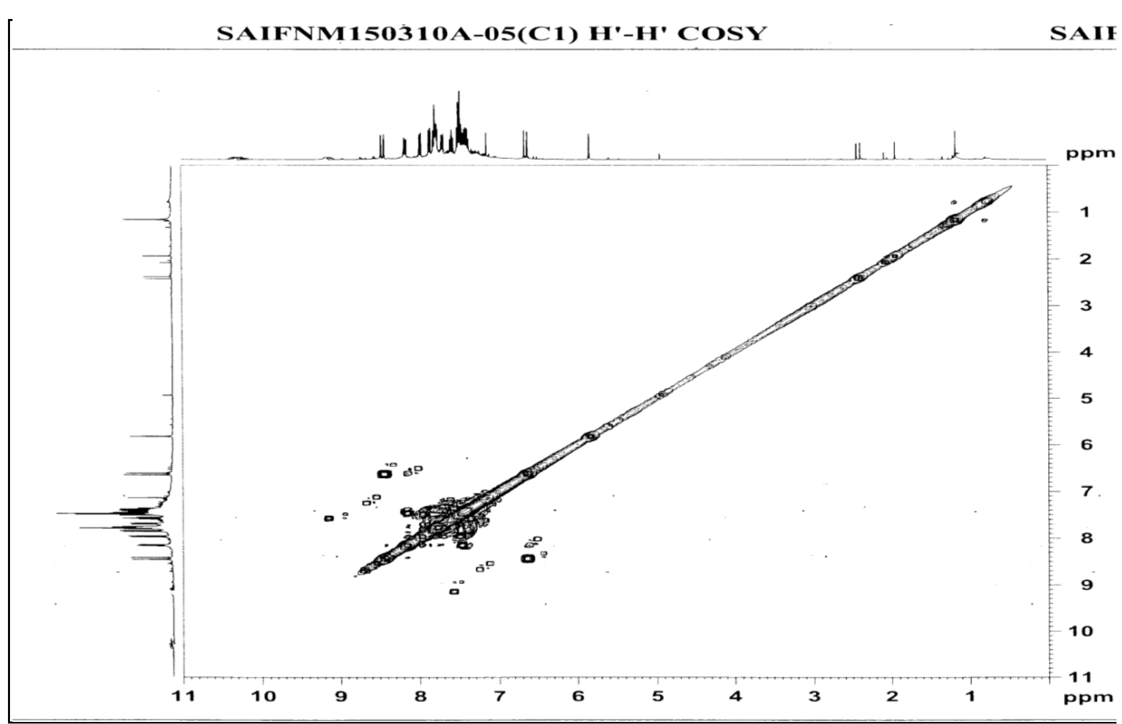


Fig.1.1.8 2D H' – H' COSY NMR spectrum of 1b

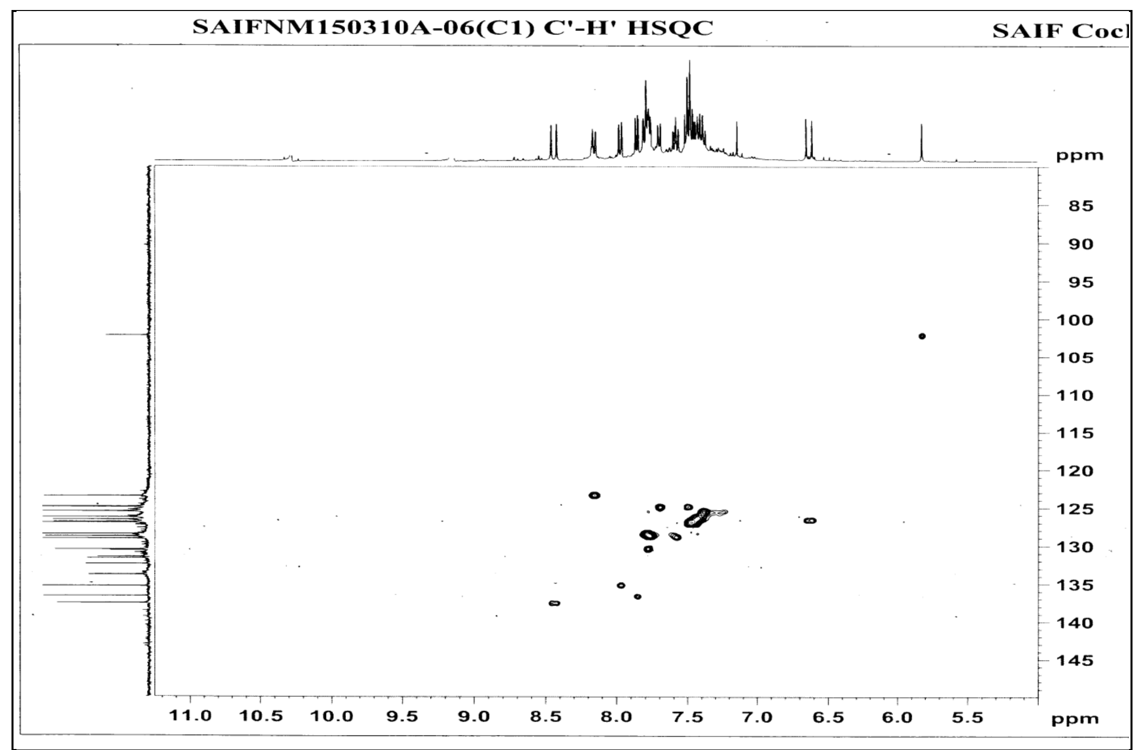


Fig.1.1.9 2D C' – H' COSY NMR spectrum of 1b

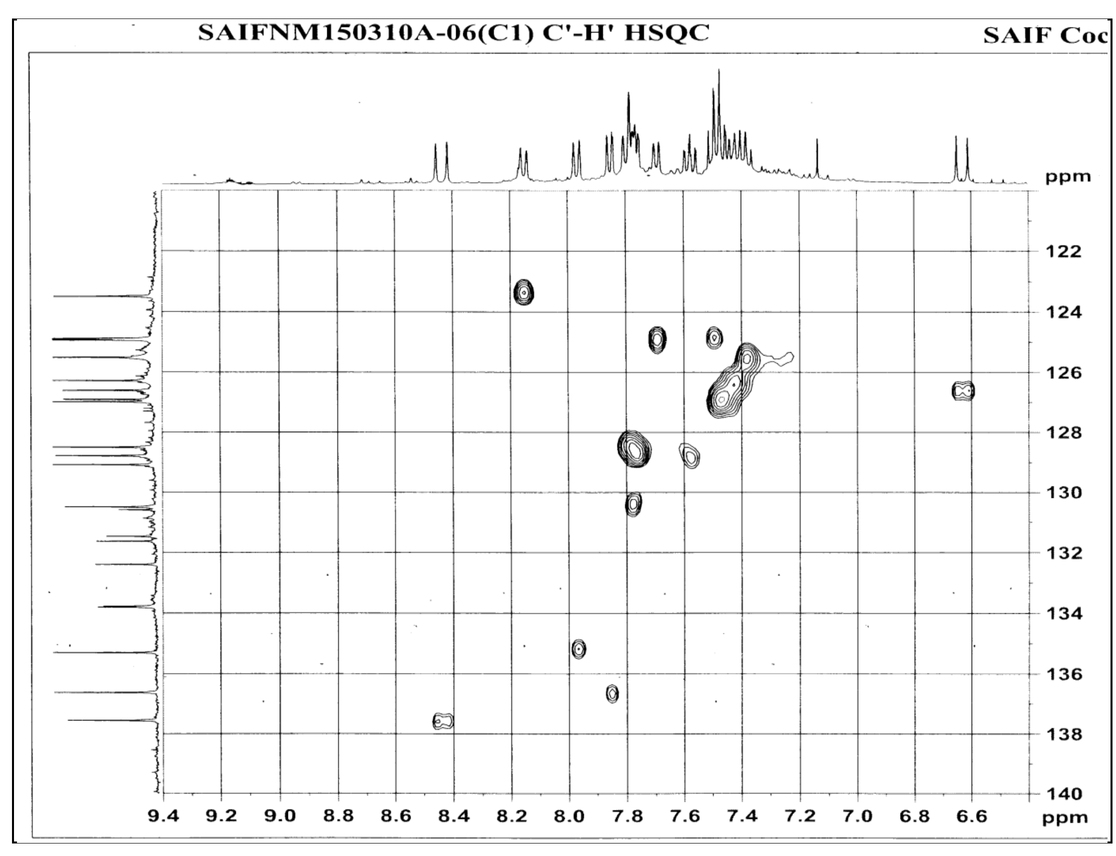


Fig.1.1.10 2D C' – H' COSY NMR spectrum of 1b

Mass spectra

The most important application of mass spectra is in the determination of molecular weight of compounds. The molecular ion peaks as well as fragment ion peaks are observed in the spectra. The mass spectra also gives idea about the various fragmentation modes of the compound. The mass spectra of both compounds showed intense molecular ion peaks. Elimination of important groups like CH_2,C_2H_2,C_2H_2O,$CH_2=C=O$ from the molecule gives different fragments .Important peaks appeared in the spectra of compounds can be conveniently accounted by the fragmentation pattern as shown in (Scheme 1.1.2) and the values are brought out in Table 1.1.7.

Table 1.1.7 Mass spectral data of 1a & 1b

Fragments*	Ligands	M+/M+1 ion	A	B	C	D	E	F	G	H
Mass pattern	**1a**	305	165	145	105	123	90	115	214	186
	1b	332	173	159	115	128	106	127	227	202

*The alphabets corresponds to the fragments given in **Scheme 1.1.2**

The (M+1) ion of 1a is observed at 305 and the M+ ion of 1b is observed at 332. Other important peaks of 1a &1b are due to fragment ion peaks which can be explained from their fragmentation patterns.Mass spectrum of 1a and 1b are given in **Fig.1.1.11** and **Fig.1.1.12** respectively**.** In the mass spectrum of 1a,there is an intense molecular ion peak at 305.The base peak is observed at 145 which is due to [Ar-CH=CH-CO]+(Ar=2-methyl phenyl).The peak at 115 is due to [Ar-CH=CH]+ and at 105 is due to [Ar-CH]+. In the mass spectrum of 1b,the molecular ion peak is observed at 332.The base peak is observed at 159 and can be assigned to the peak of [Ar-CH=CH-CO]$^{+}$, (Ar=2,5-dimethyl phenyl). The peak at 299 is due to the removal of 2 oxygen atoms from the molecular ion.The peak at 173 is due to[Ar-CH=CH-CO-CH_2]+.

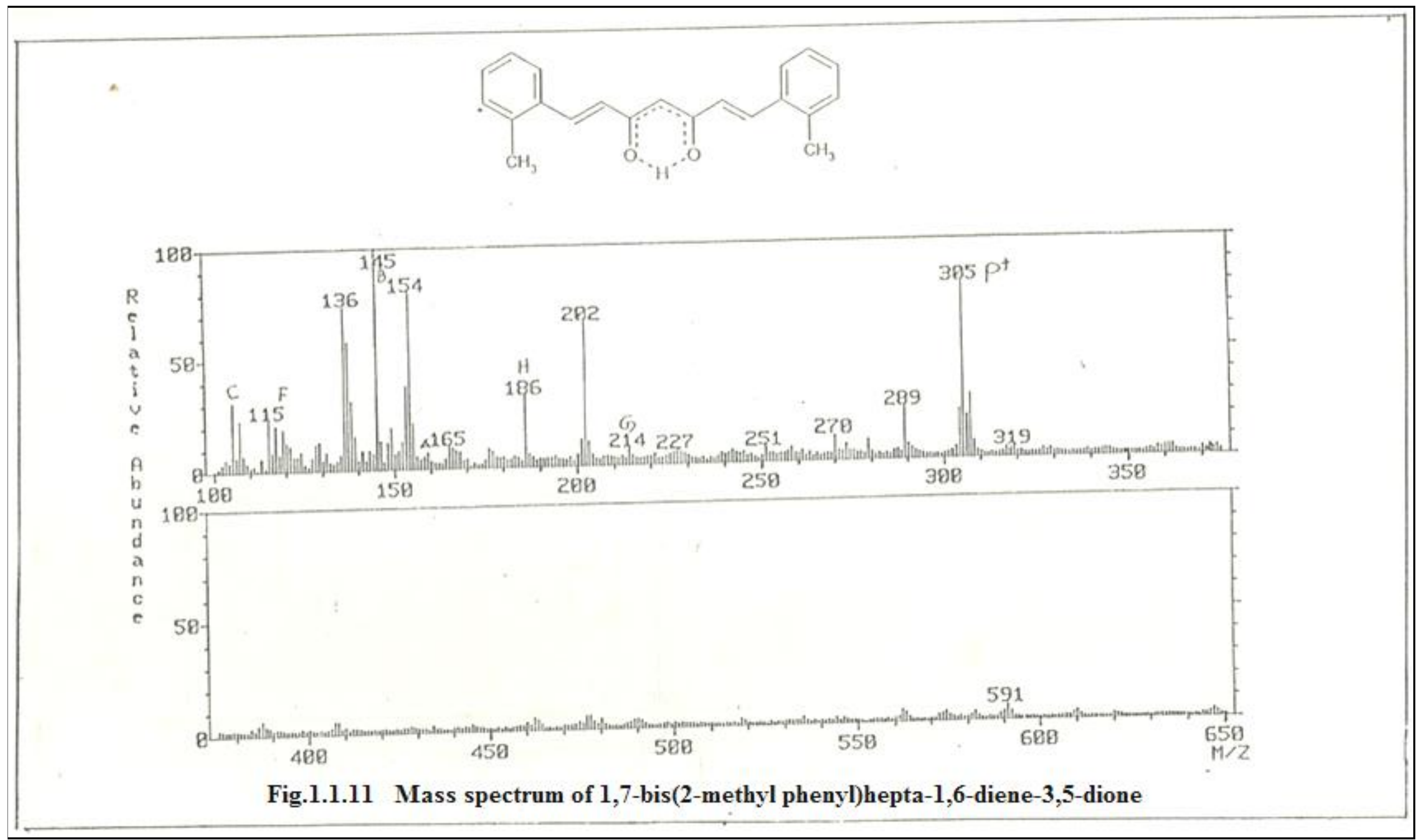


Fig.1.1.11 Mass spectrum of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione

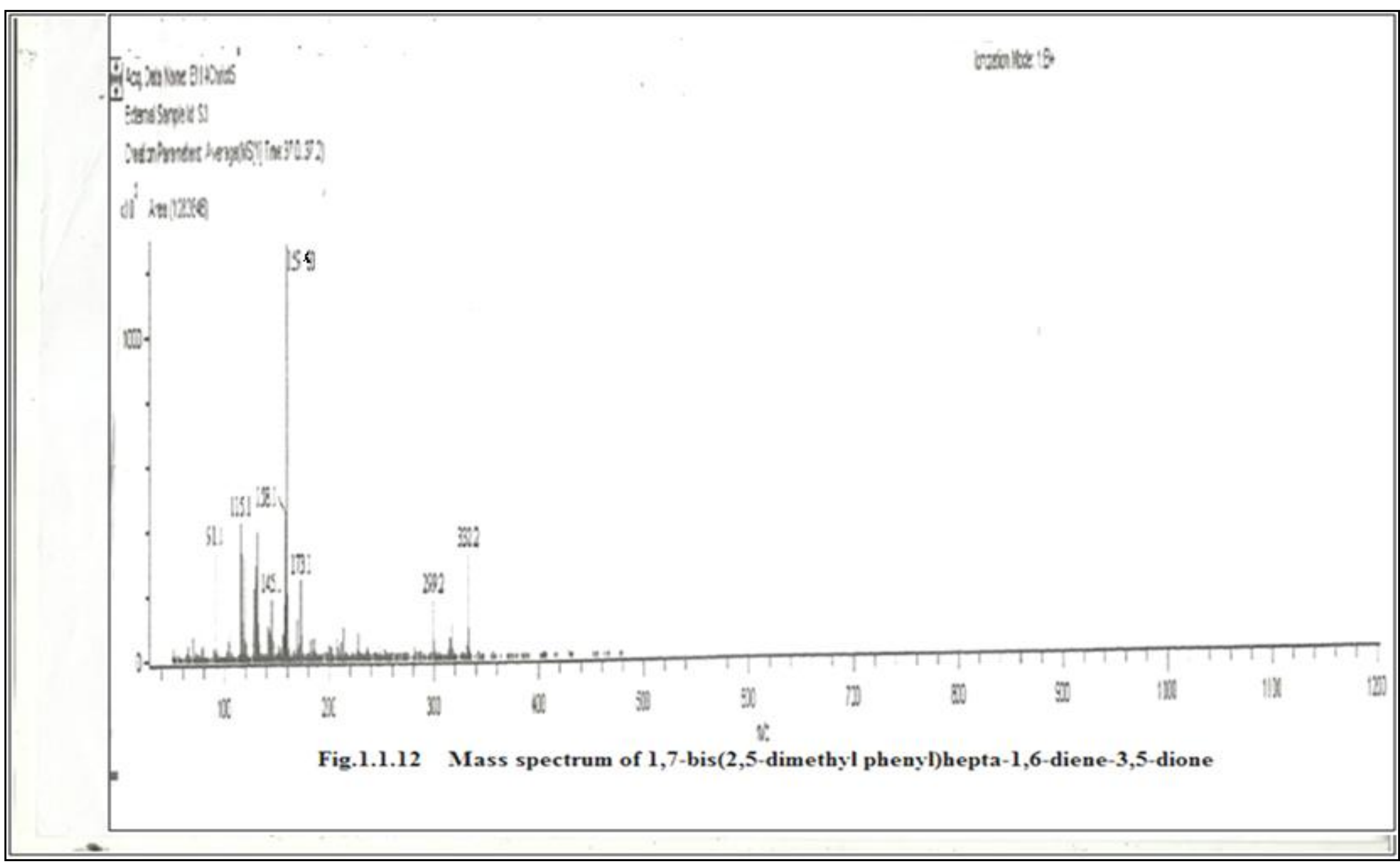
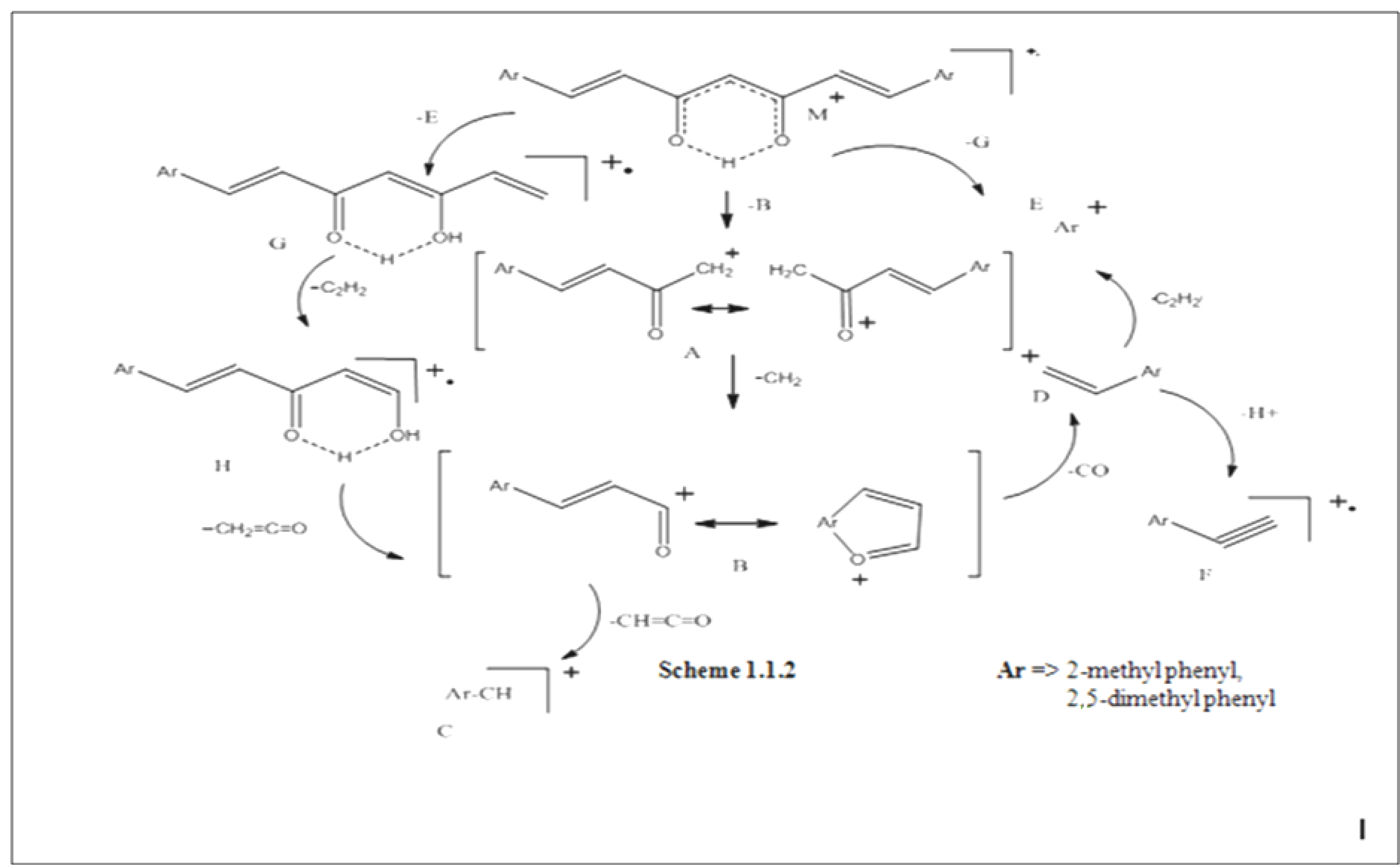


Fig.1.1.12 Mass spectrum of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione


Ar
Ar
M
-E
-G
-B
Ar
G
OH
H
E
Ar
Ar
CH2
H2C
Ar
-C2H2
-C2H2
A
Ar
-CH2
D
Ar
-H+
H
OH
H
Ar
-CO
−CH2=C=O
Ar
Ar
B
F
-CH=C=O
Scheme 1.1.2
Ar => 2-methylphenyl,
2,5-dimethylphenyl
Ar-CH
C

SECTION-II

SYNTHESIS AND CHARACTERISATION OF TRANSITION METAL CHELATES OF METHYL SUBSTITUTED 1,7-DIARYL HEPTANOIDS

1.2.1 Synthesis of metal complexes of methyl substituted 1,7-diaryl heptanoids

Copper(II),Zinc(II),Nickel(II) and Oxovanadium(IV)complexes of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(1a) and 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione(1b) were synthesized by the following general method.

To a refluxing solution of the diketone (0.002 mol) in methanol(25ml),an aqueous solution of metal salt (0.001mol)was added and the reaction mixture was refluxed for nearly 2 hrs and cooled to room temperature. The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol.

Preparation of Cu(II) complex of the ligands

The Cu(II) complexes were prepared by adding a methanolic solution of copper(II)acetate (25 ml, 0.001mol) to a solution of **1a &1b** (25 ml, 0.002 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature. The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol.

Preparation of Zn(II) complex of the ligands

The Zn(II) complexes were prepared by adding a methanolic solution of zinc acetate (25 ml, 0.001mol) to a solution of **1a&1b** (25 ml, 0.002 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature. The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol.

Preparation of Ni(II) complex of the ligands

The Ni(II) complexes were prepared by adding a methanolic solution of nickel(II) acetate (25 ml, 0.001mol) to a solution of **1a &1b** (25 ml, 0.002 mol) in methanol and repeating the above procedure.

Preparation of Oxovanadium(IV) complex of the ligands

The VO(IV)complexes were prepared by adding a methanolic solution of vanadium(IV)oxide sulphate (25 ml, 0.001mol) to a solution of **1a&1b** (25 ml, 0.002 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature. The precipitated complex was filtered, washed with 1:1, methanol: water mixture and recrystallised from hot methanol.

The reaction involved in the formation of complexes is represented below in Scheme 1.2.1

M (acetate)$_2$ M=(Cu,Zn,Ni)
Vanadium(IV) oxide sulphate M=(VO (IV))
+
2 [Ar–CH=CH–CO–CH$_2$–CO–CH=CH–Ar] → M complex (bis-ligand, O,O-chelated)

Scheme 1. 2.1

1.2.2 Characterisation of metal complexes of methyl substituted 1,7-diaryl heptanoids

Transition metal chelates (Cu, Zn,Ni, Vanadyl) of ligands **1a & 1b** were characterized using physical,analytical and spectral datas. Elemental analysis (C, H and metal percentages), physical data & spectral data of metal complexes of 1a &1b are given in Table 1.2.1 and Table 1.2.2 respectively. The datas given below suggest a ML_2 stochiometry for all complexes prepared. Magnetic moment measurements show that Ni(II)complexes are diamagnetic. Cu(II),VO(IV) chelates show paramagnetic moment.

Table 1.2.1 Analytical and spectral data of metal complexes of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione

Metalchelates	M.P. (°C)	Elemental analysis (%) Found/(calculated)			μ_{eff} BM	UV λmax (nm)	Characteristic IR stretching bands (cm^{-1})		
		C	H	Metal			(C=O)	(C-C-C)	(M-O)
Cu(II)	161	75.28 (76.43)	5.67 (5.81)	9.48 (10.01)	1.76	279 389	1598	1522	464, 415
Ni(II)	165	75.82 (76.33)	5.71 (6.39)	8.82 (9.04)	----	280, 390	1595	1516	465, 426
Zn(II)	160	75.06 (76.21)	5.65 (6.34)	9.74 (10.20)	----	282 392	1592	1520	465, 433
VO(IV	167	73.15 (74.88)	5.01 (5.65)	7.95 (7.49)	1.78	281, 393	1585	1521	456, 428

Table 1.2.2 Analytical and spectral data of metal complexes of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione

Metal chelates	M.P. (^{o}C)	Elemental analysis (%) Found/(calculated)			UV λmax nm	Characteristic IR stretching bands (cm^{-1})		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu(II)	175	75.9 (76.085)	6.23 (6.340)	8.65 (8.75)	284, 394	1608	1523	460, 430
Ni(II)	179	76.4 (76.59)	6.02 (6.38)	8.04 (8.14)	286, 395	1602	1513	465, 435
Zn(II)	182	74.93 (75.88)	6.21 (6.323)	8.89 (8.99)	289, 397	1610	1526	470, 420
VO(IV)	183	75.5 (75.72)	6.21 (6.310)	6.81 (6.98)	290, 398	1611	1532	479, 438

UV spectra

The UV absorption bands of the ligands were almost unaffected by complexation with metal ions. The spectra of complexes closely resembles the spectra of respective ligands. So there is no much change in the structure due to complex formation. There is a bathochromic shift of absorption maxima to longer wavelength which indicate the involvement of the carbonyl moiety in chelate formation. For comparison, the UV spectra of ligand 1b and its Cu(II) complex is shown in Fig 1.2.1

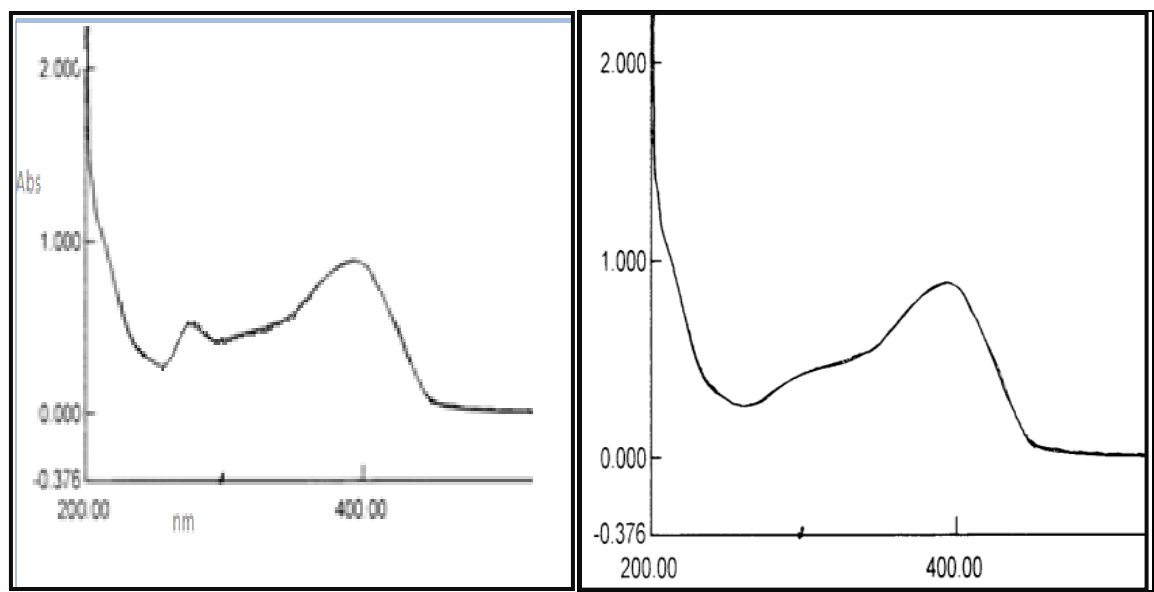


Fig 1.2.1 UV spectra of 1b and its Cu(II) complex

IR spectra

The absence of a strong band in the region 1650-1800 cm^{-1} is one characteristic feature of the metal complex. But the peak due to intramolecularly hydrogen bonded carbonyl group which is present at ~ 1630 cm^{-1} disappeared and a new band appeared at ~ 1595 cm^{-1}. The new band can be assigned to the metal coordinated carbonyl group .The replacement of enolic proton by a metal ion is also evident from the absence of the broad band in the region of 2600 -3500 cm^{-1} present in the ligand. The IR spectra of Cu(II) complex of **1b** is depicted in Fig.1.2.2

The spectra of the complexes also showed two medium intensity bands in the region of 400 – 490 cm^{-1} due to νM-O vibrations. This also gives evidence for complex formation. There is no change in the nature of alkenyl carbon as evidenced by the peak in the region at ~ 1520 cm^{-1}.Trans CH=CH vibrations are observed in the region 968 cm^{-1}.

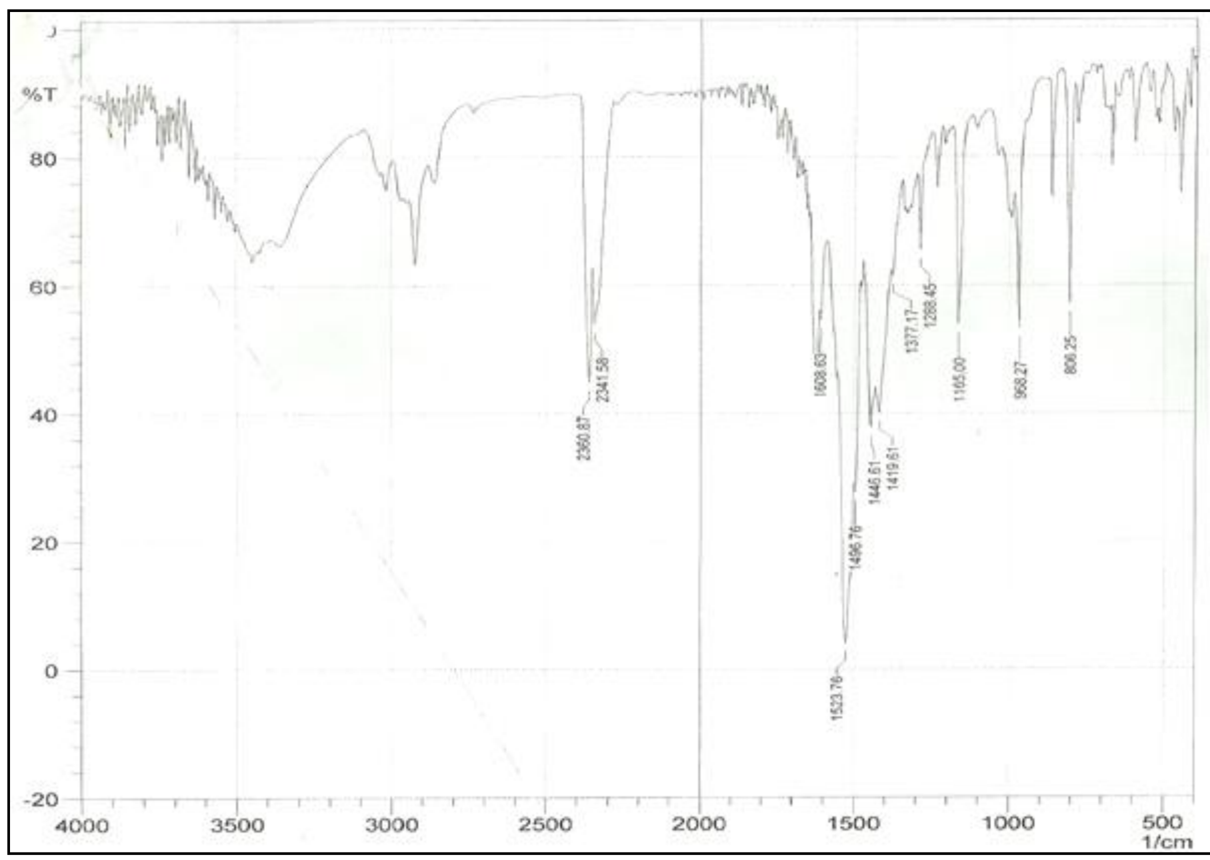


Fig 1.2.2 IR spectrum of Cu(II) complex of 1b

^{1}H NMR spectra

The main feature of NMR spectra of metal complex is the absence of singlet signal at $\delta \sim$ 16ppm which was due to the enolic proton present in the ligands. This indicates the replacement of enolic proton by metal atom in metal complexes. The phenyl and alkenyl protons are not altered much since they are not involved in metal complexation. There is a slight shift of methine signals to the downfield of the spectra. Thus the spectra of ligand and complexes are much similar except those of enolic proton. The **^{1}HNMR spectra** of Ni(II) complex of ligand 1a is given in Fig 1.2.3.

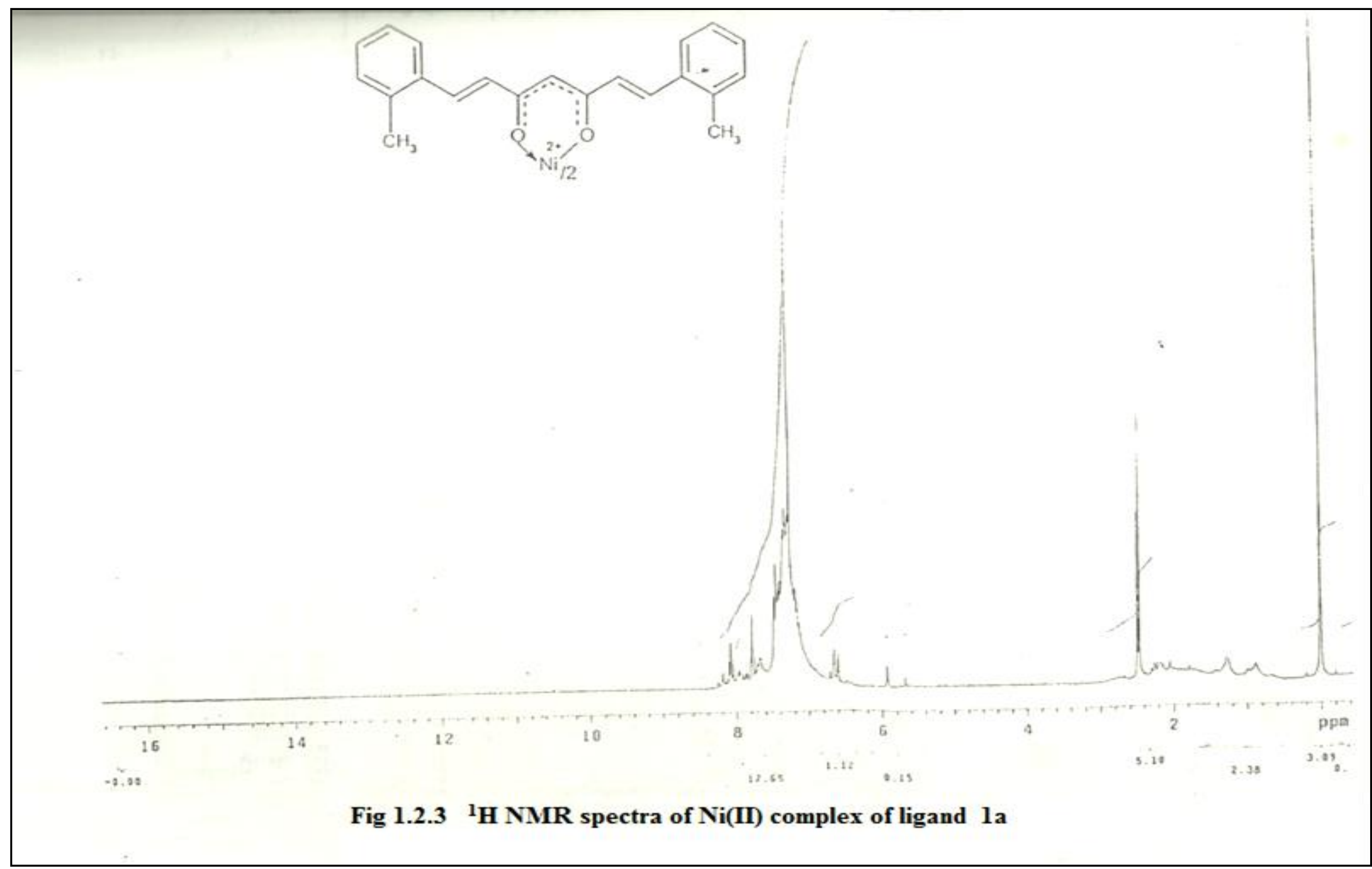


Fig 1.2.3 ^{1}H NMR spectra of Ni(II) complex of ligand 1a

Mass spectra

In their mass spectra, all the complexes showed relatively intense peaks at m/z corresponding to ML_2 stochiometry, 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,the molecular ion peak is observed in the spectra. Smaller groups like O, OH, CH etc. are also eliminated.

It was found that some fragments rearrange to form stable cyclic species as shown in the Scheme. The mass spectral analysis shows that stepwise removal of aryl groups is a characteristic feature of all the complexes. Peaks due to [ML]+ ,L+ and fragments of L+ are also detected in the spectrum .The fragmental patterns of the metal chelates **1a** & **1b** follow the **Scheme 1.2.2.** Mass spectrum of **Cu(II) complex of 1a** is given in **Fig.1.2.4** and **Ni(II) complex of 1b** is given in **Fig.1.2.5**. The mass spectral details of metal chelates of the ligands are tabulated in Table.1.2.3 & Table 1.2.4.

Table 1.2.3 Mass spectral fragmental pattern of metal chelates of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione

Fragments	Metal chelates	M+/ (M+1)/ (M+2) ion	A	B	C	D	E
Mass Pattern	Cu(II)	671	489	369	121	307	186
	Zn(II)	669	487	367	121	305	182
	Ni(II)	664	482	362	121	300	179
	VO(IV)	672	490	370	121	308	188

*The alphabets corresponds to the fragments given in **Scheme 1.2.2**

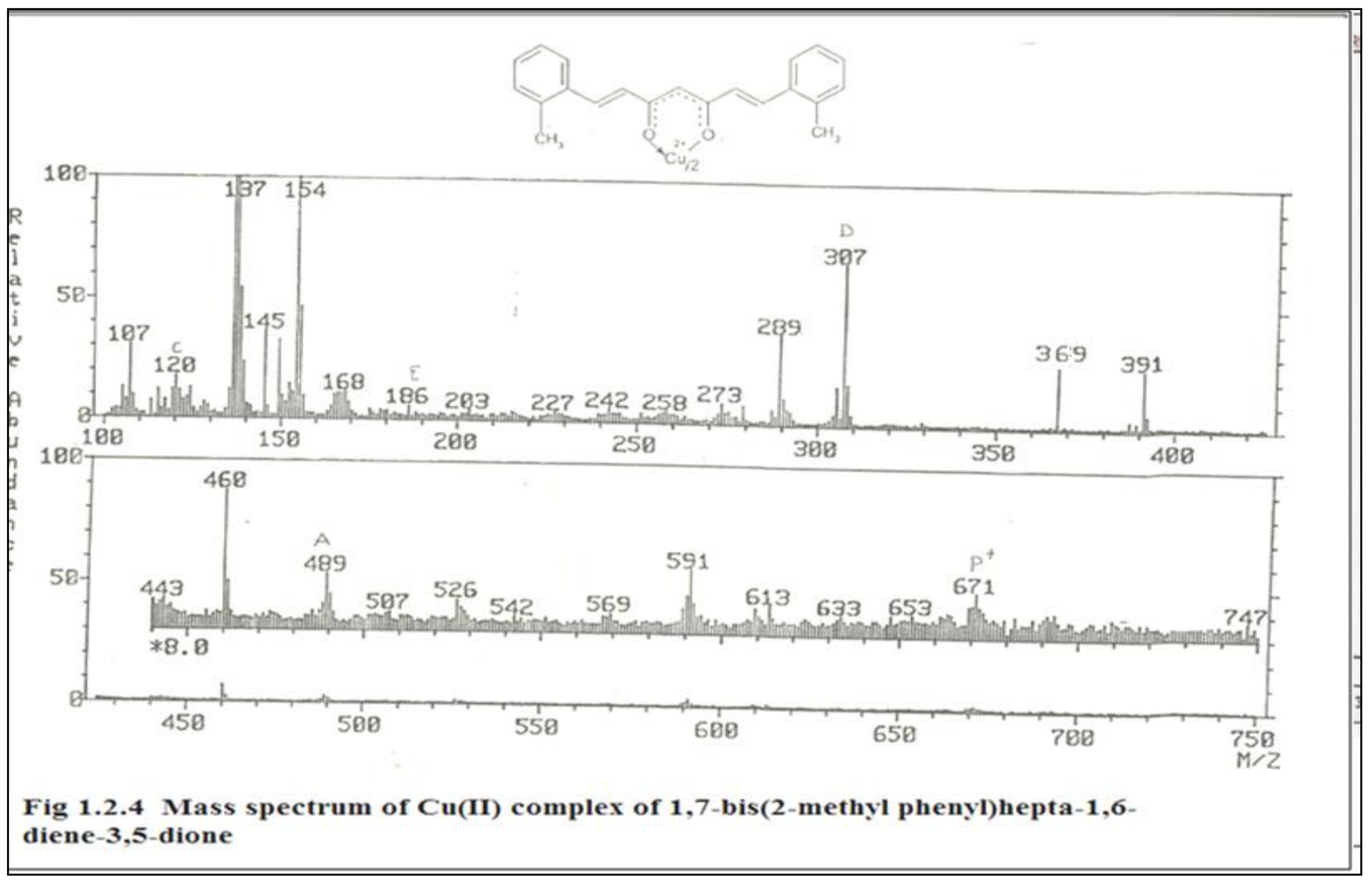


Fig 1.2.4 Mass spectrum of Cu(II) complex of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione

The peaks in the mass spectrum of Cu(II) complex of compound 1a may be explained with the help of Scheme given below.The peak at 671 is due to M+2 ion peak.The peaks at 489 and 307 are due to the removal of two and four aryl groups respectively from the molecular ion peak.The peak at 591 is due to the removal of Cu and oxygen from molecular ion.The removal of C_2H_2 group from the fragment ion (A) gives a peak at 460.The peak at 154 is the base peak.The peaks at 154,145,137 are the fragment ion peaks of the ligand which can be identified in the mass spectrum of the ligand.The peak at 120 is due to the cyclic species given in the scheme.

Table 1.2.4 Mass spectral fragmental pattern of metal chelates of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione

Fragments	Metal chelate	M+/ (M+1)/ (M+2) ion	A	B	C	D	E
Mass Pattern	Cu(II)	726	516	395	121	306	185
	Zn(II)	728	518	397	121	308	187
	Ni(II)	721	511	390	121	301	180
	VO(IV)	729	519	398	121	309	188

*The alphabets corresponds to the fragments given in **Scheme 1. 2.2**

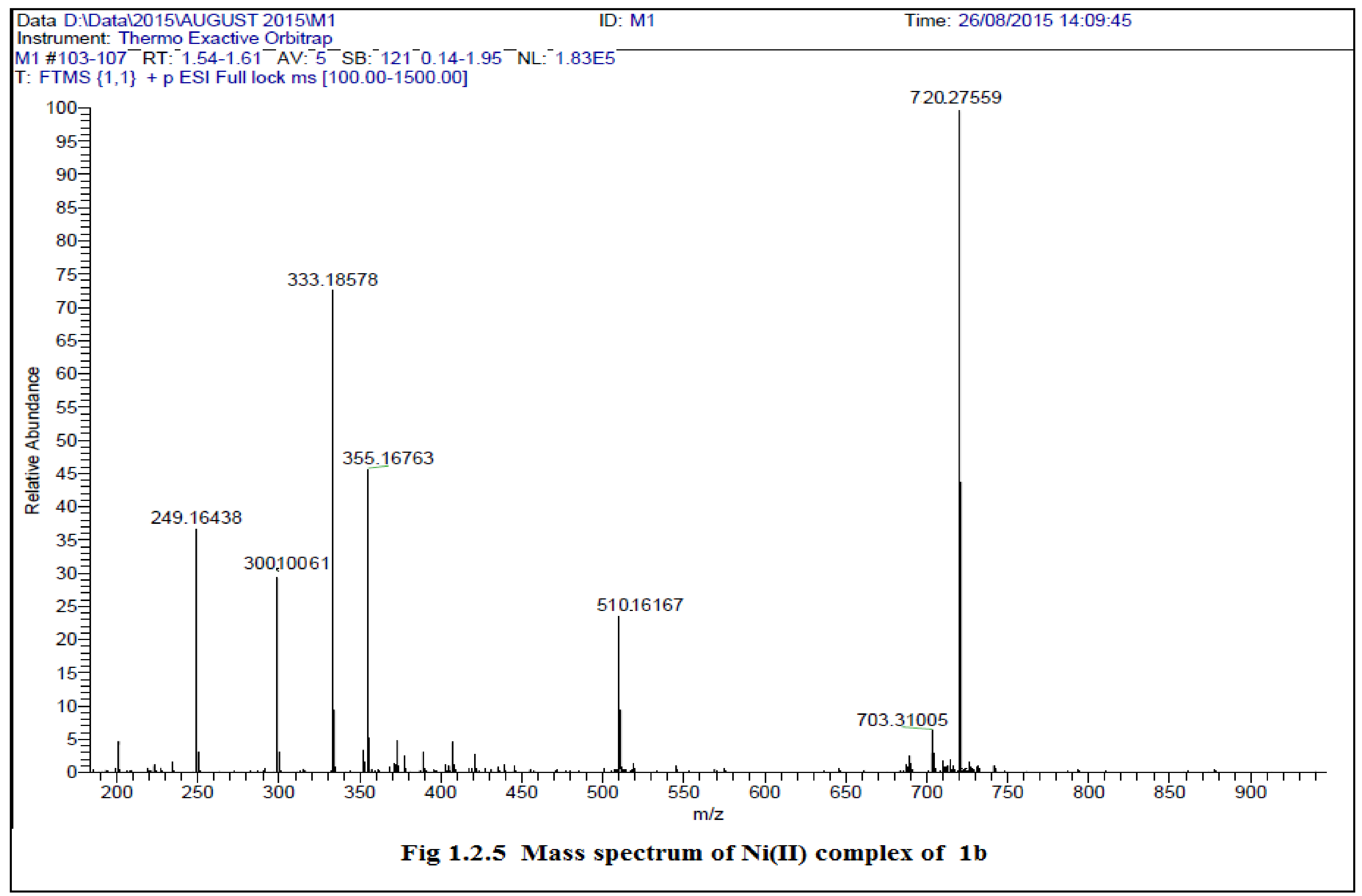


Fig 1.2.5 Mass spectrum of Ni(II) complex of 1b

In the mass spectrum of Ni(II) complex of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione ,a very intense molecular ion peak is observed at 720.The next intense peak is seen at 333 which is due to the ligand.The peak at 510 is due to the fragment ion formed by the removal of 2 Aryl groups (Ar=2,5-dimethyl) from the molecular ion.The peak at 301 is formed when 4 aryl groups are removed from the molecular ion.

Scheme 1.2.2

The observed UV, IR, ^{1}HNMR and Mass spectral data clearly reveals that metal chelates of Cu,Zn,Ni &Vanadyl are having ML_2 stoichiometry(metal ligand ratio is 1:2).The confirmed structure of metal chelates is given below.

n = 2 for Cu(II),Zn(II),Ni(II),VO(IV)
Ar = methyl substituted phenyl

THERMOGRAVIMETRIC ANALYSIS

Thermogram of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione and its Cu(II) complex are represented below in Fig.1.2.6 and Fig.1.2.7 respectively.Thermogravimetric analysis were done in the temperature range 39^0 to 735^0 and at liquid nitrogen temperature.The thermogram of the ligand shows a two stage decomposition pattern .The compound is stable upto 150 ^{0} and then the decomposition begins slowly with a sharp drop in the mass (31.5%) up to about 337.76 ^{0}.This corresponds to the removal of an aryl group from the compound.The second stage of decomposition continues to a temperature of 550 ^{0}with a mass loss of 68% which is due to the removal of second aryl group.The peak temperature in DTG is at 393.10 ^{0}.The final product was found to be an aryl group.

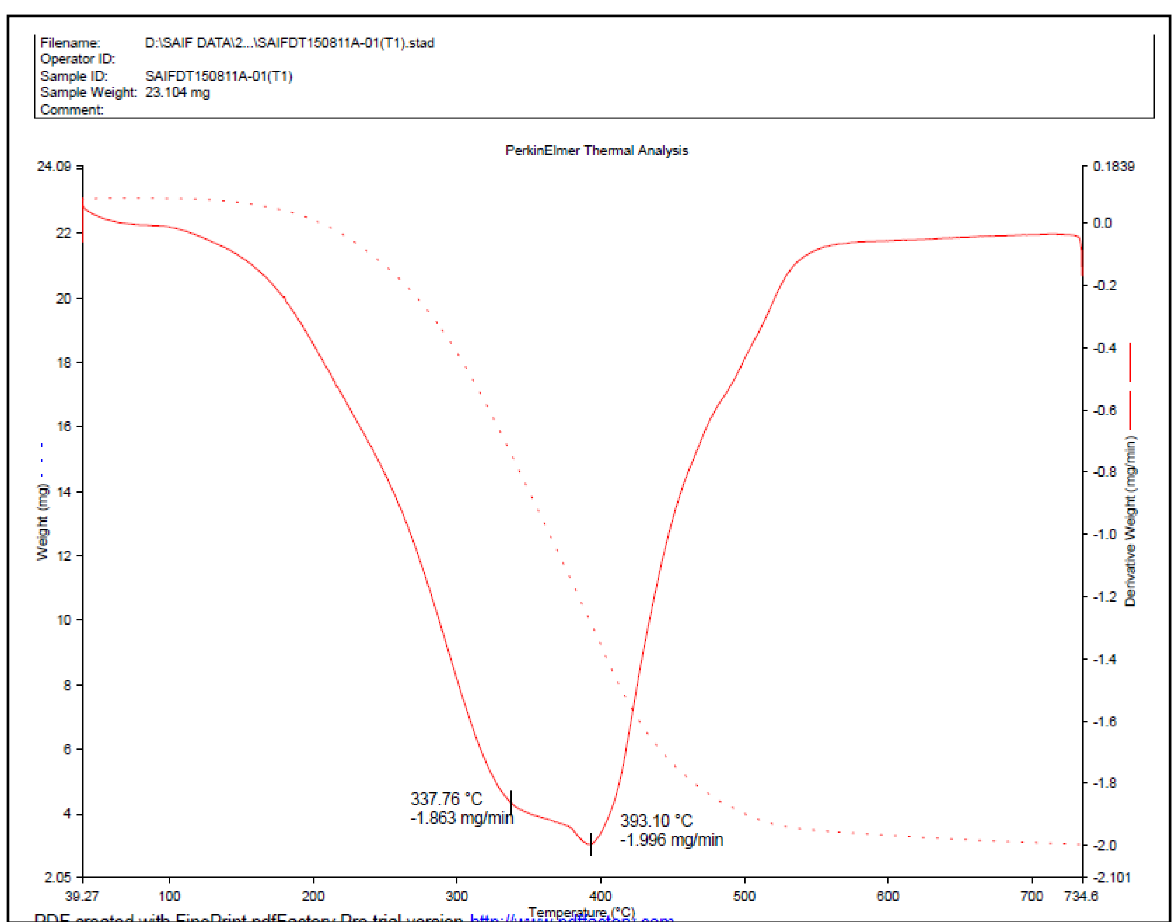


Fig.1.2.6 Thermogram of 1b

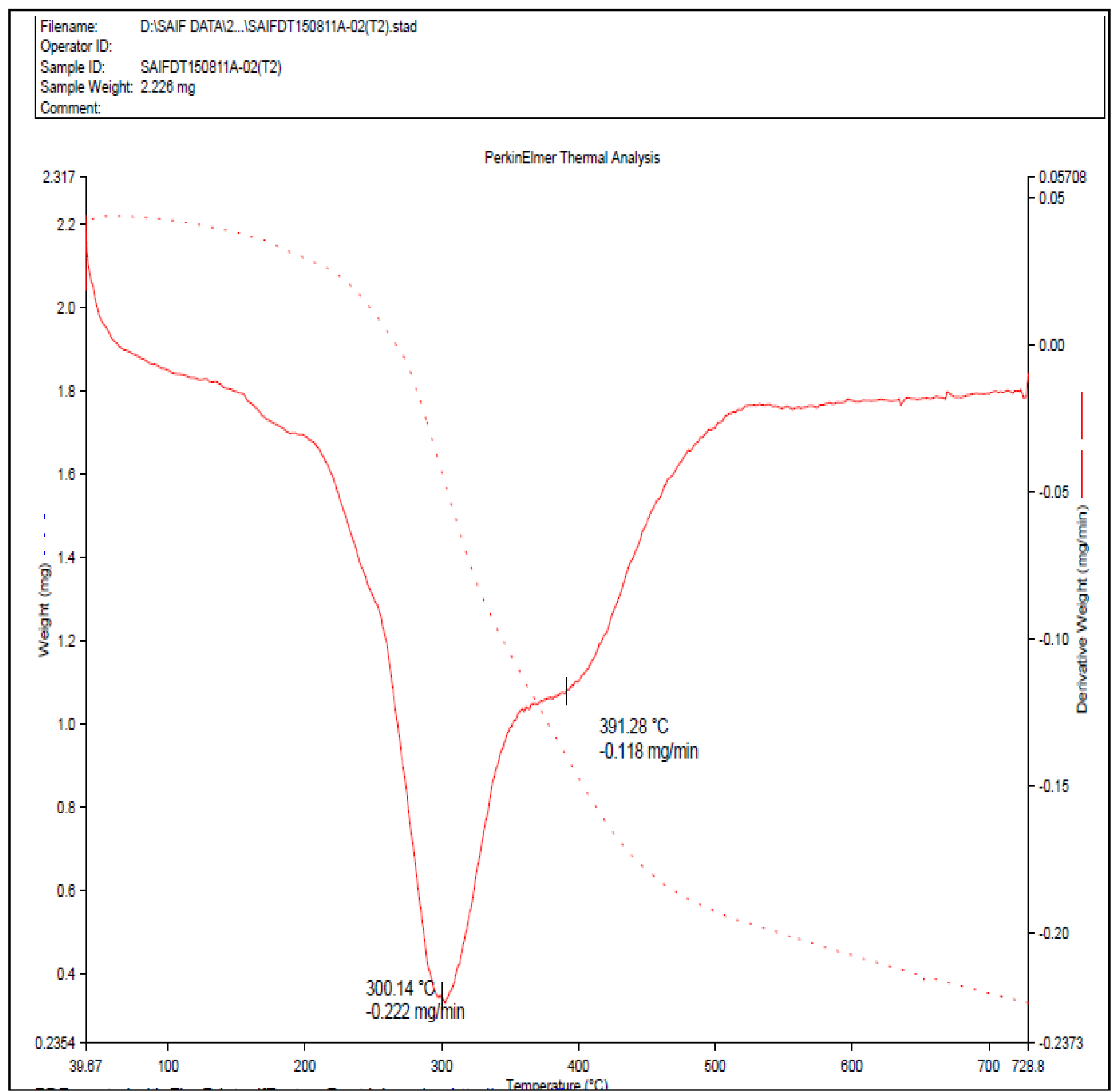


Fig.1.2.7 Thermogram of Cu(II) complex of 1b

The thermogram showed a two stage decomposition pattern.The first stage of decomposition is between 220^0 and 350^0 with a peak temperature at 300^0 C.The loss of one ligand occurred in this step.The % mass loss (45.6%) during decomposition of one of the ligand from the complex agrees well with the theoretical value.The second stage of decomposition begins at $350\ ^0$ and continues upto $450\ ^0$ with a peak temperature of $391\ ^0$.This is due to the decomposition of the second ligand according to the mass loss percentage(91.1%).The end product appears to be CuO since the mass loss from TG curve agrees well with theoretical value.

ESR Spectrum of Cu(II) complex of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione

The basic object of ESR studies in the transition metal complexes is to obtain as much information as possible about the metal-to-ligand bond, the unpaired electron distribution and the order of the energy levels.It has been demonstrated that the effective spin –orbit coupling constant and nuclear hyperfine structure of an ion will vary with the covalent character of metal-ligand bond.Complexes of Cu(II),a d^9 metal ion is very well studied using ESR Spectra.Cu(II) with a nuclear spin of 3/2 gives rise to a clearly resolvable nuclear hyperfine structure.

The esr spectrum of Cu(II) complex of 1b in DMF solution at 77K is given in Fig.1.2.8.In general complexed Cu^{2+} ion in solution exhibits four hyperfine lines in its esr spectrum.The $g_{//}$ and $g_{\perp}$ values were found from the spectrum to be 2.2734 and 2.06180 respectively.The observed g values indicates greater π bonding in the six membered C_3O_2Cu ring system and the metal-ligand bond has considerable covalent character.The solvent used for measuring the spectra is a strongly coordinating solvent and therefore the geometry of the complex may change.So the observed values cannot be interpreted as due to a square planar copper complex.So it is likely that the geometry of the complex will be tetragonally elongated octahedron.

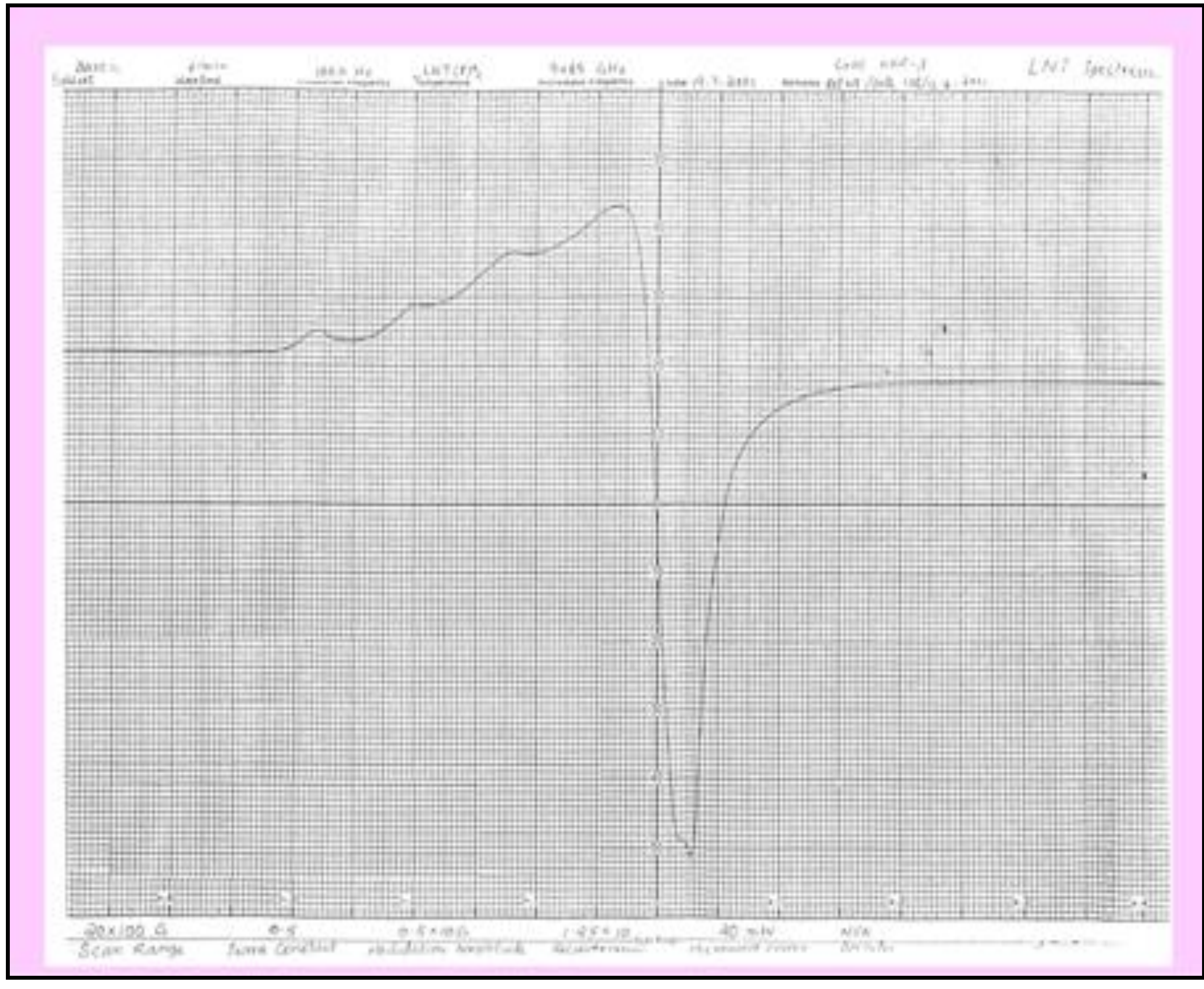


Fig.1.2.8 ESR Spectrum of Cu(II) complex of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione

SECTION III

ANTITUMOUR STUDIES OF METHYL SUBSTITUTED 1,7-DIARYL HEPTANOIDS AND THEIR TRANSITION METAL COMPLEXES

This section describes the antitumour activities of methyl substituted 1,7-diaryl heptanoids namely 1,7-bis(2-methylphenyl)hepta-1,6-diene-3,5-dione(**1a**)& 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione(**1b**)and their metal complexes [Cu(II),Zn(II) ,Ni(II)&VO(IV)]. The cytotoxicity of both ligands and their transition metal chelates were studied by different methods.The methods adopted are given below.

1)In vitro cytotoxicity study against EAC cells by Trypan blue exclusion method

2)In vitro cytotoxicity study against DLA cells by Trypan blue exclusion method

3)Determination of the effect of compounds in reducing ascites tumour development in mice(In vivo cytotoxicity study)

4)Determination of the effect of compounds on solid tumour development

1.3.1. *In vitro* Cytotoxic studies of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(1a) and their metal complexes[Cu(II),Zn(II),Ni(II) & VO(IV)]

Cytotoxic studies were done using 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(**1a**) and its metal complexes towards tumour bearing cells DLA & EAC using trypan blue exclusion method. The synthetic curcuminoid analogue and its metal chelates were dissolved in minimum quantity of DMSO.The test compounds were taken in different concentrations(10-200 µg/ml)and the solution was diluted with PBS.The cell suspension (0.1 ml stock solution containing nearly 1 million cells)was added to tubes containing different concentration of the test compounds.These assay mixture was incubated for 3 hrs at 37^0C.Then the cell suspension was mixed with 0.1ml of 1% trypan blue dye and kept for 5 mts 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 number of stained and unstained cells are counted and % cell death is found.The results of cytotoxic activity are given in **Table 1.3.1.**

Table 1.3.1. *In vitro* Cytotoxic studies of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(L_1) and their metal complexes towards EAC

Drug Con. µg/ml	% Cell death				
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$	$VO(L_1)_2$
200	26	63	44	48	53
100	19	46	30	31	39
50	14	27	18	20	26
20	8	13	9	10	11
10	2	8	4	5	6

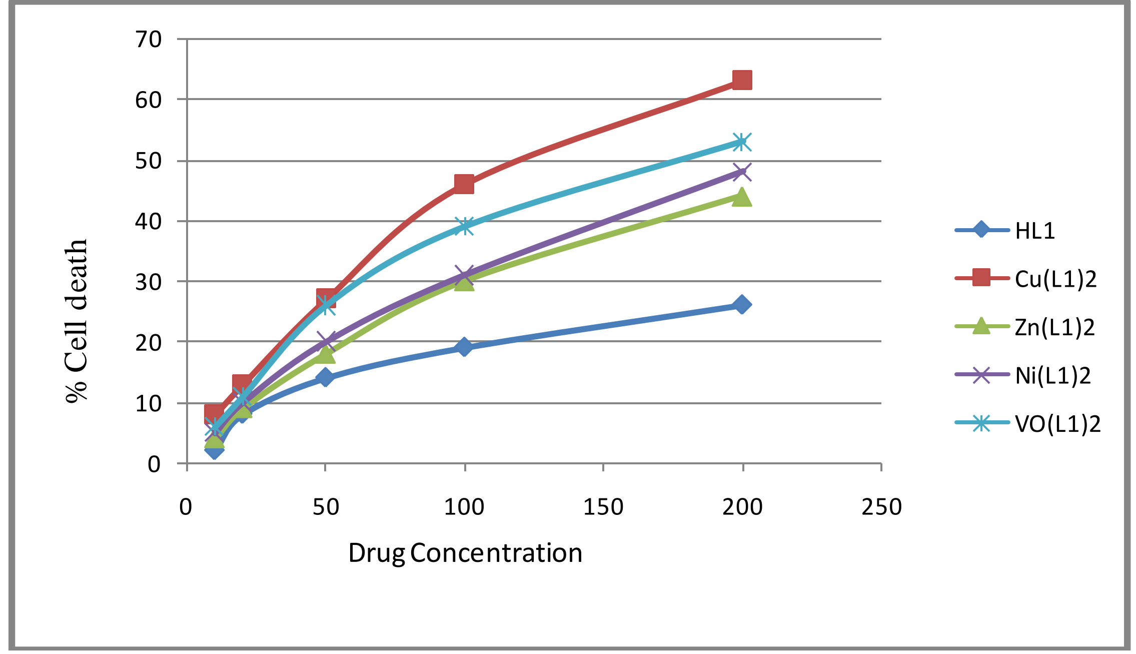


Fig.1.3.1. In vitro Cytotoxic studies of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(HL1) and their metal complexes towards EAC

Comparing the ligand and its metal complexes ,it is observed that all the metal complexes are more cytotoxic than the ligand .Among the metal complexes,Cu(II) showed enhanced cytotoxicity towards EAC cells.All the test compounds possessed maximum activity at higher concentration namely 200 µg/ml .It is observed from the results that as the concentration of the compounds increases % cell death produced increases.For a given concentration 200 µg /ml the % cytotoxicity of ligand,Cu(II),Zn(II),Ni(II)& VO(IV) complexes were 26,63,44,48 and 53% respectively.The Zn and Ni complexes showed comparable activity with respect to EAC cells.For metal complexes the cytotoxic nature follow the order Cu(II)>VO(IV)>Ni(II)>Zn(II).

In vitro Cytotoxic studies of **1a** and their metal complexes towards DLA are given in **Table.1.3.2**. & **Fig 1.3.2.**

Table 1.3.2. ***In vitro*** **Cytotoxic studies of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(L_1) and their metal complexes towards DLA**

Drug Con. µg/ml	**% Cell death**				
	L_1	**$Cu(L_1)_2$**	**$Zn(L_1)_2$**	**$Ni(L_1)_2$**	**$VO(L_1)_2$**
200	31	70	47	50	58
100	23	50	32	35	45
50	18	29	20	25	27
20	10	15	12	13	14
10	2	9	6	7	8

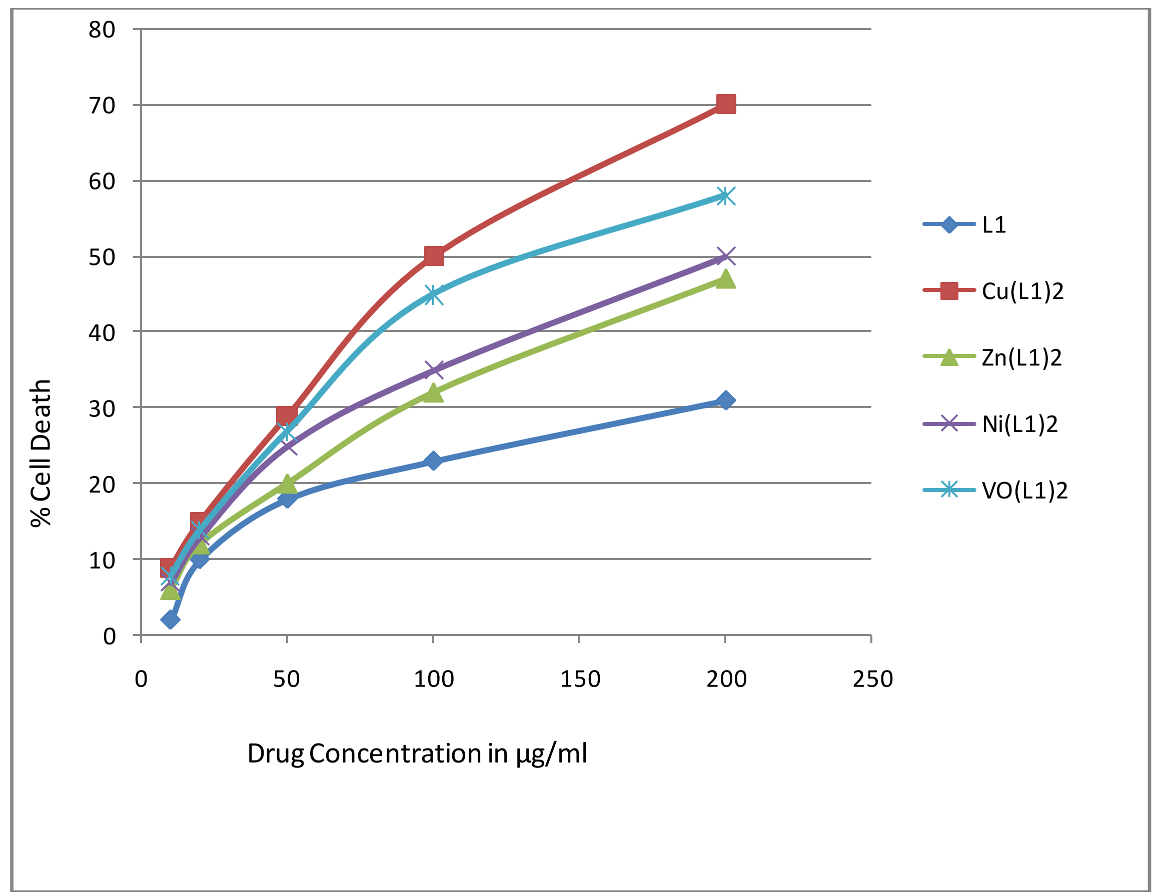


Fig 1.3.2 In vitro Cytotoxic studies of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(L_1) and their metal complexes towards DLA

Results in Table 1.3.2 shows that the ligand (1a) is little more cytotoxic towards DLA cells compared to EAC cells.The ligand showed 31% cytotoxicity towards DLA cells at a concentration of 200µg/ml where as the % cytotoxicity towards EAC cells were 26% at that particular concentration.The metal complexes of the ligand also showed a comparable increase in % cell death similar to the ligand.Here also metal complexation has increased % cell death.Cu(II) complex showed maximum activity with 70% cell death and its activity was nearly more than twice that of ligand.VO(IV)complex stood second in activity and Zn(II) complex showed minimum activity.The % cell death produced by ligand and complexes was less at lower concentration.

1.3.2. In vitro Cytotoxic studies of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(1b) and their metal complexes[Cu(II),Zn(II),Ni(II) & VO(IV)]

In vitro Cytotoxic studies of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione (**1b**) and its metal complexes were conducted with EAC and DLA cells using Trypan blue exclusion method as described in 1.3.1.The results are given in **Table1.3.3** and **Fig. 1.3.3.**

Table 1.3.3. ***In vitro*** **Cytotoxic studies of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L_2) and their metal complexes towards EAC**

Drug Con. µg/ml	% Cell death				
	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$	$VO(L_2)_2$
200	20	80	34	38	43
100	10	66	20	21	40
50	5	47	8	13	20
20	4	35	5	6	7
10	2	15	4	5	5

The ligand 1b with two methyl groups showed less cytotoxic activity when compared with the ligand 1a with one methyl group on the phenyl ring.The Cu(II) complex of ligand 1b gave 80% cell death at higher concentrations. The activity of the Cu(II) complex is about four times that of the ligand.The complex was quite effective in producing cell death.The Zn(II),Ni(II) and VO(IV) complexes showed comparable activities and their cytotoxic nature was greater than ligands.

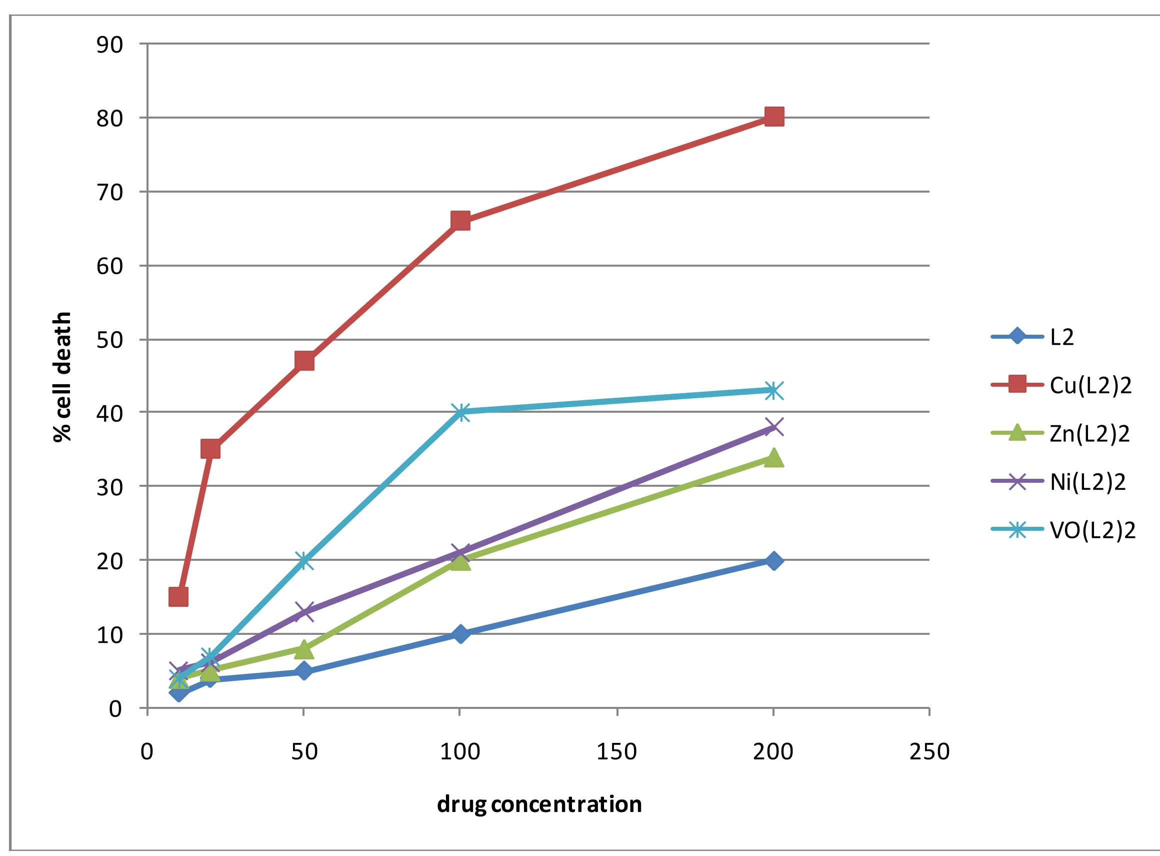


Fig1.3.3. ***In vitro* Cytotoxic studies of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione (L_2) and their metal complexes towards EAC**

Table1.3.4. In vitro Cytotoxic studies of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L_2) and their metal complexes towards DLA

Drug Con. µg/ml	% Cell death				
	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$	$VO(L_2)_2$
200	18	78	31	36	40
100	10	64	16	18	20
50	5	45	12	14	16
20	4	32	6	8	9
10	2	13	4	5	7

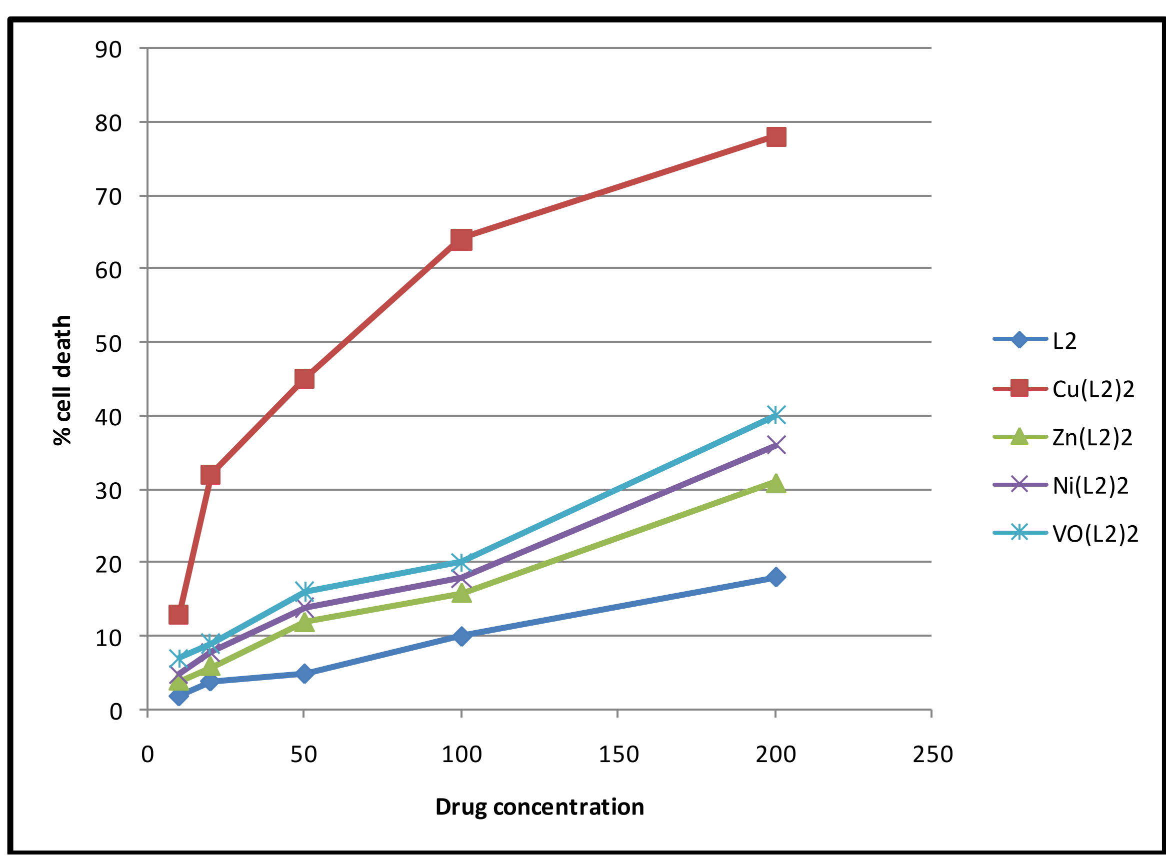


Fig1.3.4. ***In vitro*** **Cytotoxic studies of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L_2)and their metal complexes towards DLA**

The ligand 1b was not very effective against DLAcells.The % cell death was only 18% for the ligand where as for Cu(II) complex it was 78%.It is found that the metal copper has an inevitable role in cytotoxicity.All the metal complexes were more cytotoxic than the ligands.The VO(IV) complexes showed cytotoxicity of 40% which is twice that of ligand.The Zn(II) complex showed minimum activity.

IN VIVO ANTITUMOUR STUDIES OF 1,7-BIS(2,5-DIMETHYL PHENYL)-1,6-HEPTADIENE-3,5-DIONE AND THEIR Cu(II) AND VO(IV) METAL COMPLEXES

In vivo antitumour studies were conducted in mice with the ligand 1,7-bis(2,5-dimethyl phenyl)-1,6-heptadiene-3,5-dione(**1b**) and its Cu(II) & VO(IV) complexes.These two metal complexes were found to be active against DLA and EAC cells in invitro studies conducted.So they were selected for this particular study and the results are discussed in **Table 1.3.5.** The three compounds were given to tumour bearing mice groups (11 groups, each with 5 animals)as drug.All the test compounds were injected intraperitoneally and their effect in reducing ascites tumour development in mice were studied.The no. of days survived by the control group,the animals given standard drug,and the animals treated with test compounds and their % increase in life span is given in the table below.

Table 1.3.5 Effect of 1,7-bis(2,5-dimethyl phenyl)-1,6-heptadiene-3,5-dione(L2) and their metal 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±1.10	
2.Standard drug		5/5	30.6±0.489	76.87
3.L_2	20	5/5	20.2±2.83	16.76
4. L_2	10	5/5	19.0±1.85	9.8
5. L_2	5	5/5	18.5±1.16	6.9
6.$Cu(L_2)_2$	20	5/5	28.4±1.04	64.16
7. $Cu(L_2)_2$	10	5/5	25.4 ± 1.16	46.28
8. $Cu(L_2)_2$	5	5/5	21.2 ± 1.78	22.54
9.$VO(L_2)_2$	20	5/5	22.9±0.52	32.36
10. $VO(L_2)_2$	10	5/5	21.4±1.16	23.69
11. $VO(L_2)_2$	5	5/5	19.8±1.50	14.40

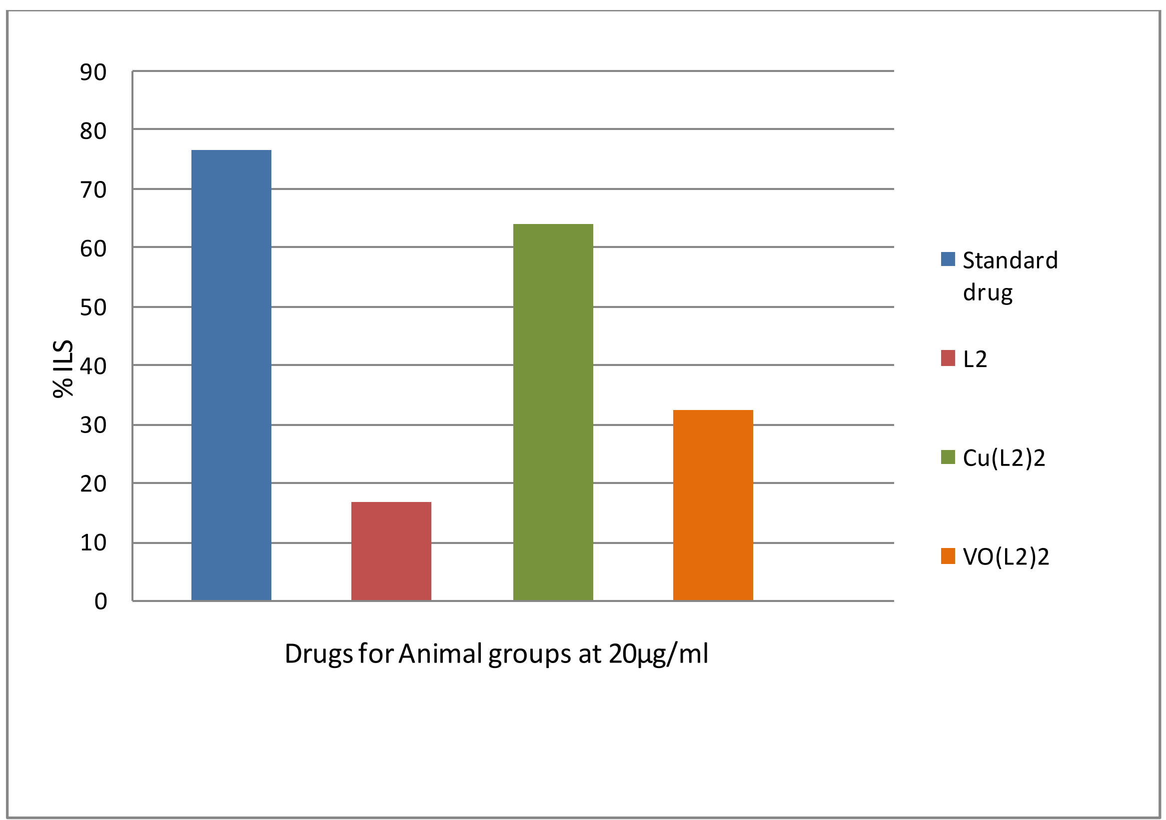


Figure 1.3.5 Effect of 1,7-bis(2,5-dimethyl phenyl)-1,6-heptadiene-3,5-dione(L2) and their metal complexes on ascites tumour reduction

The test compounds namely1,7-bis(2,5-dimethyl phenyl)-1,6-heptadiene-3,5-dione(L_2) and their metal complexes Cu(II) & VO(IV) were given as drug in different concentrations namely 5,10,20µg/ml.The no. of days survived by control group is 17.3±1.1 where as for standard drug cyclophosphamide it is 30.6±0.489.There was an increase in life span (% ILS)of 76.87% for the animals injected with std.drug.The ligand produced only 16.76% ILS at a concentration of 20µg/ml.But the Cu(II) complex could produce an increase in life span of 64.16% at the same concentration which is nearly four times that of ligand.This is comparable with the increase in life span produced by the std.drug.The animals treated with VO(IV) complex survived for 22.9 days and produced a % ILS of 32.36 at higher concentration.Comparing the ligand,Cu(II) and Vanadyl complexes it was observed that Cu(II) complex was most effective in increasing the life span of tumour burden mice.

INVIVO CYTOTOXIC STUDY ON SOLID TUMOUR DEVELOPMENT

1.3.3 Effect of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione (1a) and 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione (1b) and their Cu(II) complexes on solid tumour development

The ligands 1a and 1b and their copper complexes were used to find the effect on solid tumour development in mice.Solid tumours were induced in groups of mice by subcutaneous injection of DLA cells on the right hind limb.One group was kept as control,one group was treated with standard drug and other groups were simultaneously injected with the test compounds(200µmol/Kg body weight) for 10 days.Tumour diameter was measured every third day for 1 month and tumor volume was calculated.The results of the study are given below.

Table 1.3.6 Effect of Compounds on solid tumour

Compounds	Tumour volume on 31st day
Control group	5.042 cm^3
1a (L_1)	4.08 cm^3
1b (L_2)	4.68 cm^3
Cu $(L_1)_2$	3.65 cm^3
Cu $(L_2)_2$	3.05 cm^3
Std.drug	1.982 cm^3

All the compounds produced a significant reduction of solid tumour volume in mice.Compared to ligands ,their respective Cu(II) chelates were more effective in bringing about reduction in solid tumour volume.The measured tumour volume was 5.042 cm^3 for the control group on the 31 rst day and it were 4.08 cm^3 and 4.68 cm^3 for 1a and 1b respectively.Comparing with that of the control group,the ligands produced a decrease in

volume of 0.962 cm^3 and 0.362 cm^3 respectively. Among the ligands ,1a was more effective than 1b in reducing the tumour volume.The tumour volumes on day 31 for copper complexes of 1a and 1b were 3.65 cm^3 and 3.05 cm^3 respectively.The decrease in tumour volume was 1.392 cm^3 and 1.992 cm^3 respectively with respect to control group.The Cu(II) complex of 1b had shown a pronounced effect in reducing tumour volume.

SECTION-IV

ANTIBACTERIAL STUDIES OF METHYL SUBSTITUTED 1,7-DIARYL HEPTANOIDS AND THEIR Cu(II),Zn(II)&Ni(II)METAL COMPLEXES

1.4.1. Antibacterial studies of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione (1a) and 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione (1b) and their Cu(II),Zn(II) and Ni(II) complexes

The compounds and their complexes were evaluated for their antibacterial activities.The invitro antibacterial studies were performed on three types of bacterial strains namely Escherichia Coli, Klebsiella Pneumoniae & Bacillus Subtilis using Agar well diffusion method.The test compounds showed varying degree of inhibition against different bacterial strains.All synthesized compounds have shown to be susceptible to excellent potency against the different bacterial strains. The results of the antibacterial activity of methyl substituted 1,7-diaryl heptanoids and their complexes revealed that the ligands and their complexes possess comparable antibacterial activity to that of standard drug streptomycin (**Table 1.4.1.** & **Table1.4.2.**).The activity is expressed in terms of diameter of zone of inhibition in mm. In all the cases metal complexes possess better antibacterial activity than that of ligands, which means that metal complexation enhance the activity.

The ligand 1b was very active against E coli bacteria and produced a zone of inhibition with diameter 16mm.It exhibited only some degree of activity against Bacillus Subtilis and moderate activity against Klebsiella Pneumoniae.The ligand 1a had shown comparable activities against all the three bacterial strains.So comparing the ligands ,1b exhibited greater antibacterial activity.The activity of metal complexes followed the order Cu(II)>Zn(II)>Ni(II).The copper and zinc complexes of ligand 1b had shown enhanced activity and produced a zone of inhibition of 19mm and 18 mm respectively which is

comparable with the diameter of zone of inhibition produced by the standard drug streptomycin ie 20mm.

Table 1.4.1. Antibacterial studies of 1a (L_1) and their Cu(II),Zn(II) and 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	11	15.5	13.5	12
Klebsiella	10	14.5	12.5	10
Bacillus	8.5	13	11	7.5
Standard	20	20	20	20

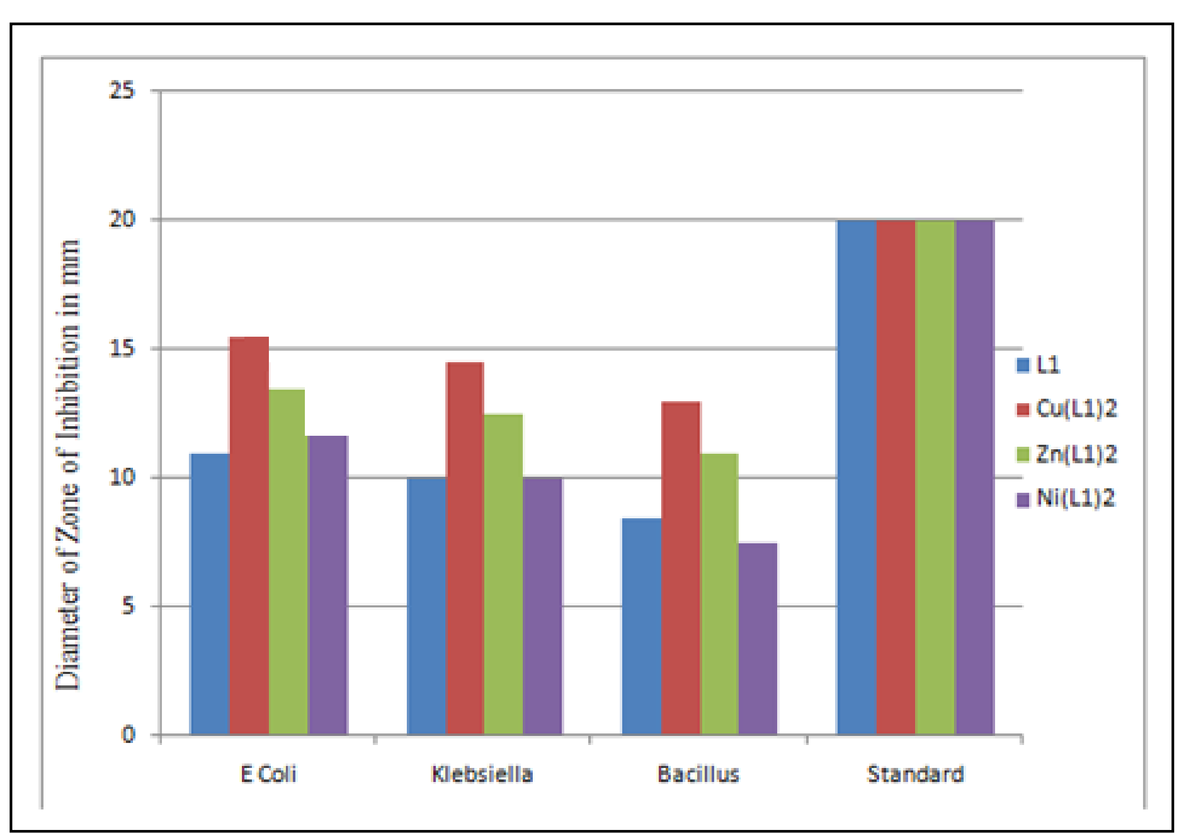


Fig.1.4.1. Antibacterial studies of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione (L_1) and their Cu(II),Zn(II) and Ni(II) complexes.

Table 1.4.2. Antibacterial studies of 1b (L_2) and their Cu(II),Zn(II) and 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	16	19	18	16.5
Klebsiella	10	18	16	12
Bacillus	7.5	14	15	11
Standard	20	20	20	20

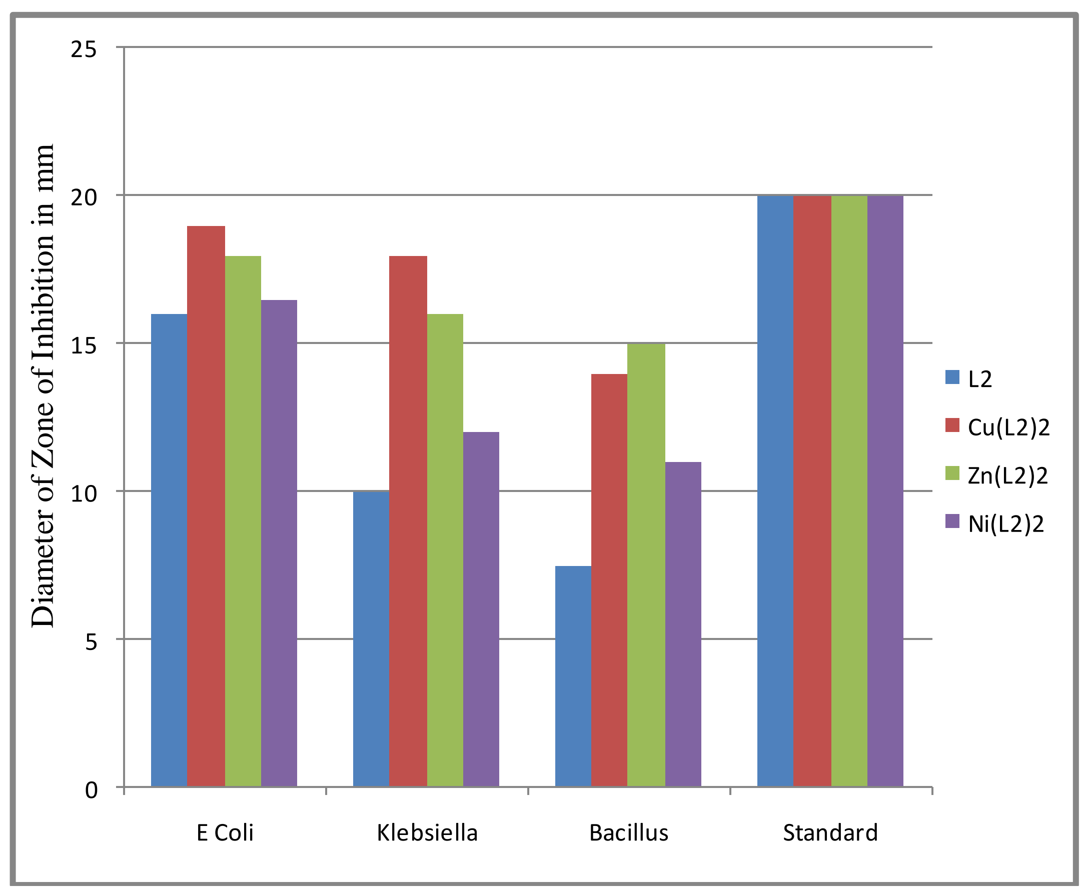


Fig.1.4.2. Antibacterial studies of 1,7-bis(2,5-dimethyl phenyl)hepta-1,6-diene-3,5-dione(L_2) and their Cu(II),Zn(II) and Ni(II) complexes

SECTION-V

ANTIFUNGAL STUDIES OF METHYL SUBSTITUTED 1,7-DIARYL HEPTANOIDS AND THEIR Zn(II)&VO(IV)METAL COMPLEXES

1.5.1. Antifungal Activity of methyl substituted 1,7-diaryl heptanoids (1a, & 1b) and their Zn(II) and VO(IV) complexes

Invitro antifungal activities of methyl substituted 1,7-diaryl heptanoids (1a & 1b) and their metal complexes namely Zn(II) &VO(IV)were investigated against three fungal cultures namely Aspergillus Niger, Penicillium Chrysogenum and Alternaria Alternate by Kirby Baurer method.The test compounds were prepared in different concentrations [100, 250, 500µg/ml] by dissolving in 2% DMSO solvent.This method was standardized using flucanazole drug. A control disc was used with DMSO only and without drug.For compounds effective against fungal cultures,the growth of fungus is inhibited as zone.The antifungal activities are measured in terms of zone of inhibition in mm.The results of the studies are given in Table **1.5.1.** and **1.5.2**.

Table 1.5.1. Antifungal studies of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione(L_1 and its Zn(II) & Vanadyl complexes

Fungi	Diameter of zone of inhibition in mm								
	L_1			$Zn(L_1)_2$			$VO(L_1)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	4.5	7	8.5	5	8	9.5	6.5	10	11
Penicillium	6.5	8	10	6.5	8	11	7	11	12
Alternaria	5	6.5	8	5.5	9	10	7.5	12	12.5

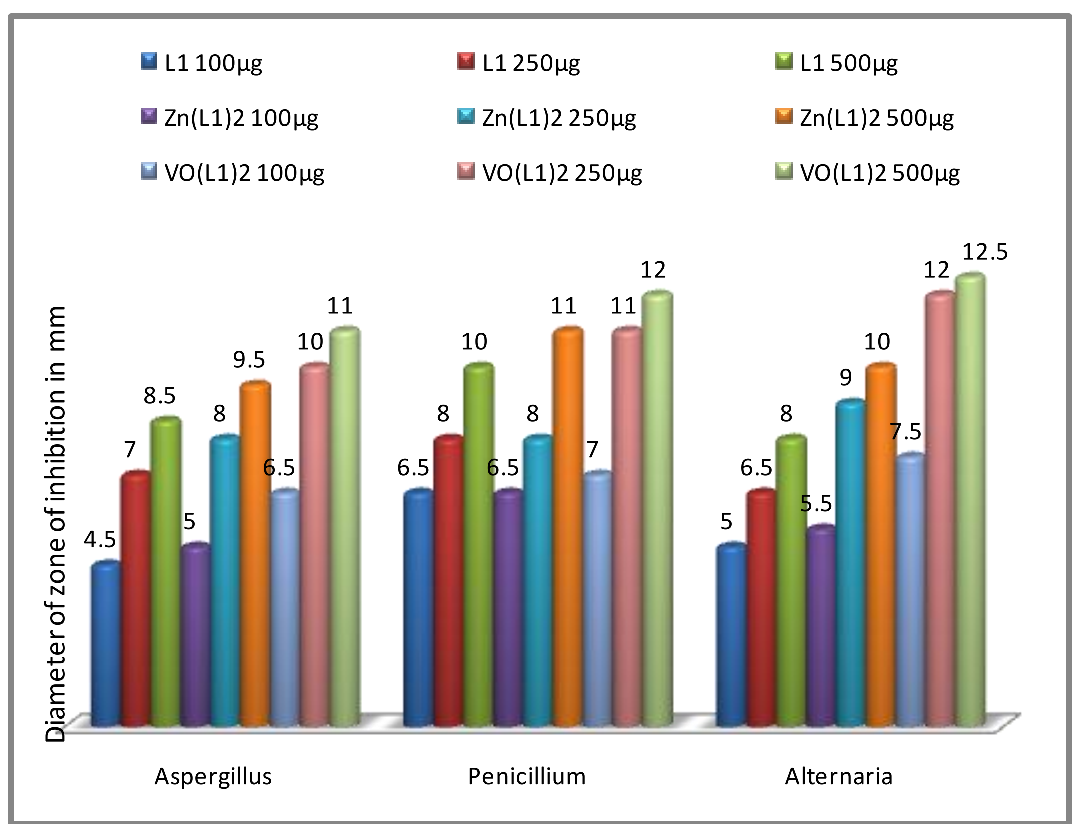


Figure 1. 5.1. Antifungal studies of 1,7-bis(2-methyl phenyl)hepta-1,6-diene-3,5-dione L_1 and its Zn(II) & Vanadyl complexes

Table 1.5.2 Antifungal studies of 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione (L_2) and its Zn(II) & Vanadyl complexes

Fungi	Diameter of zone of inhibition in mm								
	L_2			$Zn(L_2)_2$			$VO(L_2)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	8	10.5	12	10	11	14	12	14	16
Penicillium	11	13	14.5	12	15	17	14	17	19
Alternaria	10.5	12.5	15	11	13	16	13	16	17

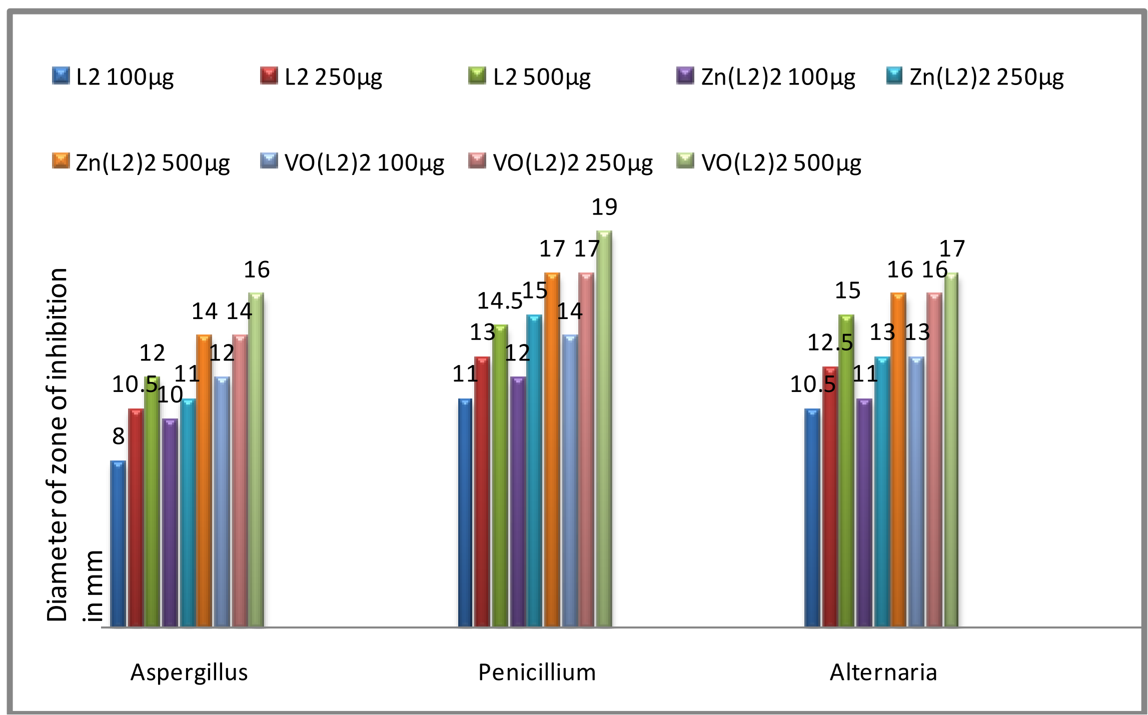


Figure 1.5.2 Antifungal studies of 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione (L_2) and its Zn(II) & Vanadyl complexes

For all the tested compounds they show maximum antifungal activity at a higher concentration of 500µg/ml.It is observed that antifungal nature increases with the concentration of the compounds.The ligand 1a exhibited a zone of inhibition of 10mm against Penicillium where as its activity against Alternaria and Aspergillus is comparable ie zone of inhibition produced is 8.5 and 8 mm respectively at higher conc.The ligand 1b exhibited more antifungal activity against Alternaria with a zone of inhibition of 15mm.Comparing the ligands,1b had shown more antifungal activity towards all fungi species than 1a. Comparing Zn(II) and VO(IV) complexes of both ligands, it was observed that vanadyl complexes exhibited more antifungal activity.The Zn(II) complexes gave inhibitory activity against fungal cultures which was only slightly greater than the ligands.But the VO(IV) complexes of both ligands especially of 1b had appreciable antifungal activity against all fungal cultures.It produced a maximum zone of inhibition of 19mm against Penicillium which is comparable with the zone of inhibition produced by the std.drug (21mm).

CHAPTER-II

SYNTHESIS, CHARACTERISATON AND BIOCHEMICAL ACTIVITIES OF 1,7-DI(THIOPHEN-2-YL)HEPTA-1,6-DIENE-3,5-DIONE AND 1,7-BIS(3-METHYL THIOPHEN-2-YL) HEPTA-1,6-DIENE-3,5-DIONE AND THEIR TRANSITION METAL CHELATES WITH Cu (II), Zn(II), Ni(II) & OXOVANADIUM(IV)

SECTION-1

SYNTHESIS AND CHARACTERISATON OF 1, 7-DI (THIOPHEN-2-YL)HEPTA-1,6-DIENE-3,5-DIONE AND 1,7-BIS (3-METHYL THIOPHEN 2-YL) HEPTA-1,6-DIENE-3,5-DIONE

2.1.1 Synthesis of 1,7–dithiophenyl heptanoids

This chapter deals with the synthesis and characterization of two curcuminoid analogues with heterocyclic ring(thiophenyl ring).The compounds synthesized are 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (**2a**) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (**2b**) .They were prepared by the condensation of heterocyclic aldehydes (thiophene-2-carboxaldehyde and 3–methyl thiophene–2-carboxaldehyde) with acetylacetone-boric oxide complex in ethyl acetate medium in presence of tributyl borate and n – butyl amine.The reaction usually leads to α,β- unsaturated 1,3-diketones with heterocyclic rings attached to it.Studies on ligands with a heteroaryl ring system instead of a phenyl ring in the unsaturated diketone moiety has not been much. The products 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (**2a**) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (**2b**) were purified by column chromatography over silica gel (60 – 120 mesh) using 4:1 (v/v) chloroform:acetone mixture as the eluent and recrystallised twice from hot benzene to get pure crystalline material. The product formation can be represented in a schematic way (**Scheme 2.1.1).**

$2a - R_1 = H$

$2b - R_1 = CH_3$

B_2O_3/Tri(sec-butyl)borate

n-$BuNH_2$

Scheme 2.1.1

The compounds 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (**2a**) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (**2b**) are black in colour,crystalline in nature and obtained in good yield nearly 75%. They show sharp melting point and are soluble in organic solvents like ethylacetate, acetone,ethanol,chloroform etc.The aldehydes used for synthesis,structures of the ligands prepared,its systematic name and yield are given in Table **2.1.1**.

Table 2.1.1 Synthetic details of ligands

Compounds	Aldehyde used for Synthesis	Structure of Ligands	Systematic name	Yield%
2a	Thiophene - 2-aldehyde		1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione	75
2b	3-methyl-thiophene-2-aldehyde		1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione	70

The observed C, H percentage and molecular weight determination (Table 2.1.2) together with mass spectral data of the compounds clearly suggest the formation of bis-condensation product in which two equivalent of aldehyde condensed with one equivalent of acetyl acetone as shown in **Scheme 2.1. 1.**

Table 2.1.2 Analytical & UV spectral data of 1,7–dithiophenyl heptanoids

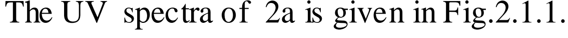
Compounds	M.P.(°C)	Elemental analysis (%)		Molecular weight	UV λmax (nm)
		C	H		
		Found/(Calculated)			
2a	114	61.63(62.5)	3.84(4.16)	287(288)	233, 331
2b	143	63.75(64.55)	4.47(5.06)	314(316)	247, 341

2.1.2. Characterisation of 1,7–dithiophenyl heptanoids

The 1,7–dithiophenyl heptanoids **2a** & **2b** synthesized were characterized by various spectral techniques like UV, IR, ^{1}HNMR, ^{13}C NMR and Mass spectral techniques.The spectral techniques used are discussed below.

UV spectra

The UV spectra of the compounds are characterized by the presence of two absorption maxima (Table 2.1.2)the low energy band and high energy band in the spectra corresponds to $n\rightarrow\pi*$ and $\pi\rightarrow\pi*$ transitions respectively which are present in 1,3- diketones. The $\pi\rightarrow\pi*$ transitions are generally intense while $n\rightarrow\pi*$ transitions are weak. The value at 230-290nm are due to $\pi\rightarrow\pi*$ transition and at 330-370nm are due to $n\rightarrow\pi*$ transitions.The ligand 1a showed two broad bands at 233 and 331nm respectively due to $\pi\rightarrow\pi*$ and $n\rightarrow\pi*$ transitions. The ligand 1b gave two bands at 247 and 341nm respectively due to $\pi\rightarrow\pi*$ and $n\rightarrow\pi*$ transitions and has shown a bathochromic shift.


The UV spectra of 2a is given in Fig.2.1.1.

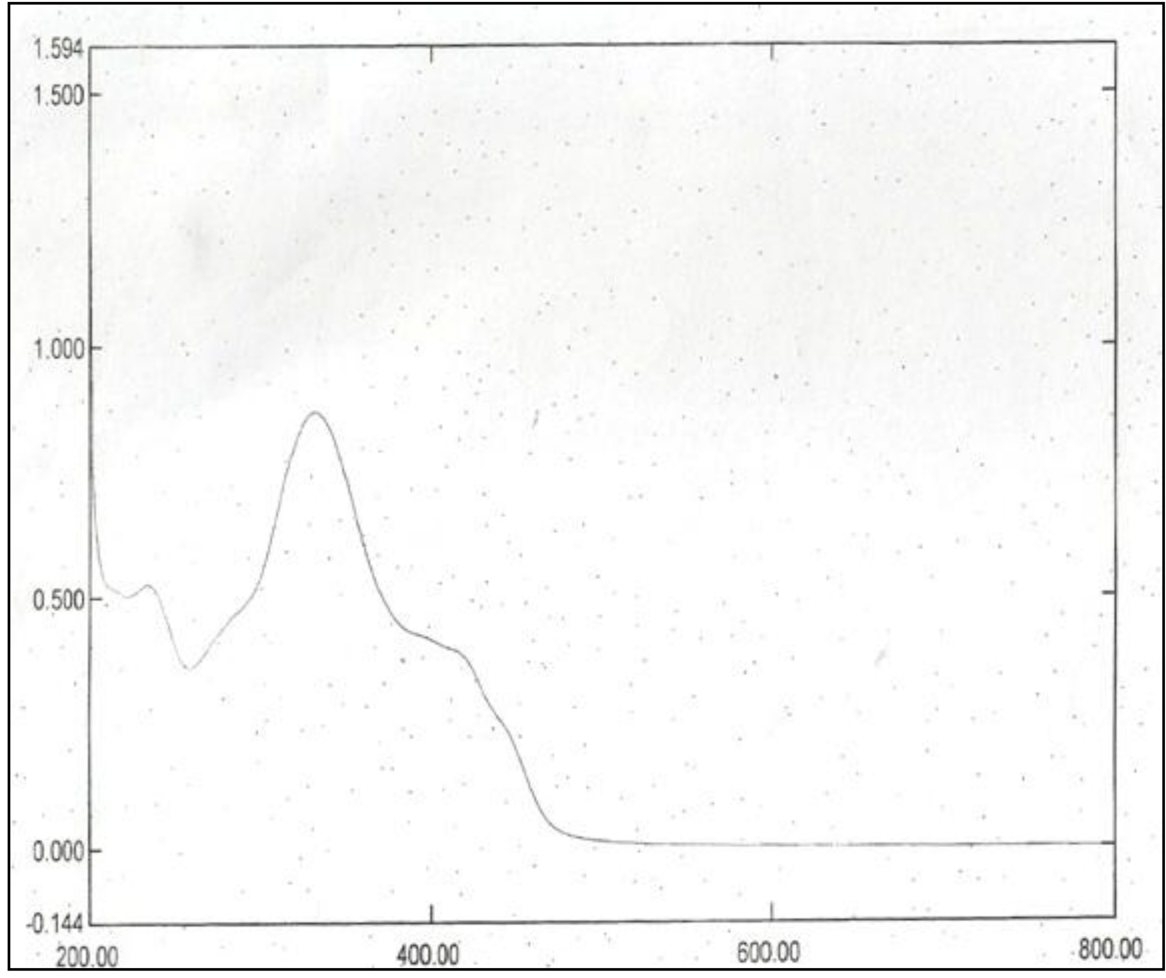


Fig.2.1 .1 UV spectrum of 1 ,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a)

IR spectra

Importance of IR spectra in establishing the keto-enol tautomers of β-diketones has been well established.The spectra mainly gives idea about the nature of the carbonyl group present,whether free or in the hydrogen bonded form. Presence of α,β – unsaturation in the compound can be very well established using IR spectroscopy .The IR spectral datas of compounds 2a & 2b are given in (Table 2.1.3).

Table 2.1.3 IR spectral data (cm^{-1}) of 1,7- dithiophenyl heptanoids

Compounds		Probable IR assignments
2a	**2b**	
1641	1652	ν (C=O) chelated
1585	1591	ν(C=C) thiophenyl
1546	1534	ν(C-C) alkenyl
1517	1521	ν_{as}(C-C-C) chelate ring
1411	1410	ν_{s}(C-C-C) chelate ring
1145,1037	1179,1023	β (C-H) chelate ring
972	961	ν(CH=CH) trans

The values corresponding to ν(C=O) are 1641 and 1652 cm^{-1} for 2a & 2b respectively. Usually free carbonyl group gives stretching frequency at ~ 1710 cm^{-1}. There is no peak in that region indicating that the C=O group is not in the keto form,instead it is in the enolic form due to peaks in the range ~ 1650 cm^{-1}. The shift is a result of internal hydrogen bonding.Resonance also contributes to the lowering of carbonyl frequency in the enol form. Thus the observed position and intensity of these bands indicate that the compound exists in strong intra molecular hydrogen bonded enolic form.A weak, broad O-H stretch is observed for the enol form at 3200-2400 cm^{-1}. There are a number of medium intensity vibrations observed in the region 1550-1600 cm^{-1} due to various ν(C=C) vibrations of the thiophenyl group. Other IR peaks due to ν(C-C) alkenyl, ν_{as}(C-C-C) chelate ring, ν_{s}(C-C-C) chelate ring & β (C-H) chelate ring are present in the spectra. The IR spectra of these compounds are also characterized by the trans ν(CH=CH) vibrations occurring at 972 & 961 cm^{-1} respectively for **2a** & **2b**. The IR spectra of 2a & 2b are given below. IR spectrum of **2a** is given in Fig.2.1.2 and **2b** in Fig.2.1.3.

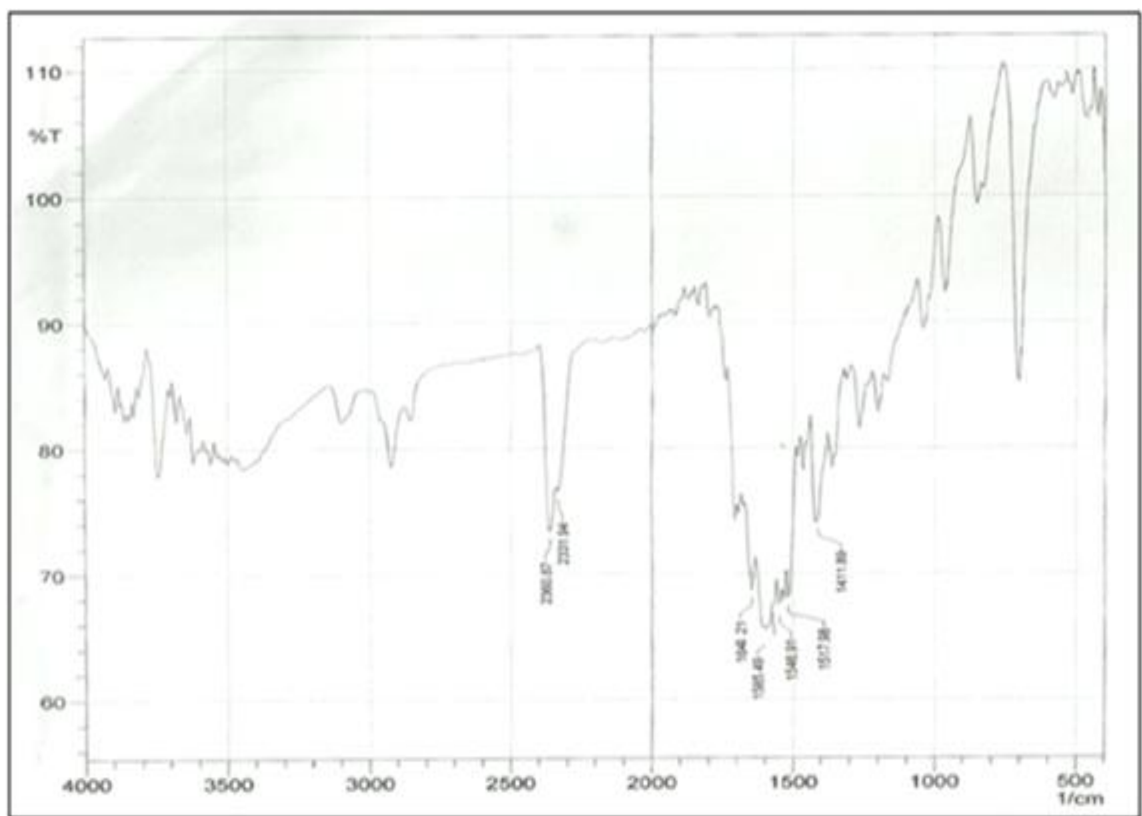


Fig.2.1.2. IR spectrum of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a)

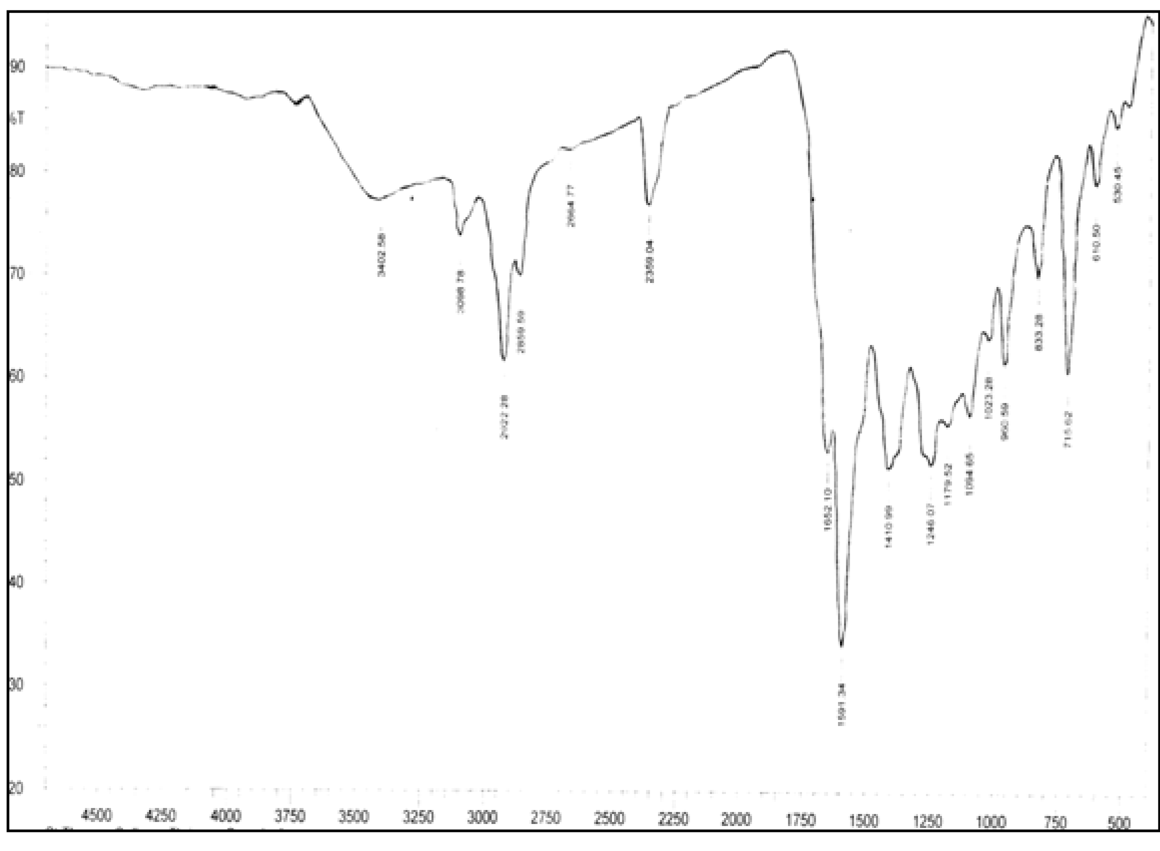


Fig.2.1.3. IR spectrum of 1,7-di(3-methylthiophen-2-yl)hepta-1,6-diene-3,5-dione (2b)

^{1}H NMR spectra

The different types of protons have characteristic values of chemical shifts in ^{1}H NMR spectra .The numerical value in ppm of the chemical shift for a proton gives a clue regarding the type of proton originating the signal.The 1,7- dithiophenyl heptanoids show specific peaks corresponding to enolic, methine, alkenyl and thiophenyl protons(Table.2.1.4). Compounds **2a** & **2b** displayed a one proton singlet at ~ 16ppm assignable to strong intra molecularly hydrogen bonded enolic proton . Another one proton singlet at ~ 5.9 ppm corresponds to the strong intra molecularly hydrogen bonded methine proton.The spectral data suggests the structure given below.

Enolic structure of 1,7- dithiophenyl heptanoids

Table 2.1.4 Characteristic ^{1}H NMR spectral data of 1,7- dithiophenyl heptanoids

Compounds	Chemical shifts (δ ppm)				
	Enolic	Methine	Alkenyl	Thiophenyl	Methyl
2a	16.1	5.8	6.7-7.9	7.0-7.1	-
2b	16.15	5.9	6.9-7.6	7.0-7.3	2.4

Other signals appearing in the ^{1}H NMR spectra are that of the thiophenyl protons present in the range δ 7 .0 – 7.3ppm and alkenyl protons in the range of 6.7 – 7.9ppm. The methyl group on the thiophenyl ring of compound **2b** showed an additional peak at 2.4ppm as expected. The ^{1}H NMR spectra of **2b** is given in Fig.2.1.4.

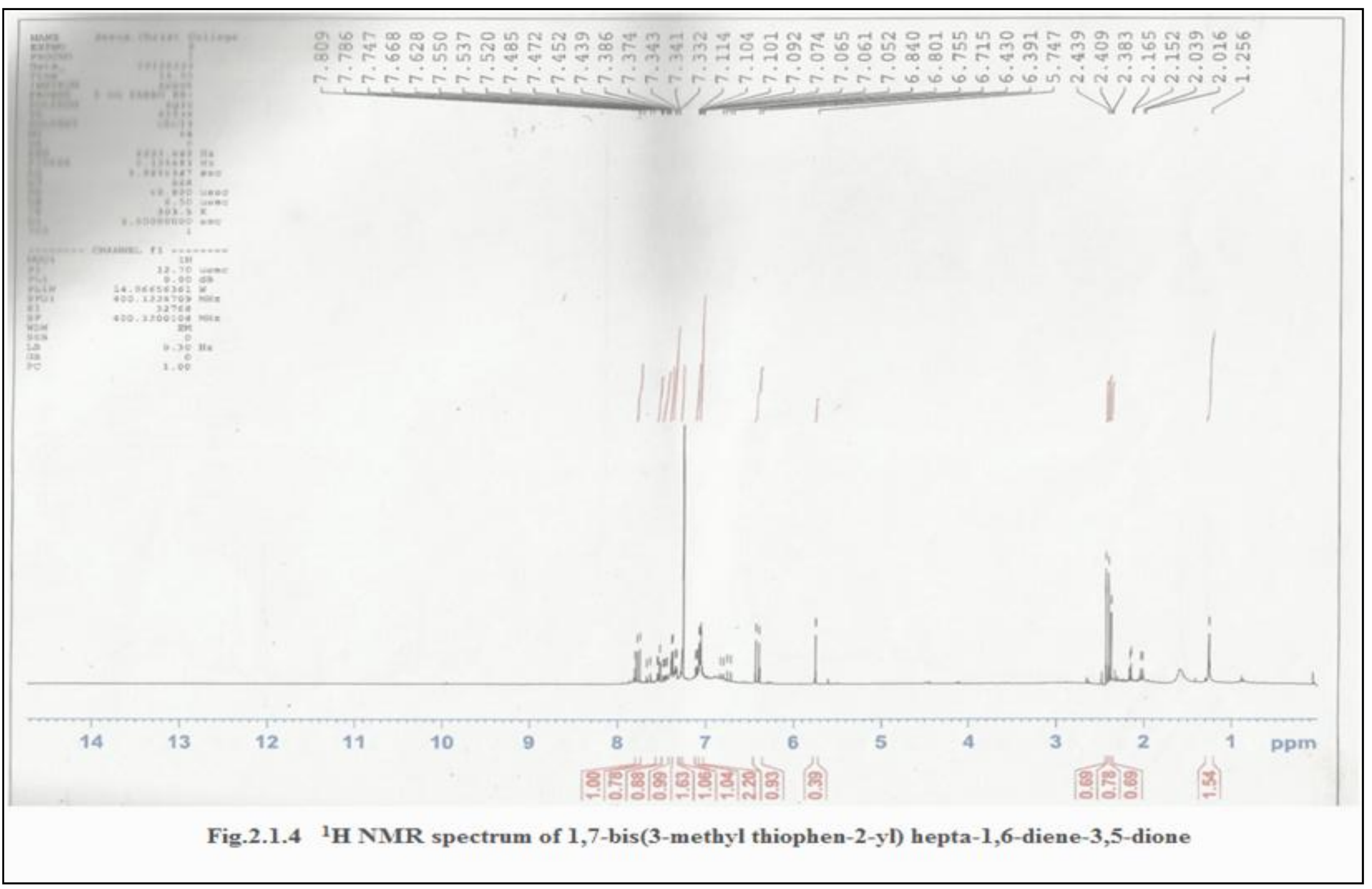


Fig.2.1.4 ^{1}H NMR spectrum of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione

^{13}C NMR spectra

The ^{13}C NMR spectral data of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (**2a**) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (**2b**) are given in Table 2.1.5 & 2.1.6.

Structure representing different carbon atoms in 2a.

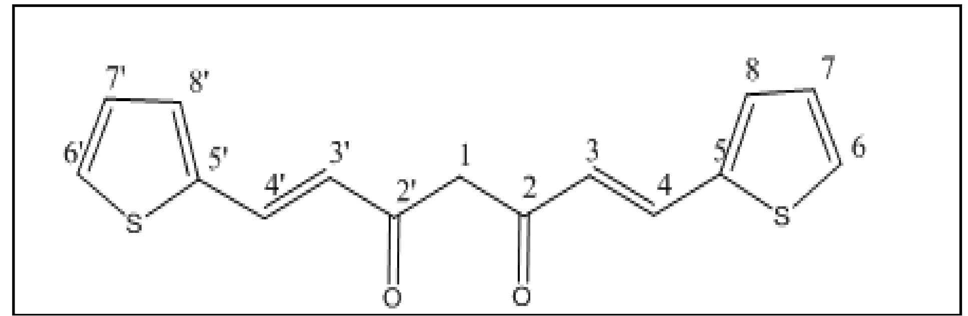


Table 2.1.5 ^{13}C NMR spectral data of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (chemical shift in ppm)

C1	C2,C2'	C3,C3'	C4,C4'
106.80	190.99	140.26	129.32
C5,C5'	C6,C6'	C7,C7'	C8,C8'
143.2	133.67	131.73	127.45

In ^{13}**C** spectra ,nearly every non equivalent carbon atom in an organic molecule gives rise to a peak with a different chemical shift.It helps in the identification of chemically and magnetically distinct carbons. The peak corresponding to C1 (methine) is present at a position ~ at 107ppm in 2a & ~101ppm in 2b . Usually CH_2 carbon which is flanked between two carbonyl groups appears at a position nearer to 55ppm. Instead, there is a possibility of keto-enol tautomerism which makes the methine to become an alkenyl carbon. Thus there is a down ward shift in the peak of C1.

The C2 carbon of carbonyl appears at a position at~ 190ppm in 2a & ~183ppm in 2b. In ^{13}C NMR,the carbons of carbonyl groups have the largest chemical shifts.The alkenyl carbon of both **2a** & **2b** are present at a position nearer to the thiophenyl ring system.They are seen at140,138ppm(C3) &129,126ppm (C4)of compounds 2a and 2b respectively. The thiophenyl carbon atoms are present between 124 – 143ppm. The carbon (C5)which is attached to the alkenyl carbon atom is downshielded and present at a position 143ppm and 135ppm respectively. The ^{13}C NMR spectrum of **2b** is given in Fig.2.1.5. In the ^{13}C NMR spectrum of **2b** the carbon to which the methyl group is attached is shifted to 134.92ppm. Also in **2b** the methyl group is present at a position ~ 20ppm.

Structure representing different carbon atoms in 2b.

Table 2.1.6 ^{13}C NMR spectral data of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (chemical shift in ppm)

C1	**C2,C2'**	**C3,C3'**	**C4,C4'**	**C5,C5'**
101.82	183.50	138.35	126.78	135.72
C6,C6'	**C7,C7'**	**C8,C8'**	**C9,C9'**	
134.92	130.8	124.85	20.98, 19.33	

Mass Spectra

In mass spectrum, ions are detected as a function of their m/z (mass to charge)ratio.The value `of m/z at which the molecular ion appears on the mass spectrum helps to find the molecular weight of the compound.The molecular ion undergoes fragmentation producing a series of molecular fragments called fragment ions.These ions appear at m/z values corresponding to their individual masses.The mass spectrum of **2a** & **2b** are represented in Fig.2.1.6 &2.1.7 respectively. The mass spectrum of **2a** shows a molecular ion (M+2) peak at 290 and a base peak (most intense peak)at 109 which is due to fragment F in Scheme 2.1.2. Removal of different groups from the molecular ion, according to the scheme, leads to smaller fragments which can be easily identified in the spectrum and are given in Table 2.1.7. Mass peaks due to the elimination of small groups like S, O, OH, CO, C_2H_2, CH_2=C=O,CH=C=O,CH_2,C_2H_2O etc. are also present in the spectra. In the mass spectrum of **2a** several small fragments are observed apart from the fragmental pattern described in Scheme 2.1.2. A strong peak is observed at 246 due to the removal of a sulphur and CH group. Removal of additional CH groups leads to the peaks at 229, 217 and 206(G).

Table 2.1.7 Mass spectral fragmental pattern of 1,7-dithiophenyl heptanoids

Fragment	Ligand	M+/ (M+1)/ (M+2) ion	A	B	C	D	E	F	G	H
Mass pattern	2a	290	152	137	99	109	83	109	206	184
	2b	317	166	151	111	123	103	122	218	193

*The alphabets corresponds to the fragments given in **Scheme .2.1.2**

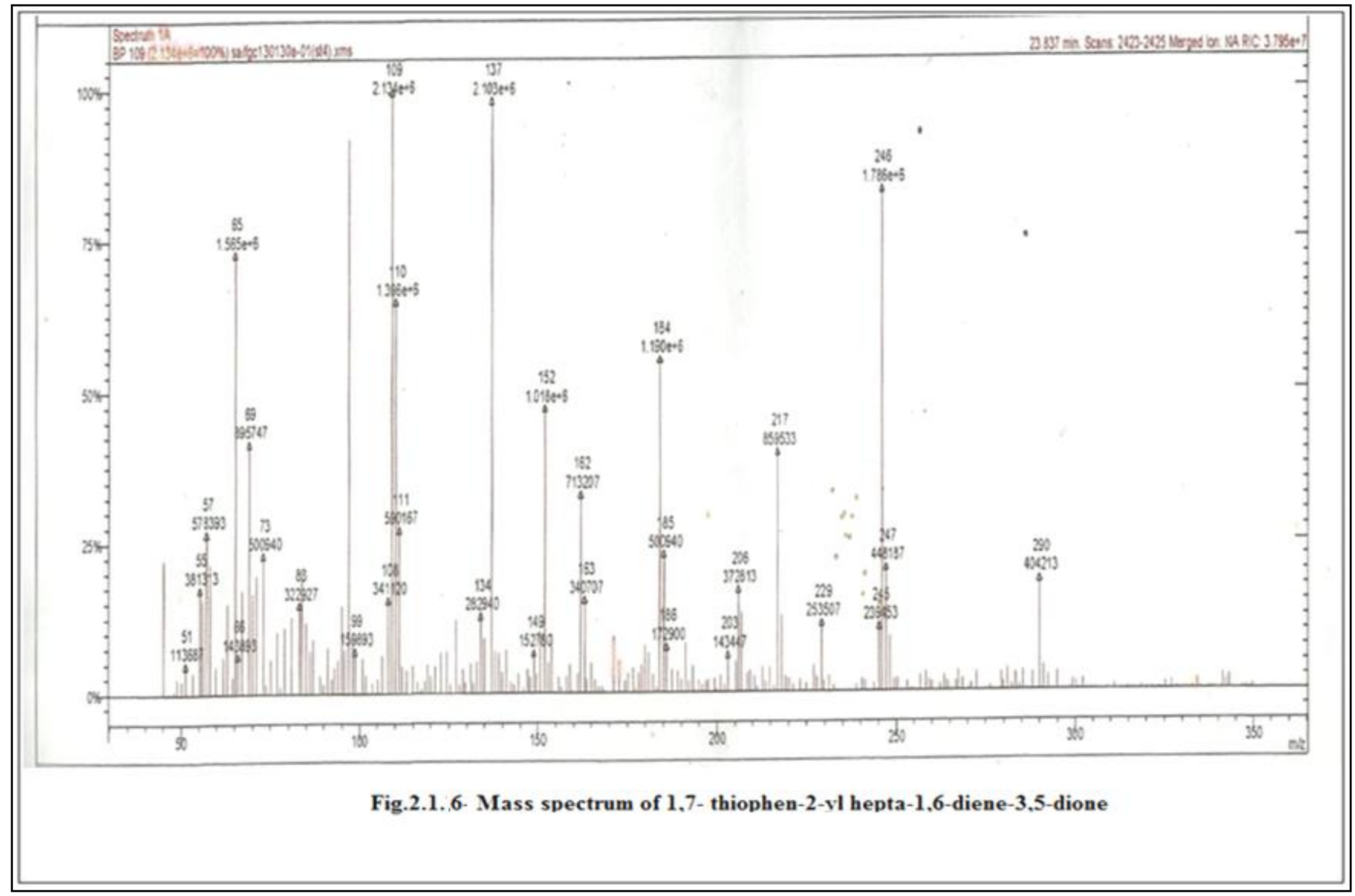


Fig.2.1.6- Mass spectrum of 1,7- thiophen-2-yl hepta-1,6-diene-3,5-dione

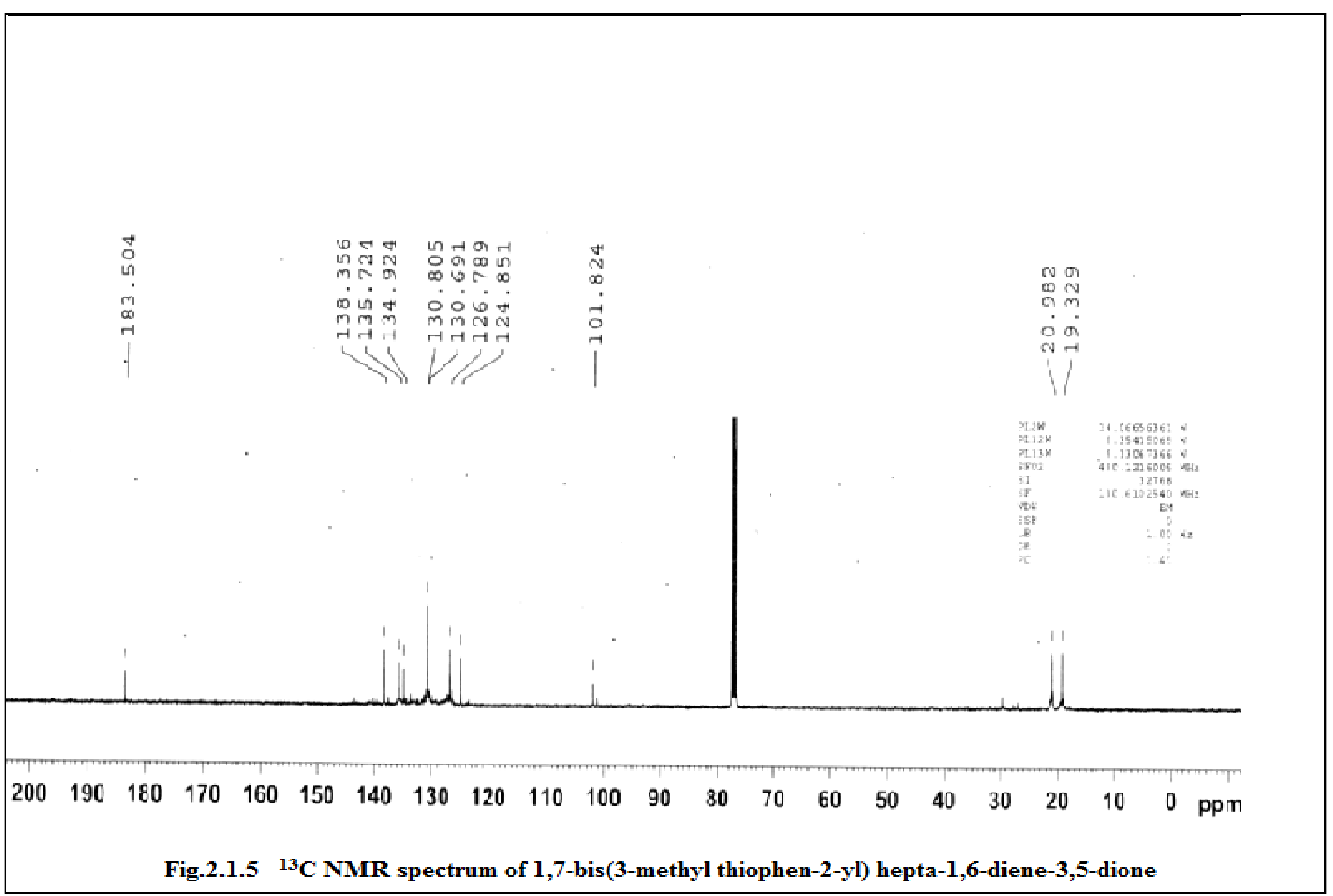


Fig.2.1.5 ^{13}C NMR spectrum of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione

The mass spectrum for **2b** shows a much less intense molecular ion(M+1) peak at 317.The fragment peak at 151 due to B(Ar-CH=CH-C=O)$^+$ is the most intense in the mass spectrum.The next intense peak is observed at m/z 111 which is due to fragment C(Ar-CH$^+$).All other peaks in the spectrum can be explained from the fragmentation pattern given in scheme.Removal of 3-methyl thiophenyl group,CH_2,C_2H_2O,C_2H_2,CH_2=C=O,CH=C=O etc give rise to different fragment ions.A strong peak is observed at 275 which is due to the removal of one S and CH group. Again removal of the second S and CH group leads to the peak at 233. The mass spectrum of **2b** is given in Fig.2.1.7.

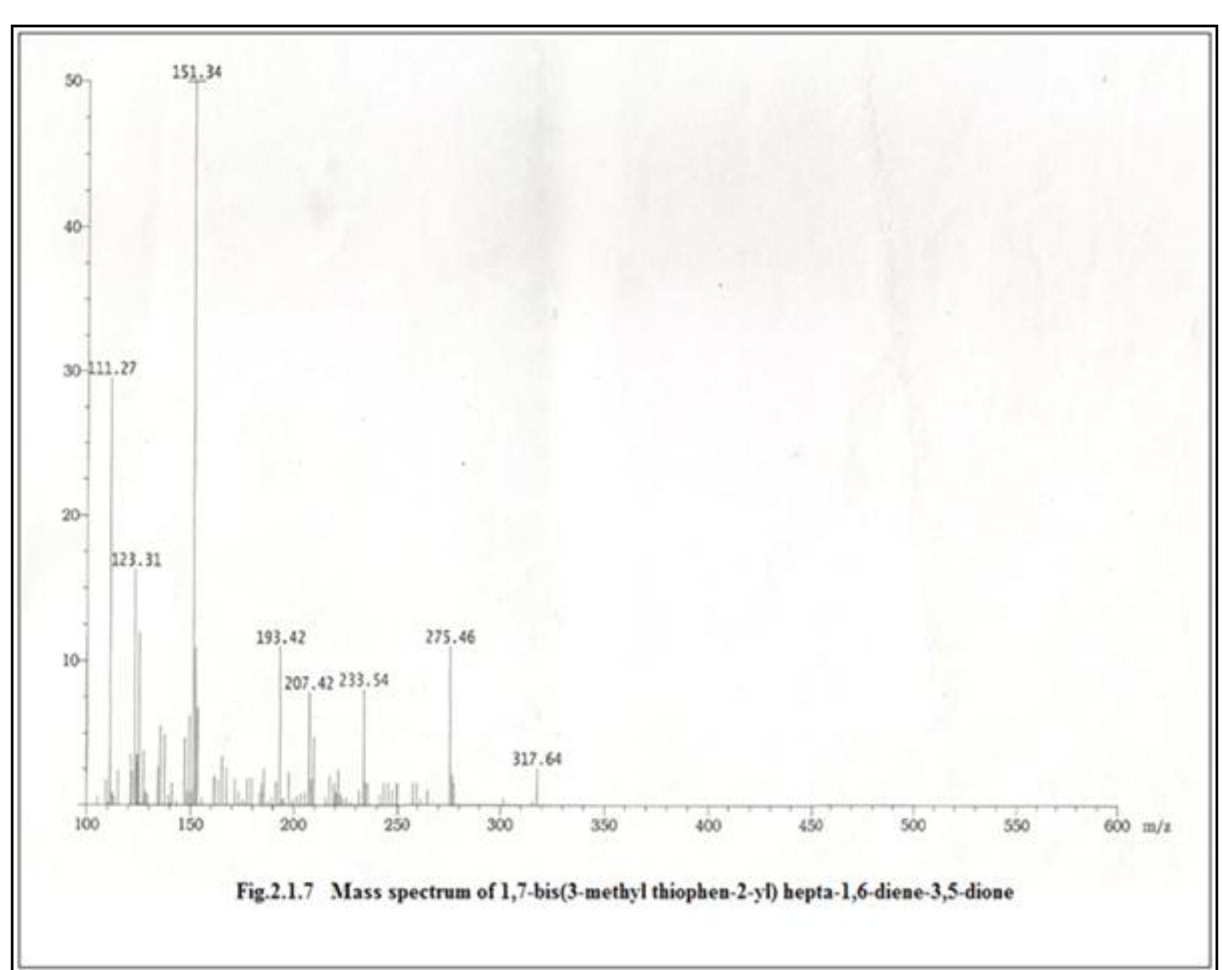


Fig.2.1.7 Mass spectrum of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione

Scheme 2.1.2 **Ar** = Thiophen-2-yl, 3-methyl thiophen-2-yl.

Thermogravimetric analysis

Thermogravimetric studies of1,7- diheteroaryl heptanoids (Table 2.1.8) gives some important results regarding the structure of molecules. The thermogram of 2a shows a one stage decomposition pattern as shown in Fig.2.1.8. The compound is stable upto a temperature of 210°C and then decomposition begins slowly with a sharp drop in the mass upto a

temperature of 427°C. The peak temperature in DTG is found at 347.5°C. There appears a shoulder in the DTG curve at a temperature of 266°C. The sample was heated upto a temperature of 740°C and the remaining portion was found to be an aryl group.

Table 2.1.8 Thermogravimetric studies of 1,7- diheteroaryl heptanoids

Compound (mol. mass)	Temp. range in TG (°C)	Peak Temp. (°C)	Mass loss %		Pyrolysis %	Final product
			TG	Theoretical		
2a (288)	210 - 427	347.5	59.39	60.63	70.13	Thiophenyl
2b (316)	236 - 469	365	56.35	57.13	73.62	3-methyl Thiophenyl

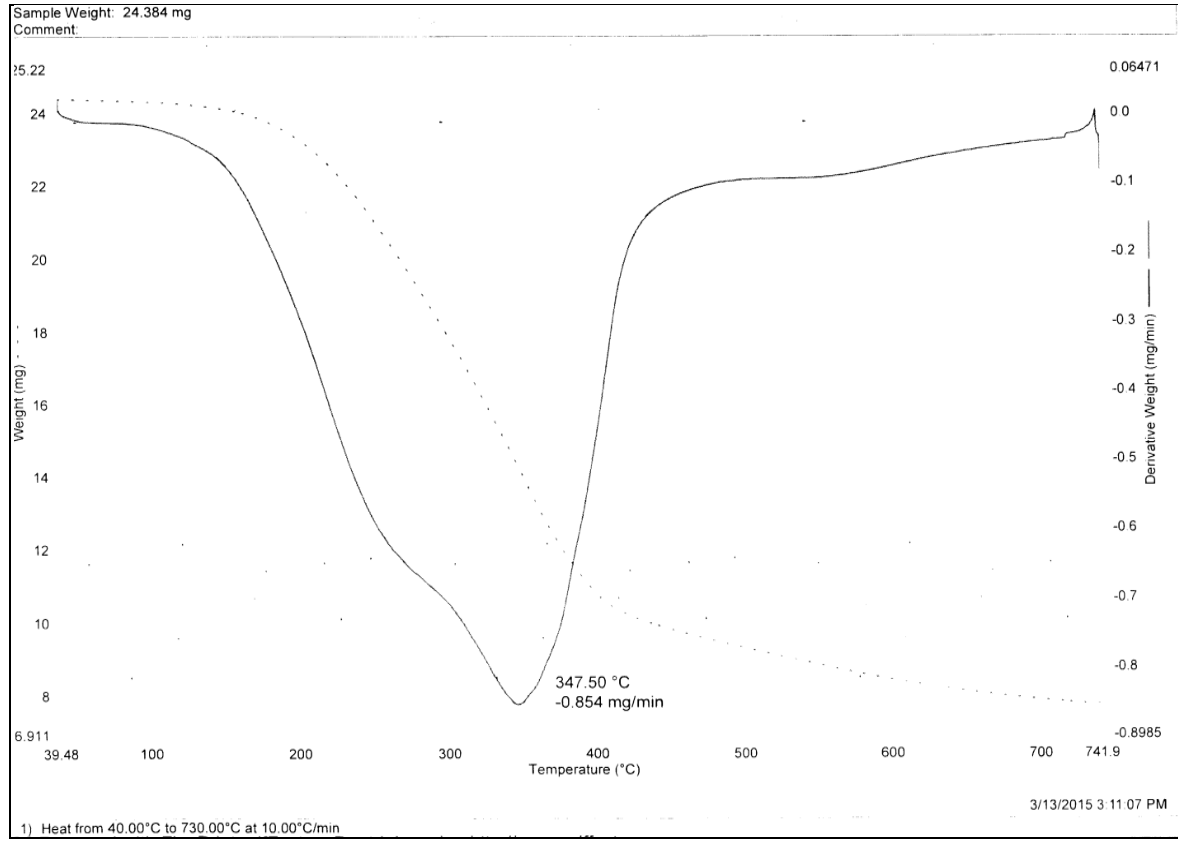


Fig.2.1.8 Thermogravimetric studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione

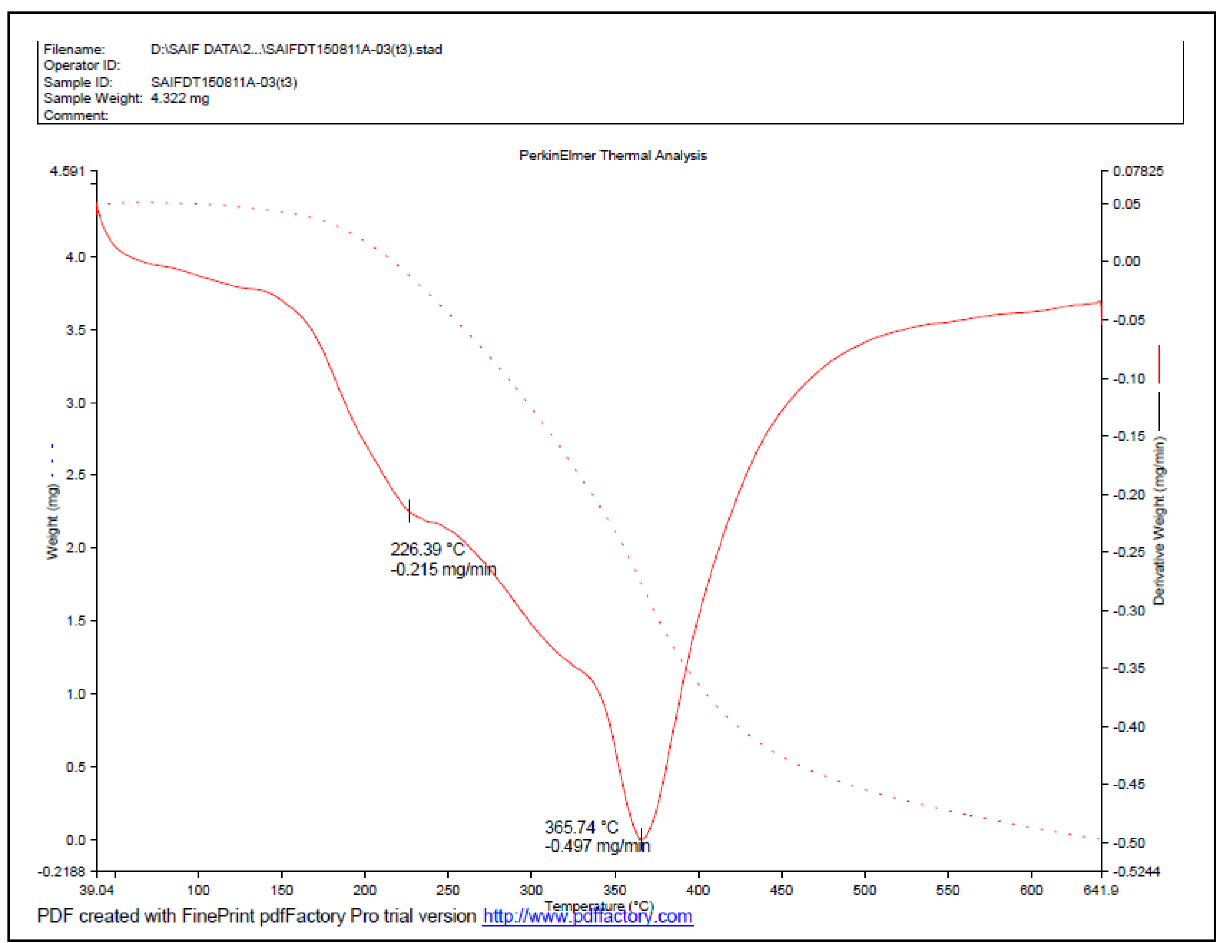


Fig.2.1.9 Thermogram of 1,7-di(3-methylthiophen-2-yl)hepta-1,6-diene-3,5-dione

SECTION-II

SYNTHESIS AND CHARACTERISATION OF TRANSITION METAL CHELATES OF 1, 7 -DITHIOPHENYL HEPTANOIDS & METHYL SUBSTITUTED DERIVATIVE

2.2.1 Synthesis of metal complexes of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (2b)

Copper(II),Zinc(II),Nickel(II)and Oxovanadium(IV)complexes of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (2b) were synthesized by the following general method.

To a refluxing solution of the diketone(0.002 mol) in methanol(25ml),a methanolic solution of metal salt (0.001mol)was added and the reaction mixture was refluxed for nearly 2 hrs and cooled to room temperature. The precipitated complex was filtered, washed with 1:1 methanol:water mixture and recrystallised from hot methanol.

Preparation of Cu(II) complex of the ligands

The Cu(II) complexes were prepared by adding a methanolic solution of copper(II)acetate (25 ml, 0.001mol) to a solution of **2a&2b** (25 ml, 0.002 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature. The precipitated complex was filtered, washed with 1:1 methanol:water mixture and recrystallised from hot methanol.

Preparation of Zn(II) complex of the ligands

The Zn(II) complexes were prepared by adding a methanolic solution of zinc acetate (25 ml, 0.001mol) to a solution of **2a&2b** (25 ml, 0.002 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature. The

precipitated complex was filtered, washed with 1:1 methanol:water mixture and recrystallised from hot methanol.

Preparation of Ni(II) complex of the ligands

The Ni(II) complexes were prepared by adding a methanolic solution of nickel(II) acetate (25 ml, 0.001mol) to a solution of **2a &2b** (25 ml, 0.002 mol) in methanol and repeating the above procedure.

Preparation of Oxovanadium(IV) complex of the ligands

The VO(IV) complexes were prepared by adding a methanolic solution of vanadium (IV)oxide sulphate (25 ml, 0.001mol) to a solution of **2a&2b** (25 ml, 0.002 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature. The precipitated complex was filtered, washed with 1:1 methanol:water mixture and recrystallised from hot methanol.

The reaction involved in the formation of complexes is represented below in Scheme 2.2.1

Scheme 2.2.1

2.2.2 Characterisation of metal complexes of methyl substituted 1,7-dithiophenyl heptanoids

Transition metal chelates (Cu, Zn,Ni,Vanadyl) of ligands **2a & 2b** were characterized using physical,analytical and spectral datas.The spectral techniques used in characterization include UV,IR,NMR and Mass spectral analysis. Elemental analysis (C, H and metal percentages) , physical data and UV ,IR spectral data of metal complexes of 2a are given in Table 2.2.1 and metal complexes of 2b are given in Table 2.2.2 respectively. The datas given below suggest a ML_2 stochiometry for all complexes prepared.

Table 2.2.1 Analytical and UV, IR spectral data of metal complexes of 2a

Metal chelate	M.P. (^{o}C)	Elemental analysis (%) Found/(calculated)			UV λmax (nm)	Characteristic IR stretching bands (cm^{-1})		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu(II)	139	56.47 (57.25)	3.45 (4.00)	9.99 (10.01)	234 336	1614	1531	490, 430
Ni(II)	141	56.89 (58.01)	3.47 (4.20)	9.27 (10.90)	233, 337	1583	1520	485, 431
Zn(II)	150	56.30 (56.91)	3.44 (3.61)	11.22 (11.90)	235 337	1616	1532	473, 420
VO(IV)	173	56.16 (57.30)	3.43 (3.52)	7.94 (8.05)	236, 334	1592	1528	484, 432

Table 2.2.2 Analytical and UV, IR spectral data of metal complexes of 2b

Metal chelates	M.P. (^oC)	Elemental analysis (%) Found/(calculated)			UV λmax nm	Characteristic IR stretching bands (cm^{-1})		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu(II)	189	58.05 (58.83)	4.25 (4.32)	9.00 (9.15)	249, 344	1589	1519	485, 442
Ni(II)	192	58.55 (59.24)	4.20 (4.35)	8.05 (8.52)	251, 346	1602	1525	486, 443
Zn(II)	194	58.50 (58.67)	4.25 (4.31)	9.01 (9.40)	250, 345	1595	1521	483, 445
VO(IV)	196	57.75 (58.54)	4.26 (4.31)	7.03 (7.30)	255, 350	1594	1510	473, 437

2.2.3. Characterization by various spectral techniques

UV spectra

The spectra of metal complexes also show two UV transitions, the $\pi \rightarrow \pi * transition$ & $n \rightarrow \pi * transition$. The UV absorption bands of the ligands were almost unaffected by complexation with metal ions. The spectra of complexes closely resembles the spectra of respective ligands. So there is no much change in the structure due to complex formation.The $n \rightarrow \pi *$ transition of the dicarbonyl chromophore of the free ligand showed a slight red shift indicating involvement of the dicarbonyl moiety in chelate formation. For comparison, the UV spectra of ligand 2a and its Zn(II) complex are shown in Fig.2.2.1

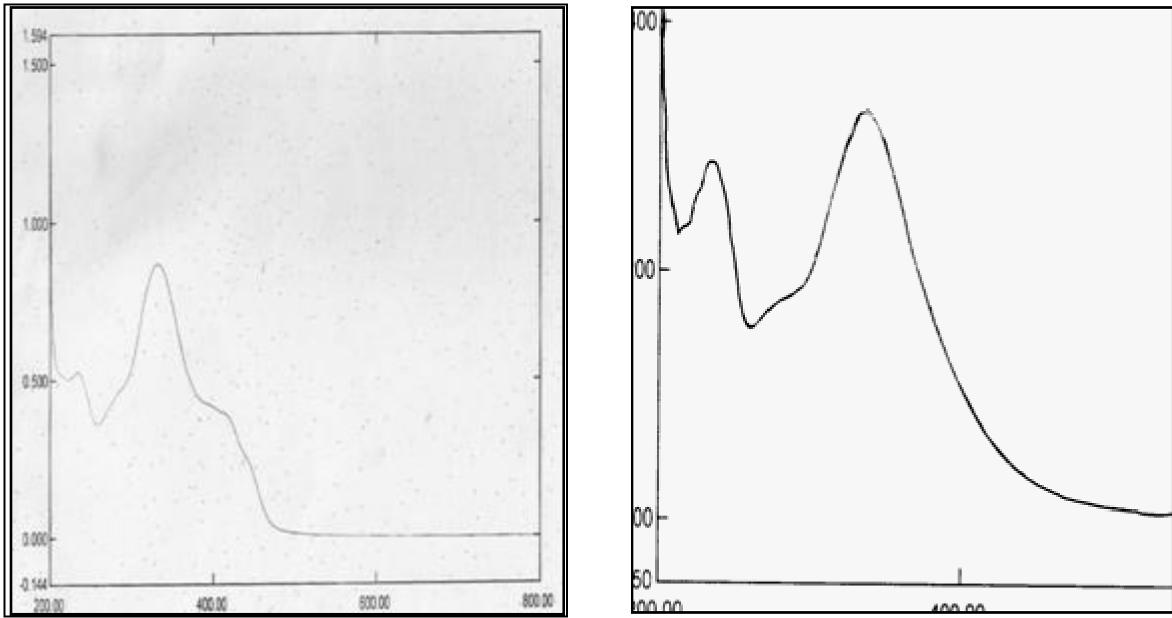


Fig.2.2.1 UV spectra of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and its Zn(II) complex

IR spectra

Instead of the intramolecularly hydrogen bonded carbonyl band at 1641 cm^{-1} for ligand 2a and the band at 1652 cm^{-1} for ligand 2b, a strong band assignable to the stretching of the coordinated carbonyl moiety appears in the region 1585 -1620 cm^{-1} in the spectra of all metal complexes.Involvement of the carbonyl group in coordination is further supported by the observation of two medium intensity bands in the region of 400 – 490 cm^{-1} due to νM-O vibrations(Metal-Oxygen). The replacement of enolic proton by a metal ion is also evident from the absence of the broad band in the region of 2600 -3500 cm^{-1} present in the ligand. All these support the formation of metal complexes. There is no change in the nature of alkenyl carbon due to metal complexation.The IR spectra of Cu(II) complex of 2a is depicted in Fig.2.2.2 and the IR spectra of VO(IV) complex of 2b is depicted in Fig.2.2.3

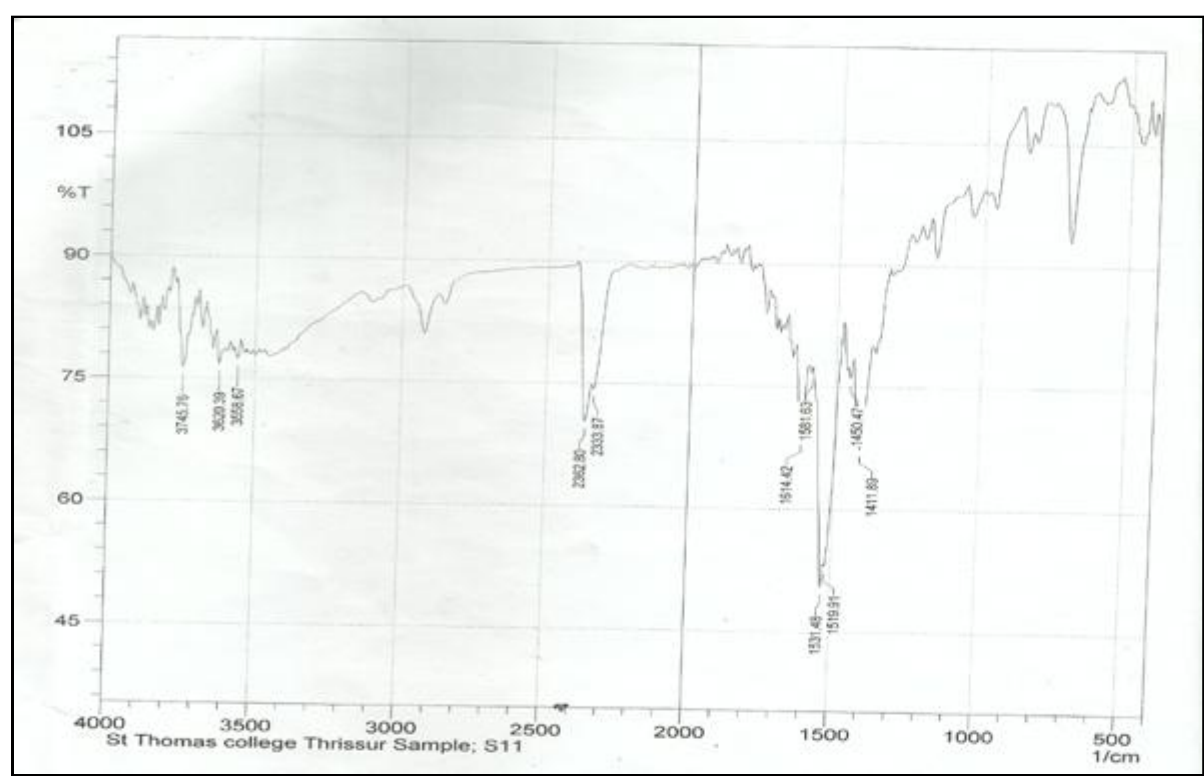


Fig.2.2.2 IR spectra of Cu(II) complex of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a)

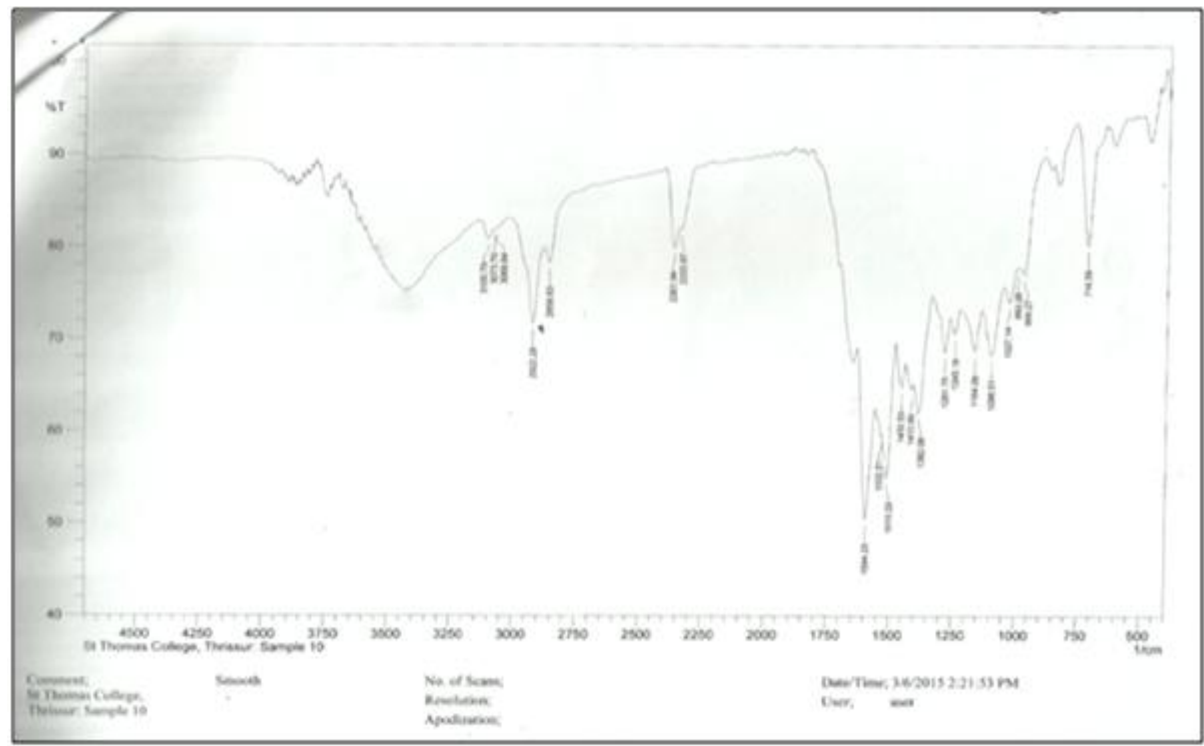


Fig.2.2.3 IR spectra of VO(IV) complex of 1,7-bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione (2b)

^{1}H NMR spectra

A characteristic feature of ^{1}H NMR spectra of metal complexes is the absence of singlet signal at δ ~ 16ppm which suggests the replacement of enolic proton in the ligand by metal atom in metal complexes. The phenyl and alkenyl protons are not altered much since they are not involved in metal complexation.However the observed downfield shift of the methine proton signals is consistent with decreased electron density around the central atom of the psuedo aromatic metal chelate ring.Thus the spectra of ligand and complexes are much similar except those of enolic proton. The **^{1}H NMR spectra** of Zn(II) complex of ligand 2b is given in Fig 2.2.4.

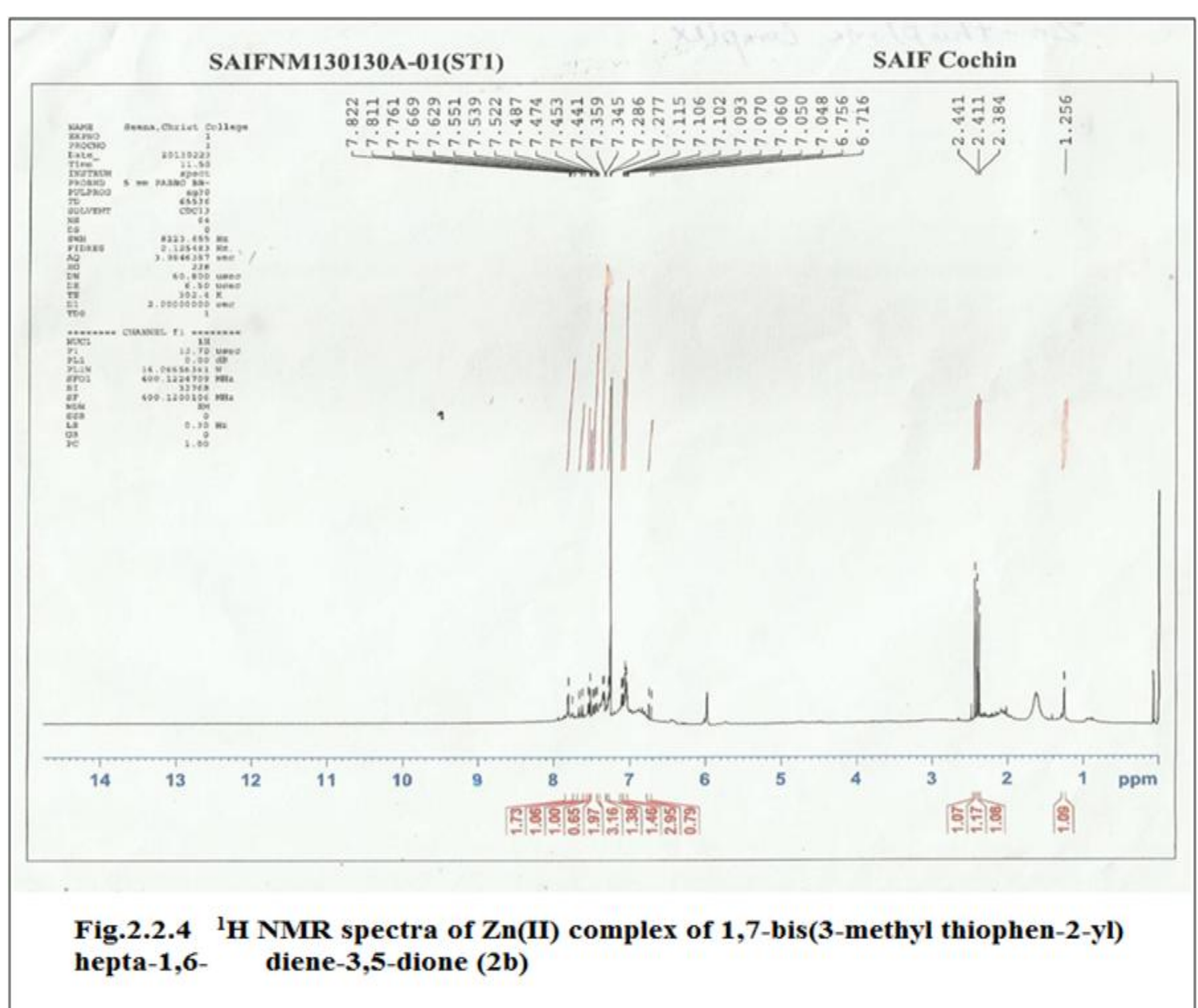


Fig.2.2.4 ^{1}H NMR spectra of Zn(II) complex of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6- diene-3,5-dione (2b)

Mass spectra

In their mass spectra,all the complexes showed relatively intense peaks at m/z corresponding to ML_2 stochiometry,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^+(fragment F in Table) and fragments of L^+ are also detected in the spectrum. It was found that some fragments rearrange to form stable cyclic species as shown in the Scheme. The fragmental patterns of the metal chelates **2a** & **2b** can be identified from **Scheme 2.2.2.** which is given below.The fragment F given below in the table corresponds to that of the ligand peak. Mass spectrum of VO(IV) complex of **2b** is given in Fig.2.2.5

Table 2.2.3 Mass spectral fragmental pattern of metal chelates of 2a

Fragments	Metal chelates	M+/ (M+1)/ (M+2) ion	A	B	C	D	E	F
Mass Pattern	Cu(II)	637	471	350	121	303	184	289
	Zn(II)	639	473	352	121	305	184	289
	Ni(II)	633	467	346	121	301	180	289
	VO(IV)	641	475	354	121	309	188	289

*The alphabets corresponds to the fragments given in **Scheme 2.2.2**

Table 2.2.4 Mass spectral fragmental pattern of metal chelates of 2b

Fragments	Metal chelates	M+/ (M+1)/ (M+2) Ion	A	B	C	D	E	F
Mass Pattern	Cu(II)	694	500	379	121	306	184	317
	Zn(II)	696	501	381	121	308	187	317
	Ni(II)	689	495	374	121	301	180	317
	VO(IV)	697	503	382	121	309	188	317

*The alphabets corresponds to the fragments given in **Scheme 2.2.2**

Scheme 2.2.2

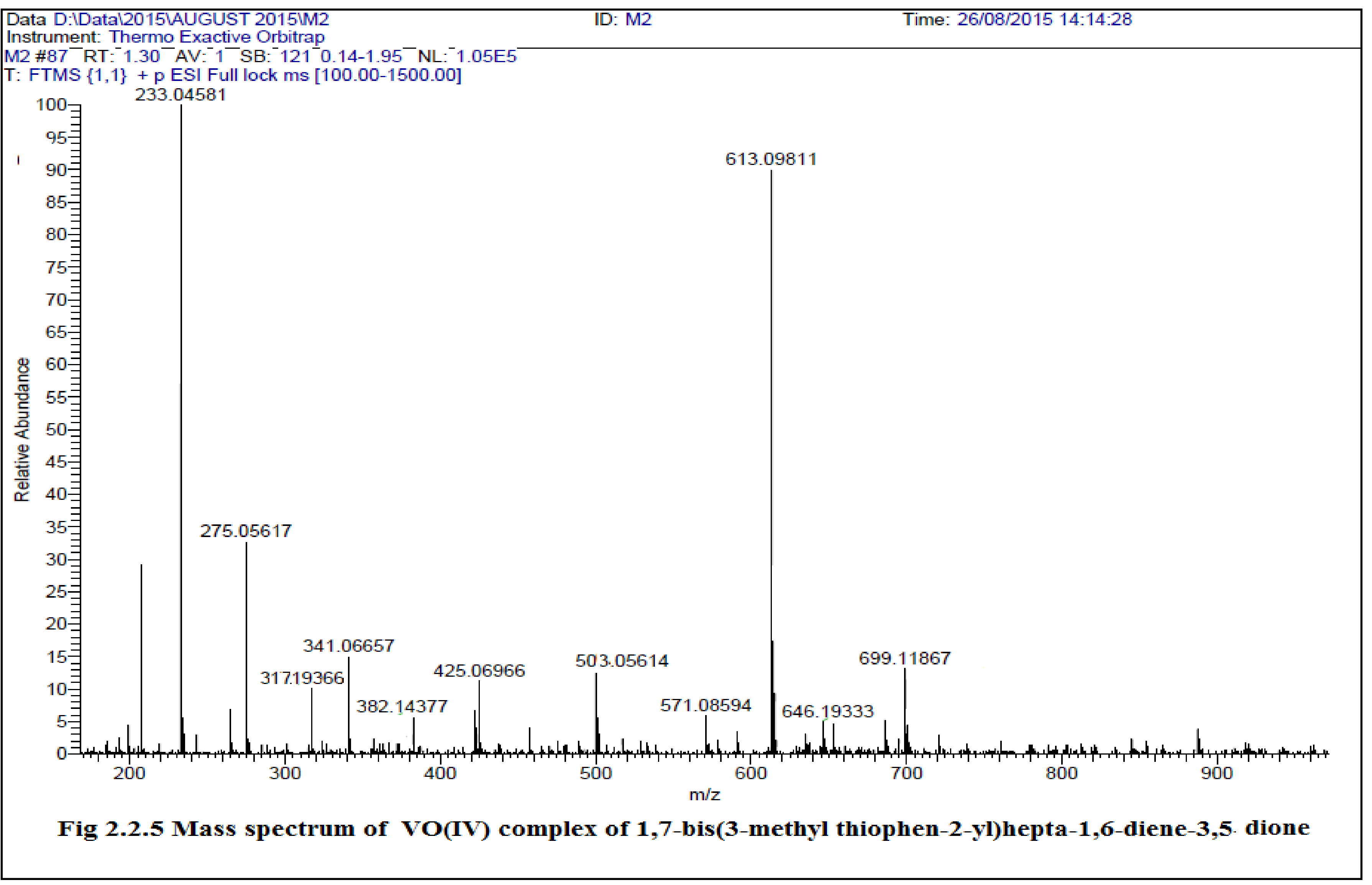


Fig 2.2.5 Mass spectrum of VO(IV) complex of 1,7-bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5 dione

In the mass spectrum of VO(IV) complex of 1,7-bis (3-methylthiophen-2-yl)hepta-1,6-diene-3,5-dione a less intense M+2 peak is observed at 699.The peak at 646 is due to the removal of Vanadium from the molecular ion.The intense peak at 613 is due to the removal of Vanadyl and one oxygen from molecular ion.The peak at 503 is due to the fragment ion formed by the removal of 2 Ar groups from molecular ion (Ar=3-methylthiophenyl).The peak due to the ligand is observed at 317.The peaks at 275 and 233 are due to fragments of ligand and are observed in the spectrum of ligand.The peak at 275 is due to the removal of one S and CH group from ligand and the base peak at 233 is due to the removal of the second S and CH group.

SECTION-III

CYTOTOXIC AND ANTITUMOUR STUDIES OF 1,7–DITHIOPHENYL HEPTANOIDS AND THEIR TRANSITION METAL CHELATES

This section deals with the cytotoxic and antitumour activities of 1,7-diheteroaryl heptanoids namely 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (**2a**) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione(**2b**) and their metal chelates Cu(II),Zn(II),Ni(II) &VO(IV). Invitro cytotoxic activity against DLA and EAC cell lines were studied.The Invivo antitumour activity was determined by using DLA cell line induced solid tumour and EAC cell line induced ascites tumour model in mice and its comparison with a std.anticancer drug cyclophosphamide.

2.3.1.Invitrocytotoxic activity:

Short term cytotoxic activity of compounds 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (**2a**) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (**2b**) and their metal chelates Cu(II),Zn(II),Ni(II) &VO(IV) were assayed 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* Cytotoxic studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and their metal complexes [Cu(II),Zn(II),Ni(II) &VO(IV)] towards EAC cells

The curcuminoid analogue 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (**2a**) and their metal complexes were used for *in vitro* cytotoxicity study towards EAC cells. All the test compounds were prepared in different concentrations namely 10,20,50,100,200 µg/ml.The cytotoxic nature of the compounds were determined in terms of % cell death produced by them.The results of the study is given in Table **2.3.1** and represented diagrammatically in Fig.**2.3.1.**

Table 2.3.1. ***In vitro*** **Cytotoxic studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione(HL_1) and their metal complexes towards EAC**

Drug Con. µg/ml	% Cell death				
	HL_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$	$VO(L_1)_2$
200	40	92	84	75	82
100	28	80	73	65	72
50	14	65	58	49	56
20	8	48	43	37	42
10	5	32	28	21	25

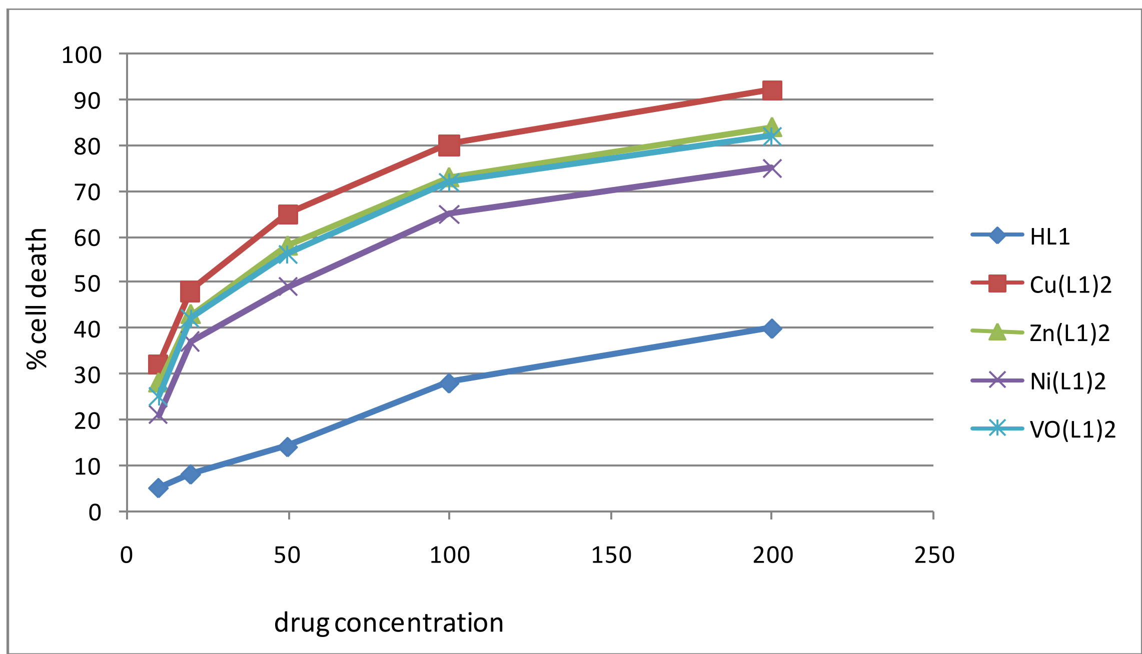


Fig.2.3.1. ***In vitro*** **Cytotoxic studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione(HL_1) and their metal complexes towards EAC**

The ligand 2a produced 40% cell death towards EAC cell lines at a concentration of 200µg/ml. At lower concentration the activity of the compound is negligible. All the metal

complexes produced greater % of cell death.They were quite active even at lower concentrations.As concentration increases the % of cell death increases.All the metal complexes showed marked cytotoxic activity.The % cell death produced by Cu(II), Zn(II),Ni(II) and VO(IV) complexes at 200µg/ml are 92, 84, 75 & 82 % respectively.The Cu(II) complex of ligand was very effective in producing a cell death of 92% indicating its potent cytotoxic nature.The Zn(II) and VO(IV) complexes showed comparable cytotoxic activity nearly 80% which is twice that of the ligand.The Ni(II)complexes possessed minimum activity among complexes,but even then it could produce 75% cell death.The results indicate that metal chelation enhance cytotoxicity of compounds considerably.

2.3.3 *In vitro* Cytotoxic studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and their metal complexes [Cu(II),Zn(II),Ni(II) &VO(IV)] towards DLA cells

The % cell death were also calculated with DLA cell lines.The results of the study in terms of % cell death is represented in Table **2.3.2** and diagrammatically in Fig. **2.3.2** .

Table.2.3.2 *In vitro* Cytotoxic studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5dione (HL_1) and their metal complexes towards DLA

Drug Con. µg/ml	% Cell death				
	HL_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$	$VO(L_1)_2$
200	38	89	80	73	79
100	23	75	72	63	69
50	12	62	55	45	54
20	8	45	40	35	39
10	3	30	27	19	23

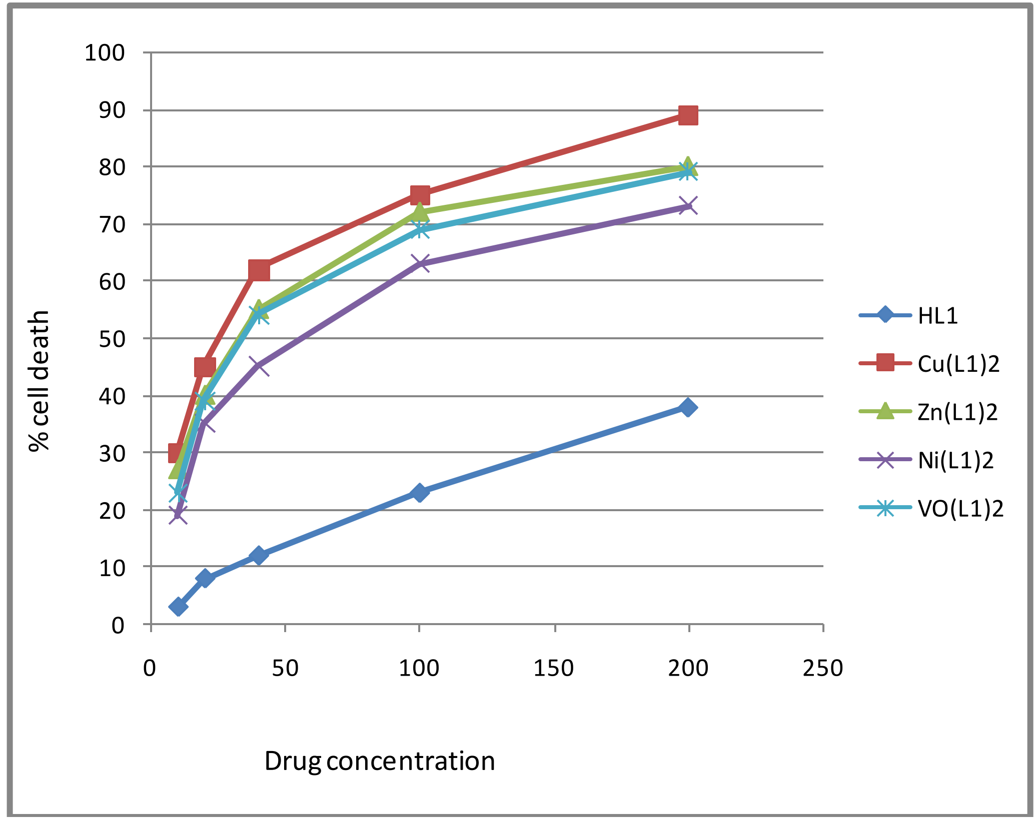


Fig.2.3.2 ***In vitro*** **Cytotoxic studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione(HL_1) and their metal complexes towards DLA**

The results of the study indicate that the activity of compound 2a and metal complexes towards DLA cells follows a similar pattern as that with EAC cells.The ligand as well as the metal complexes showed a slight decrease in activity when compared with their activities towards EAC cells.But all the metal complexes showed enhanced activity when compared with the ligand.The compound 2a produced 38% cell death where as its Cu(II) complex produced 89% cell death.The activity of metal complexes followed the order Cu(II)>Zn(II)>VO(IV)>Ni(II) and the % cell death produced by them were 89,80,79 and 73 respectively.The values show that all the metal complexes possess significant cytotoxic nature.

2.3.4. *In vitro* Cytotoxic studies of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione(2b) and their metal complexes [Cu(II),Zn(II),Ni(II) &VO(IV)] towards EAC cells

In vitro Cytotoxic studies were done using 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione(**2b**) and their metal complexes towards EAC cell lines.The observations are given in Table **2.3.3** and graphically in Fig. **2.3.3** . The ligand 2b which has a methyl group on the thiophenyl ring as compared to ligand 2a gave lesser % of cell death with EAC cells.All metal complexes possessed more cytotoxic activity than ligands.But comparing with the metal complexes of 2a,the metal complexes of 2b produced lesser % of cell death.The % cell death produced by Cu(II) complex of 2b was 80% and it is less active than Cu(II) complex of 2a.Comparing the ligand and metal complexes,the complexes were twice more active than the ligand.

Table 2.3.3. *In vitro* Cytotoxic studies of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-Dione(HL_2) and their metal complexes towards EAC

Drug Con. µg/ml	% Cell death				
	HL_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$	$VO(L_2)_2$
200	37	80	74	65	72
100	25	68	63	54	62
50	12	53	48	37	46
20	6	36	33	25	32
10	3	20	18	11	21

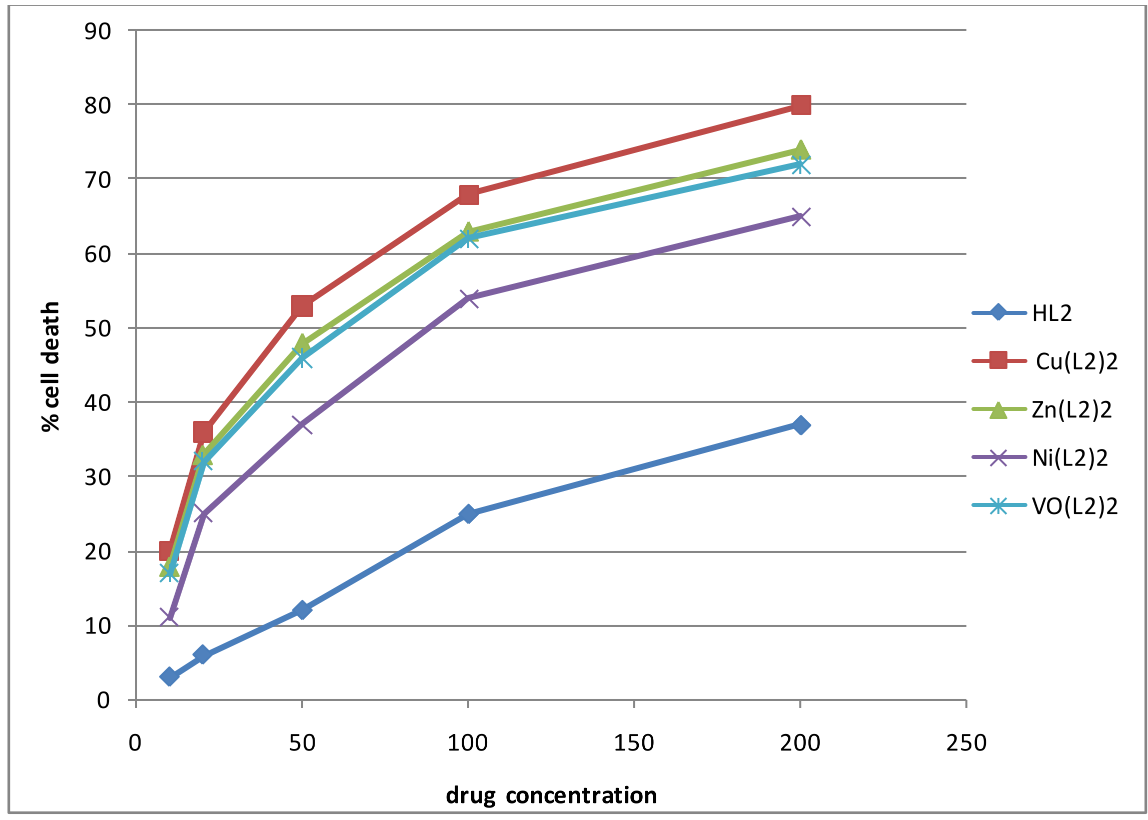


Fig.2.3.3. ***In vitro* Cytotoxic studies of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (HL_2) and their metal complexes towards EAC**

2.3.5 *In vitro* Cytotoxic studies of of 1,7-bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione(HL_2) and their metal complexes towards DLA

The results of the study are given in Table **2.3.4** and represented graphically in Fig. **2.3.4.**The ligand 2b and its metal complexes were not as effective as ligand 2a and its metal complexes in its activity against DLA cells.

All the results show that little activity was found with 10µg/ml. Also it is found that the ligand and complexes show maximum activity towards EAC cells than DLA. Even though all the metals are divalent better results are observed for Cu(II).

Table 2.3.4. ***In vitro*** **Cytotoxic studies of of 1,7-bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione(HL_2) and their metal complexes towards DLA**

Drug Con. µg/ml	% Cell death				
	HL_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$	$VO(L_2)_2$
200	34	76	70	61	68
100	21	64	59	50	58
50	12	49	44	33	42
20	6	32	29	21	28
10	4	16	14	7	17

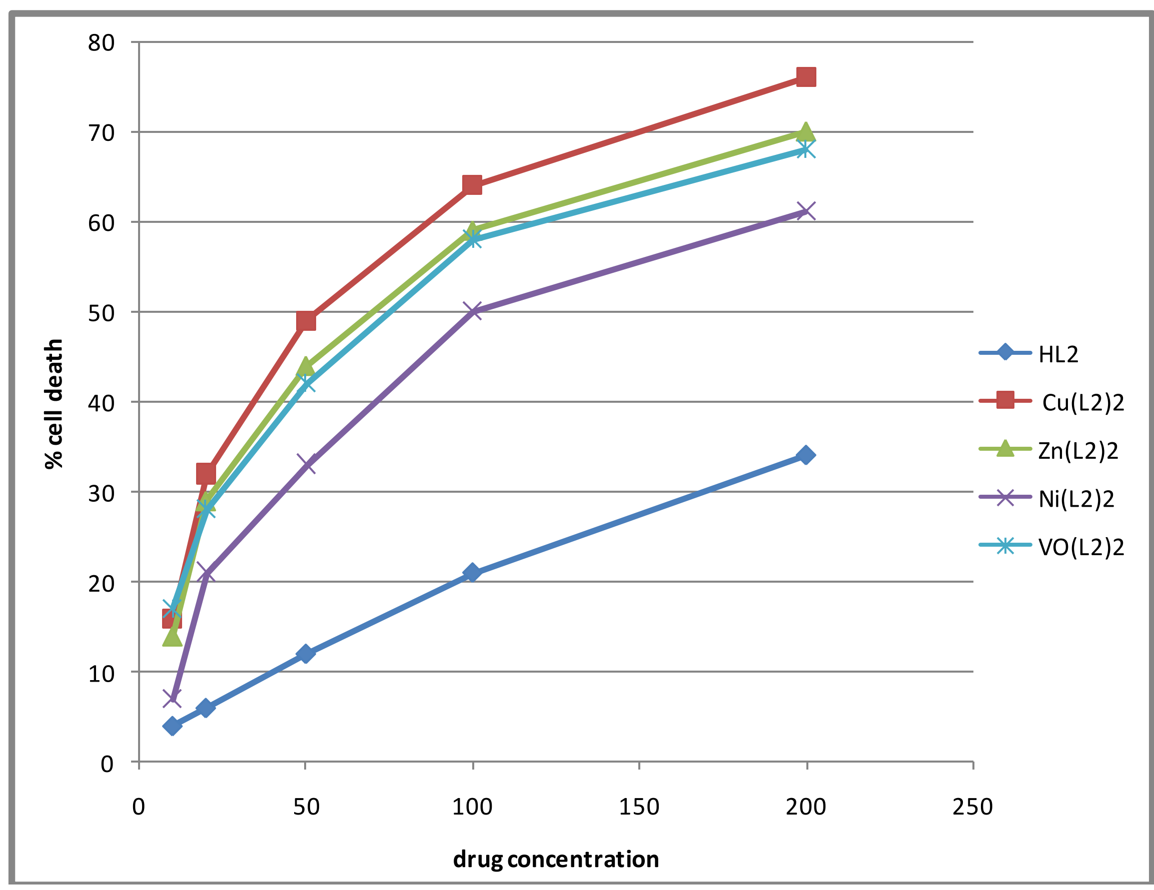


Fig.2.3.4. ***In vitro*** **Cytotoxic studies of of 1,7-bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione (HL_2) and their metal complexes towards DLA**

Moderate results of cytotoxic activity was found with 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione(**2b**) and their metal complexes towards DLA. Here, even though the values were doubled with metal chelation, comparable results are obtained with all the three metals except Cu.

Conclusion

A comparative study of the complexes of the ligands 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (**2a**) and1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione(**2b)** shows that ligand **2a** and its metal complexes gives good results than that of **2b** both towards EAC & DLA. So, out of the two hetero ligands, **2a**, the unsubstituted thiophene ligand and its complexes are more active than methyl substituted ones in the in vitro studies conducted.

IN VIVO ANTITUMOUR STUDIES OF 1,7-DITHIOPHENYL HEPTANOIDS AND THEIR Cu(II) METAL COMPLEXES

The effect of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione(**2a)** and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione(**2b**) and their Cu(II) complexes on the survival rate of ascites tumour bearing animals were studied.Swiss Albino mice (male,6-8 weeks old) weighing 28-30g were divided into 14 groups of five animals each.Viable EAC cells in 0.1ml of PBS were injected into the peritoneal cavity. **Group1**, Control: Oral administration of 0.1 ml of distilled water/animal without drug treatment. **Group 2**, Standard: Cyclophosphamide 25mg/kg body weight.**Group 3-5**: Ligand, 1,7-di(thiophen-2-yl)-1,6-heptadiene-3,5-dione**(2a)** with concentrations 20µg/ml, 10µg/ml and 5µg/ml was given as drug. **Group 6-8** Cu(II) complex of 2a as drug with concentrations 20µg/ml, 10µg/ml & 5µg/ml respectively. **Group 9-11**: Ligand 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione(**2b**) with concentrations 20µg/ml, 10µg/ml and 5µg/ml was given as drug. **Group 12-14**: Cu(II) complex of 2b as drug with concentrations 20µg/ml, 10µg/ml & 5µg/ml respectively.

2.3.6 Effect of 1,7-di(thiophen-2-yl)-1,6-heptadiene-3,5-dione(2a)and the Cu(II) complex on ascites tumour

All the test compounds were injected intraperitoneally and their effect in reducing ascites tumour development in mice were studied.The no. of days survived by the control group,the animals given standard drug,and the animals treated with test compounds and their % increase in life span is found and the results are presented in Table **2.3.5** .

Table 2.3.5 Effect of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) and its Cu(II) complex 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±1.10	
2.Standard drug		5/5	30.6±0.489	76.87
3.L_1	20	5/5	26.8±2.9	54.9
4. L_1	10	5/5	26.4±3.6	52.21
5. L_1	5	5/5	24.4±3.26	41.04
6.$Cu(L_1)_2$	20	5/5	30.2±1.04	74.61
7. $Cu(L_1)_2$	10	5/5	30.1±1.16	73.98
8. $Cu(L_1)_2$	5	5/5	28.8±1.78	66.50

The treatment with test compounds namely 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) and its Cu(II) complex in different concentrations namely 5,10,20µg/ml increased the average life span of tumour bearing animals. The no. of days survived by control group is 17.3±1.1 where as for standard drug cyclophosphamide it is 30.6±0.489.At 5,10,20µg/ml concentrations,the ligand 2a increased the survival rate of animals by 24.4 ± 3.26, 26.4 ± 3.6,26.8 ± 2.9 days respectively.The ligand produced 54% ILS at a concentration of 20µg/ml.But the Cu(II) complex could produce an increase in life span of 74%,73% and 66% at 5,10,20µg/ml concentrations respectively.The Cu(II) complex has significantly increased the life span of ascites tumour bearing animals.The % ILS due to Cu(II) complex is comparable to the results obtained with standard drug.

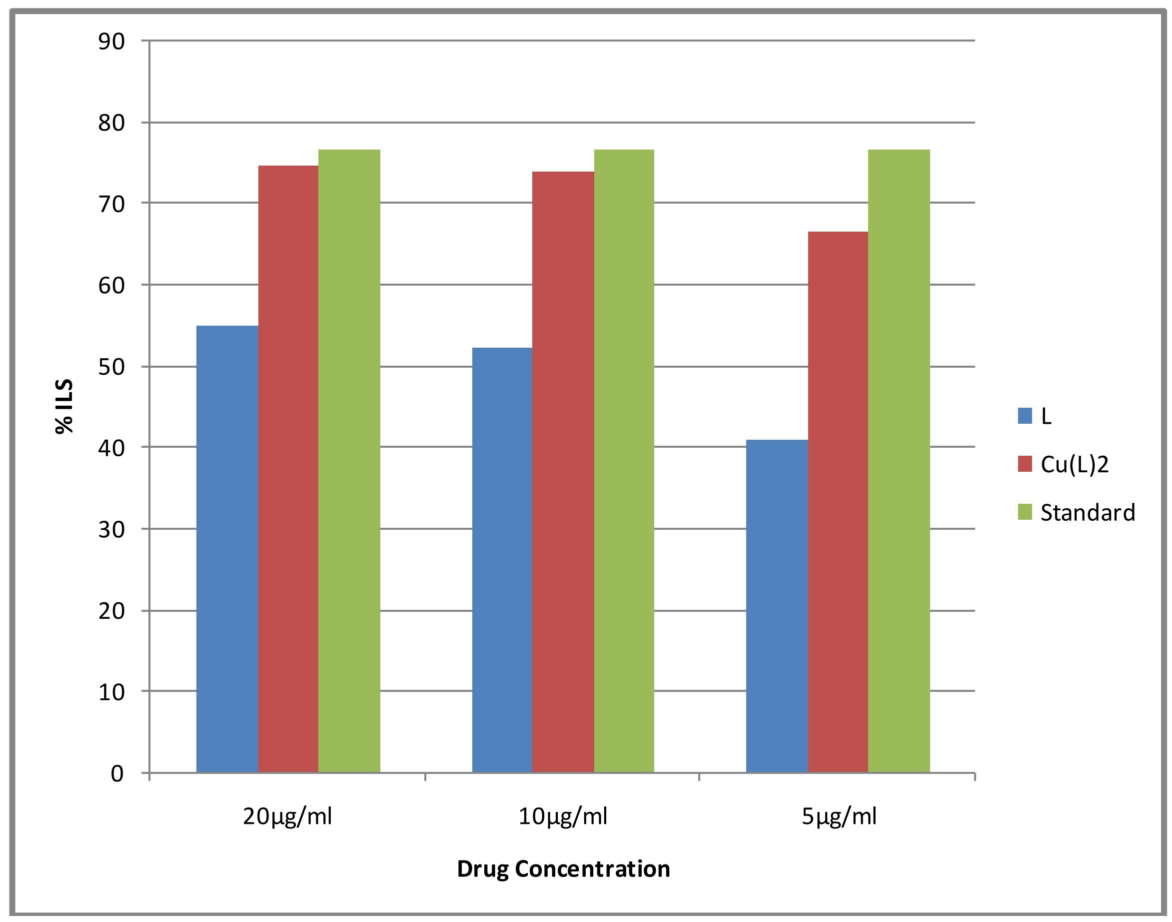


Fig.2.3.5 The % ILS with different conc. of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) and its Cu(II) complex

2.3.7 Effect of 1,7-bis(3-methyl thiophen-2-yl)-1,6-heptadiene-3,5-dione(2b) and the Cu(II) complex on ascites tumour

All the test compounds were injected intraperitoneally and their effect in reducing ascites tumour development in mice were studied.The no. of days survived by the control group,the animals given standard drug,and the animals treated with test compounds and their % increase in life span is found and the results are presented in Table **2.3.6** and in Fig.**2.3.6**

Table 2.3.6 Effect of 1,7- bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione (L2) and its Cu(II) complex 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±1.10	
2.Standard drug		5/5	30.6±0.489	76.87
3.L_2	20	5/5	25.0±3.7	44.17
4. L_2	10	5/5	24.4±3.26	41
5. L_2	5	5/5	21.0±2.09	21.38
6.$Cu(L_2)_2$	20	5/5	29.2±1.04	68.81
7. $Cu(L_2)_2$	10	5/5	28.8±1.16	66.58
8. $Cu(L_2)_2$	5	5/5	27.0±1.78	56.06

The mice with EAC induced ascites tumour survived for a period of 17.3±1.10 days.The administration of standard drug cyclophosphamide increased the life span to 30.6±0.489days.The %ILS produced by the ligand at 5,10,20 µg/ml are 21,41 and 44% respectively where as for Cu(II) complex at the same concentrations the %ILS are 56, 66, 68% respectively.All the compounds exhibited greater activity at higher concentrations.The Cu(II) complex was quite active even at lower concentration.The activity of the complex is comparable with the std.drug.

Comparing the ligands 2a and 2b and their Cu(II) complexes ,the ligand 1,7- bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione (L2)and its complex was not as effective as 2a and its complex in increasing the life span of animals.The Cu(II) complex of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) is found to be the most active compound in the invivo cytotoxic study conducted and is very effective in increasing the life span of EAC induced ascites tumour bearing mice.

INVIVO CYTOTOXIC STUDY ON SOLID TUMOUR DEVELOPMENT

2.3.8 Effect of compounds on solid tumour development

DLA cells were injected subcutaneously on the right hind limb of mice to produce solid tumour.Swiss Albino mice were divided into six groups.Group 1:control(treated with DLA cells), Group 2:cyclophosphamide 10mg/kg b.wt.(reference drug) + DLA cells, Group 3:ligand 2a + DLA cells,Group 4:Cu(II) complex of 2a + DLA cells, Group 5:ligand 2b+ DLA cells, Group 6:Cu(II) complex of 2b+ DLA cells.

The ligands 2a and 2b and their copper complexes were used to find the effect on solid tumour development in mice.At 24 h,after tumour inoculation, the test compounds(200µmol/Kg body weight) were injected for 10 consecutive days.The diameter of the tumour was measured using vernier calipers every third day for 1 month and tumor volume was calculated.

Table 2.3.7 Effect of Compounds on solid tumour

Compounds	Tumour volume on 31st day
Control group	5.042 cm^3
2a (L_1)	3.08 cm^3
2b (L_2)	3.98 cm^3
Cu $(L_1)_2$	2.25 cm^3
Cu $(L_2)_2$	3.05 cm^3
Std.group	1.982 cm^3

All the compounds produced a significant reduction of solid tumour volume in mice.Compared to ligands ,their respective Cu(II) chelates were more effective in bringing about reduction in solid tumour volume.The measured tumour volume was 5.042 cm^3 for the control group on the 31 rst day.The std.drug treated mice showed the reduced tumour volume 1.982 cm^3.The ligand 2a and 2b treated groups significantly decreased the tumour volume to 3.08 cm^3 and 3.98 cm^3 respectively.Comparing with that of the control group,the ligands produced a decrease in volume of 1.962 cm^3 and 1.062 cm^3 respectively. Among the ligands ,2a was more effective than 2b in reducing the tumour volume.The tumour volumes on day 31 for copper complexes of 2a and 2b were 2.25cm^3 and 3.05 cm^3 respectively.The decrease in tumour volume was 2.792 cm^3 and 1.992 cm^3 respectively with respect to control group. The decrease in tumour volume for std.drug was 3.060 cm^3 .The Cu(II) complex of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1)had shown a pronounced effect in reducing tumour volume.

SECTION-IV

ANTIBACTERIAL STUDY OF 1,7-DITHIOPHENYL HEPTANOIDS AND THEIR Zn(II),Ni(II)&VO(IV)METAL COMPLEXES

Antibacterial studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and 1,7-bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione(2b)and their metal chelates Zn(II),Ni(II) &VO(IV)

Antibacterial screening of ligands and metal complexes were carried out by using Agar well diffusion method.Bacterial cultures included in the study are Escherichia Coli, Klebsiella Pneumoniae & Bacillus Subtilis.The test compounds showed varying degree of inhibition against different bacterial strains.All synthesized compounds have shown to be susceptible to excellent potency against the different bacterial strains. The results of the antibacterial activity of synthesized ligand with a heterocyclic ring attached to the unsaturated diketo moiety part and their complexes revealed that the ligands and their complexes possess comparable antibacterial activity to that of standard drug streptomycin.The activity is expressed in terms of diameter of zone of inhibition in mm. In all the cases metal complexes possess better antibacterial activity than that of ligands, which means that metal complexation enhance the activity .

2.4.1 Antibacterial studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and their metal chelates Zn(II),Ni(II) &VO(IV)

The ligand 2a demonstrated comparable antibacterial activity against Bacillus Subtilis and Klebsiella Pneumoniae species by producing a zone of inhibition of 10 mm.The activity of the ligand was only half of the activity exhibited by the std.drug.All the complexes elicited inhibitory activities against all three bacterial strains and were more effective than ligand.The ligand 2a gave a zone of inhibition of 8.5mm against E.coli species where as its VO(IV) complex exhibited maximum inhibitory activity against E.coli species with a zone of

inhibition of 18mm which is comparable with the activity of streptomycin.The std.drug produced a zone of inhibition of 20 mm against all bacterial strains.The Zn(II) and Ni(II) complexes had shown a slight marginal increase in activity compared with the ligand.The Vanadyl complex was quite effective against all the three bacterial strains.The results of antibacterial study of 2a and its metal complexes are given in **Table.2.4.1**

Table 2.4.1 Antibacterial studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) and their metal chelates Zn(II),Ni(II) &VO(IV)

Bacteria	Diameter of zone of inhibition in mm			
	L_1	$VO(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$
E Coli	8.5	18	12	9
Klebsiella	10	14	11	10.5
Bacillus	10	14	10.5	10.5
Standard	20	20	20	20

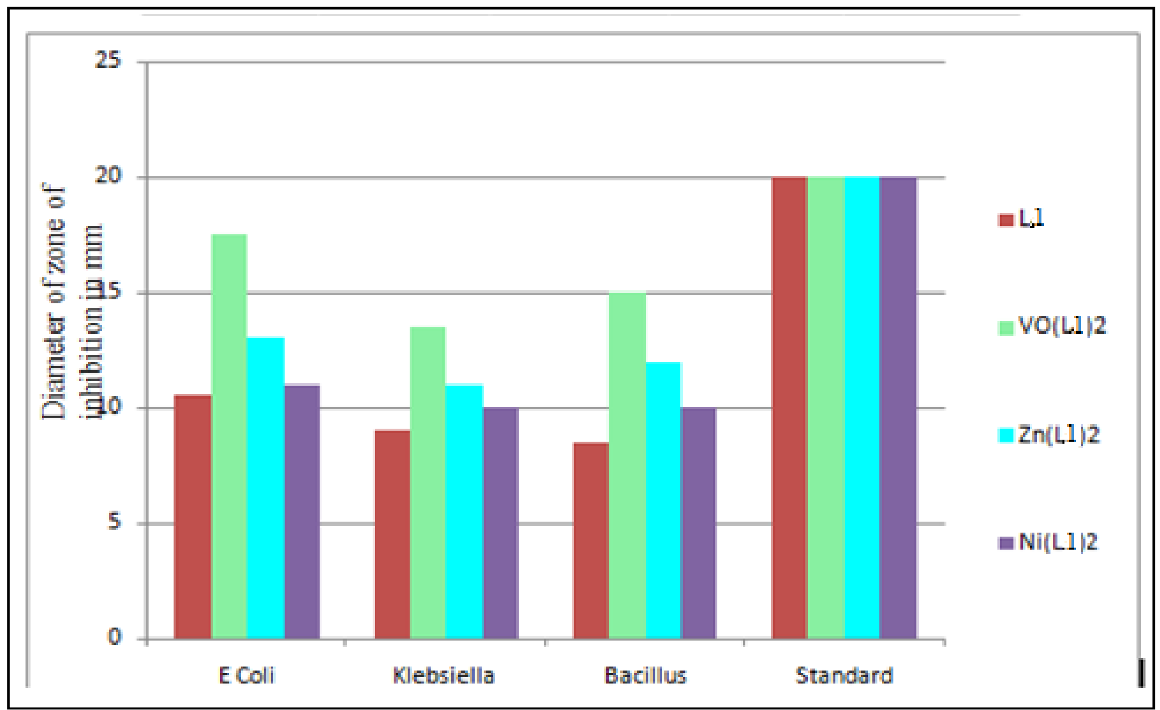


Fig. 2.4.1 Antibacterial studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) and their metal chelates Zn(II),Ni(II) &VO(IV)

2.4.2 Antibacterial studies of 1,7- di(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione (2b) and their metal chelates Zn(II),Ni(II) &VO(IV)

The ligand 2b was more active against E coli bacteria and produced a zone of inhibition with diameter 10.5mm compared with its activity against Bacillus Subtilis(8.5mm) and against Klebsiella Pneumoniae(9mm).The activity of metal complexes followed the order VO(IV)>Zn(II)>Ni(II).The vanadyl complex of ligand 2b had shown enhanced activity and produced a zone of inhibition of 17.5mm which is comparable with the diameter of zone of inhibition produced by the standard drug streptomycin ie 20mm.The vanadyl complex was active against Bacillus Subtilis and Klebsiella Pneumoniae and produced a zone of inhibition of 15mm and 13.5mm respectively.The Zn(II) complex exhibited moderate activity against all bacterial strains.The results of antibacterial study of 2b and its metal complexes are given in **Table 2.4.2** and represented graphically in **Fig. 2.4.2**

Table 2.4.2 Antibacterial studies of 1,7-di(3-methylthiophen-2-yl)hepta-1,6-diene-3,5-dione (L2) and their metal chelates Zn(II),Ni(II) &VO(IV)

Bacteria	Diameter zone of inhibition in mm			
	L_2	$VO(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$
E Coli	10.5	17.5	13	11
Klebsiella	9	13.5	11	10
Bacillus	8.5	15	12	10
Standard	20	20	20	20

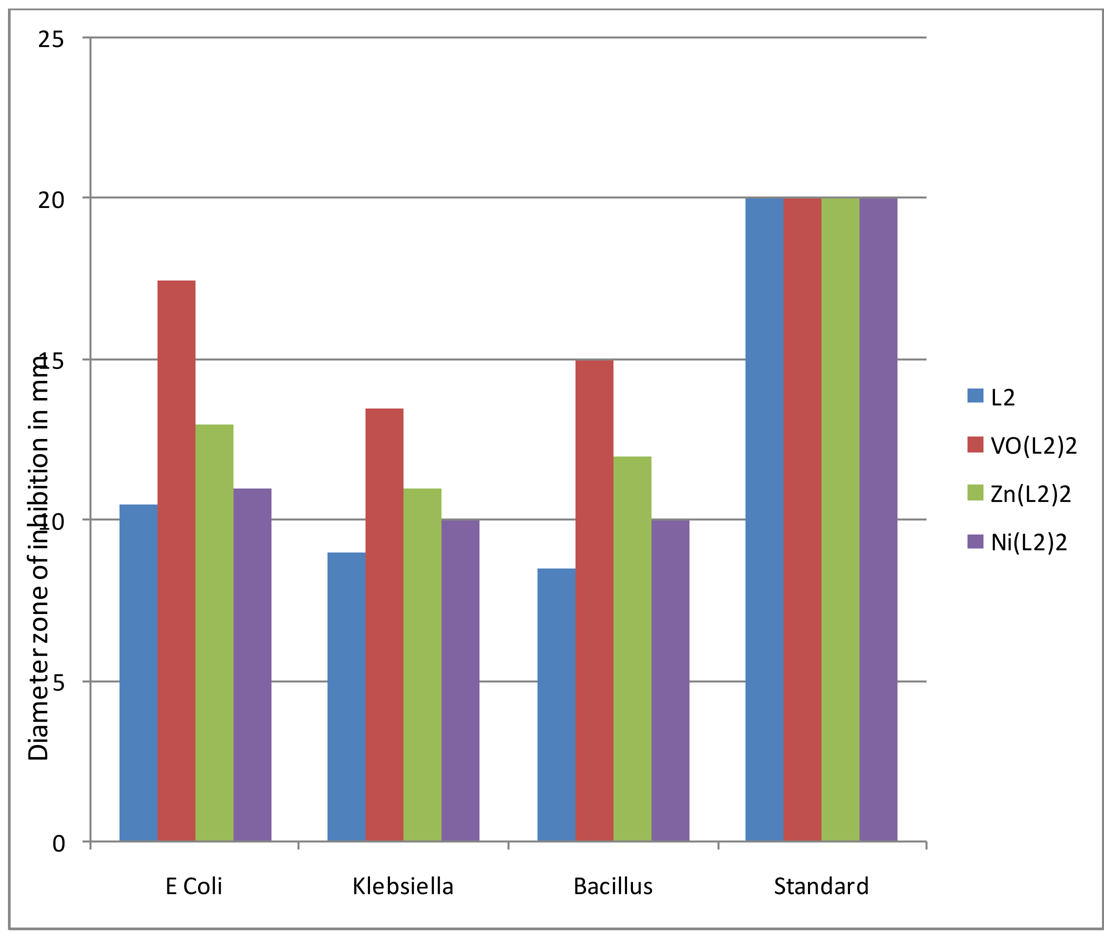


Fig 2.4.2 Antibacterial studies of 1,7-di(3-methylthiophen-2-yl)hepta-1,6-diene-3,5-dione (L2) and their metal chelates Zn(II),Ni(II) &VO(IV)

SECTION-V

ANTIFUNGAL STUDY OF 1,7-DITHIOPHENYL HEPTANOIDS AND THEIR Zn(II) &VO(IV)METAL COMPLEXES

Antifungal Activity of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and1,7-di(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione(2b) and their Zn(II) and VO(IV) complexes

The curcuminoid analogues with thiophenyl ring and their metal complexes namely Zn(II) &VO(IV)were studied for their antifungal activity against three fungal cultures namely Aspergillus Niger, Penicillium Chrysogenum and Alternaria Alternate.Kirby Baurer disc plate method was used to test the susceptibility of the fungi species to the test compounds. Different concentrations [100, 250, 500μg/ml] by dissolving in 2% DMSO solvent were used for all the test compounds and results were compared with the std.drug flucanozole.The antifungal activities are measured in terms of zone of inhibition in mm.The data of the study revealed that the synthesized 1,7-dithiophenyl heptanoids and their Zn(II) &VO(IV) complexes possess comparable antifungal activities to that of std.drug.

2.5.1 Antifungal Activity of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and their Zn(II) and VO(IV) complexes

The inhibition zone of the test compounds with the three fungi species in comparison to flucanazole(std.drug) is shown in Table **2.5.1**

Table 2.5.1. Antifungal studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L_1 and its Zn(II) & Vanadyl complexes

Fungi	Diameter of zone of inhibition in mm								
	L_1			$Zn(L_1)_2$			$VO(L_1)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	8	11	13.5	9.5	13	15	10.5	13.5	18
Penicillium	9.5	11.5	15	11	13.5	16	12	14	17.5
Alternaria	9	12.5	16	10	14	17	11	14.5	18.5

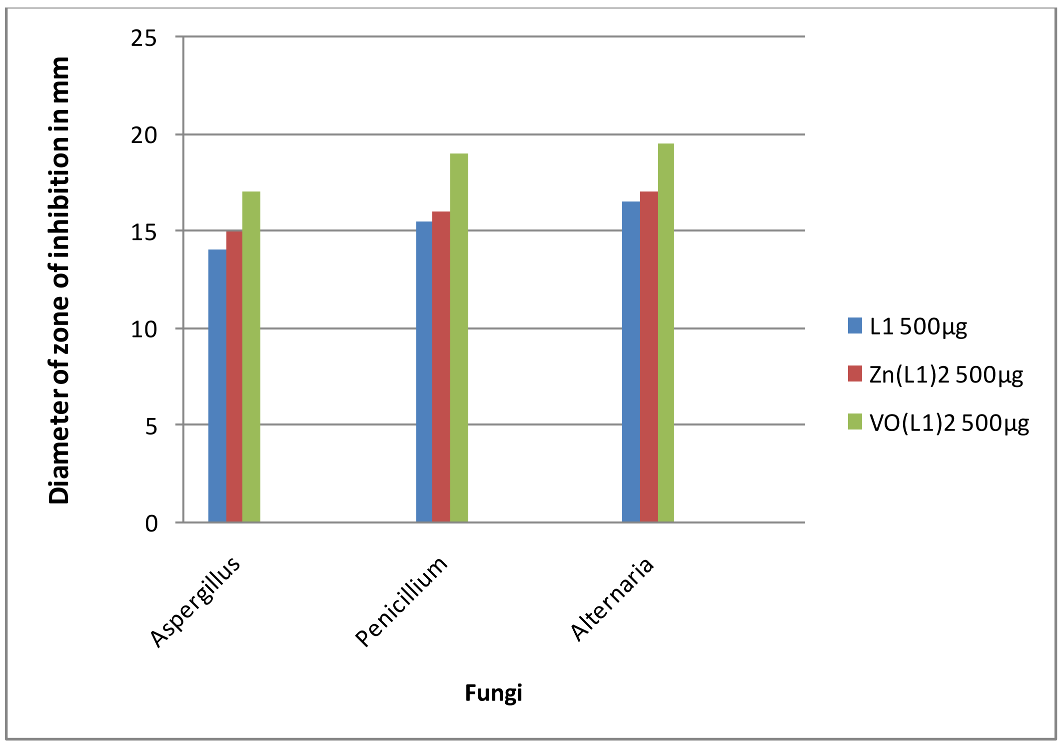


Fig. 2.5.1. Antifungal studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L_1 and its Zn(II) & Vanadyl complexes

The ligand 2a exhibited moderate antifungal activity against all organisms at a concentration of 100µg/disc.The compound was found to be very active against all species in 500µg/disc concentration.The ligand showed maximum antifungal activity with Alternaria with a zone of

inhibition of 16mm.The Zn(II) and VO(IV) complexes had shown significant activity as expected.It is observed that vanadyl complex of the ligand exhibited the most effective antifungal activity against all the three fungal cultures.The zone of inhibition produced by the VO(IV)complex is 18mm,17.5mm and 18.5mm against Aspergillus, Penicillium and Alternaria respectively.This is comparable with the zone of inhibition (21mm) produced by the std.drug.

2.5.2 Antifungal Activity of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione(2b) and their Zn(II) and VO(IV) complexes

The inhibitory effect of ligand and its metal complexes against the fungal cultures is represented in **Table 2.5.2** .For all the tested compounds they show maximum antifungal activity at a higher concentration of 500µg/ml.It is observed that antifungal nature increases with the concentration of the compounds.The ligand 2b exhibited a zone of inhibition of 15.5mm against Penicillium where as zone of inhibition produced is 16.5 and 14 mm against Alternaria and Aspergillus respectively at higher conc.The ligand 2b exhibited more antifungal activity against Alternaria . The Zn(II) complexes gave inhibitory activity against fungal cultures which was only slightly greater than the ligands.The VO(IV) complexes were quite effective against all fungi at all concentrations.The vanadyl complex of ligand 2b demonstrated promising antifungal activity producing a zone of inhibition of 19.5mm with Alternaria species.It has been found to be a potent antifungal compound.

Comparing the ligands, 2b had shown a slight increase in antifungal activity towards all fungi species than 2a.The ligand 2b has a methyl group on the thiophenyl ring compared with 2a. Comparing Zn(II) and VO(IV) complexes of both ligands, it was observed that vanadyl complexes exhibited more antifungal activity.The VO(IV) complexes of both ligands especially of 2b had appreciable antifungal activity against all fungal cultures.

Table 2.5.2 Antifungal studies of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (L_2) and its Zn(II) & Vanadyl complexes

Fungi	Diameter of zone of inhibition in mm								
	L_2			$Zn(L_2)_2$			$VO(L_2)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	8.5	11.5	14	9	11.5	15	11	13	17
Penicillium	10	12	15.5	11	14	16	14	17	19
Alternaria	11	13	16.5	12	14	17	15	17.5	19.5

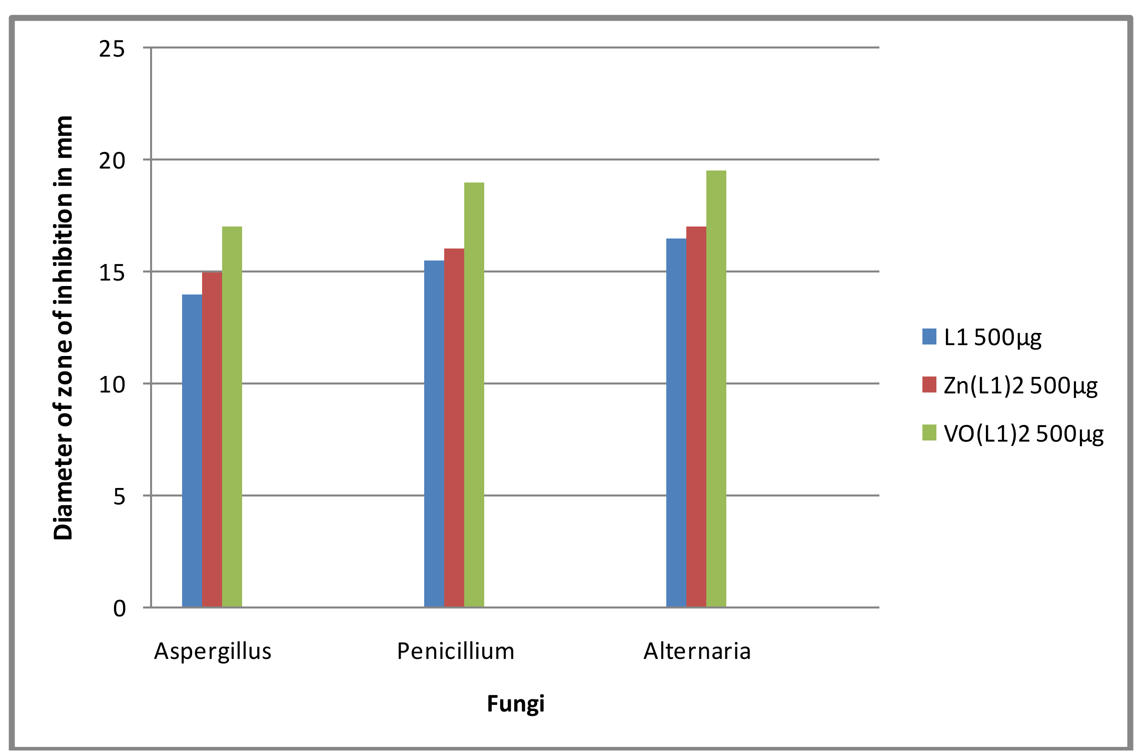


Fig. 2.5.2 Antifungal studies of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (L_2) and its Zn(II) & Vanadyl complexes

CHAPTER-III

SYNTHESIS, CHARACTERISATION AND BIOCHEMICAL ACTIVITIES OF CHLORO SUBSTITUTED 1,7-DIARYLHEPTA-1,6-DIENE-3,5-DIONES AND THEIR TRANSITION METAL CHELATES WITH Cu(II), Zn(II), Ni(II) & OXOVANADIUM(IV)

SECTION-I

SYNTHESIS AND CHARACTERIZATION OF 1,7-BIS (CHLORO-SUBSTITUTED ARYL)-HEPTA-1,6-DIENE-3,5-DIONES

This chapter deals with the synthesis and characterization of three curcuminoid analogues with chloro substituted phenyl ring. The compounds synthesized are 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(**3a**), 1,7-bis(2-chloro phenyl)hepta-1,6-diene-3, 5-dione(**3b**) and 1,7-bis(3,4-dichloro phenyl) hepta-1,6-diene-3,5-dione(**3c**). Section 1 deals with the synthesis and characterization of the ligands. Section 2 deals with the synthesis and characterization of the transition metal chelates of the ligands with Cu(II),Zn(II),Ni(II) and VO(IV). Section 3 in this chapter includes the cytotoxic study both invivo and invitro of the curcuminoid analogues and their transition metal complexes.Section 4 of this chapter deals with the Antibacterial activity of the ligands and metal complexes and Section 5 is about their Antifungal activity.

3.1.1. Synthesis of chloro analogues of curcuminoid derivatives

The chloro substituted 1,7- diaryl heptanoids were prepared by the condensation of chloro substituted benzaldehydes (4-chloro benzaldehyde, 2-chloro benzaldehyde and 3,4-dichloro benzaldehyde) with acetylacetone(1,3-diketone)in presence of boric oxide in ethyl acetate medium in presence of tri(sec-butyl) borate and n-butyl amine. The condensation reaction actually leads to mono condensation and bis condensation products.They should be separated and purified. The product formation can be represented in a schematic way (**Scheme 3.1.1**).

$3a – R_1, R_2 = H, R_3 = Cl,$

$3b – R_1 = Cl, R_2, R_3 = H$

$3c – R_1 = H, R_2, R_3 = Cl$

B_2O_3/ Tri(sec-butyl)borate

n-$BuNH_2$

Scheme 3.1.1

The products 1,7-bis(4-chlorophenyl)hepta-1,6-diene-3,5-dione(**3a**),1,7-bis(2-chloro phenyl)hepta-1,6-diene-3,5-dione(**3b**) and 1,7-bis(3,4-dichlorophenyl)hepta-1,6-diene-3,5-dione(**3c**) were purified by column chromatography over silica gel (60 – 120 mesh) using 4:1 (v/v) chloroform:acetone mixture as the eluent and recrystallised twice from hot benzene to get pure crystalline material. The aldehydes used for synthesis, structures of the compounds prepared, its systematic name, and yield are given in Table 3.1.1.

Table 3.1.1 Synthetic details of Chlorosubstituted 1,7-diarylheptanoids

Compounds	Aldehyde used for Synthesis	Structure of Ligands	Systematic name	Yield%
3a	4-chloro benzaldehye		1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione	72
3b	2-chloro benzaldehyde		1,7-bis(2-chloro phenyl)hepta-1,6-diene-3,5-dione	59
3c	3,4-dichloro benzaldehyde		1,7-bis(3,4-dichlorophenyl) hepta-1,6-diene-3,5-dione	65

All the chloro compounds are crystalline in nature, dark red in colour, show sharp melting points and are soluble in organic solvents.The elemental analysis results, MeltingPoint, molecular weight determination and UV spectral data are given in **Table 3.1.2.**

Table 3.1.2 Analytical & UV spectral data of chloro analogues of 1,7–diaryl heptanoids

Compounds	M.P.(^{o}C)	Elemental analysis (%)		Molecular weight	UV λmax (nm)
		C	H		
		Found/(Calculated)			
3a	97	65.31(66.07)	3.72(4.05)	342(345)	225, 307
3b	91	65.12(66.08)	3.61(4.05)	344(345)	230, 308
3c	118	54.32(55.07)	2.39(2.88)	412(414)	246, 332

The observed C, H percentage and molecular weight determination (Table 3.1.2) together with mass spectral data of the compounds clearly suggest that two mole aldehyde has condensed with 1 mole of the diketone as shown in **Scheme 3.1.1.**

3.1.2. Characterisation of chloro substituted 1,7–diphenyl heptanoids

The compounds 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(**3a**), 1,7-bis(2-chloro phenyl)hepta-1,6-diene-3,5-dione(**3b**), and 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione**(3c)** synthesized were characterized by UV, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral techniques. The spectral data of synthesized ligands are discussed below.

UV spectra

The UV spectra of all the synthesized ligands are characterized by the presence of two absorption maxima (Table 3.1.2) in the range 225-250nm and 300-340nm .These peaks can be assigned due to $\pi \rightarrow \pi *$ and n$\rightarrow \pi *$ transitions which are present in diketones. The value at 225-250nm are due to $\pi \rightarrow \pi *$ transition and at 300-340nm are due to n$\rightarrow \pi *$ transitions.A band of low intensity usually indicates a n$\rightarrow \pi *$ transition which is a forbidden transition and the more intense band usually indicates a $\pi \rightarrow \pi *$ transition which is an allowed transition.The UV spectra of compound 3a is reproduced below in Fig 3.1.1.

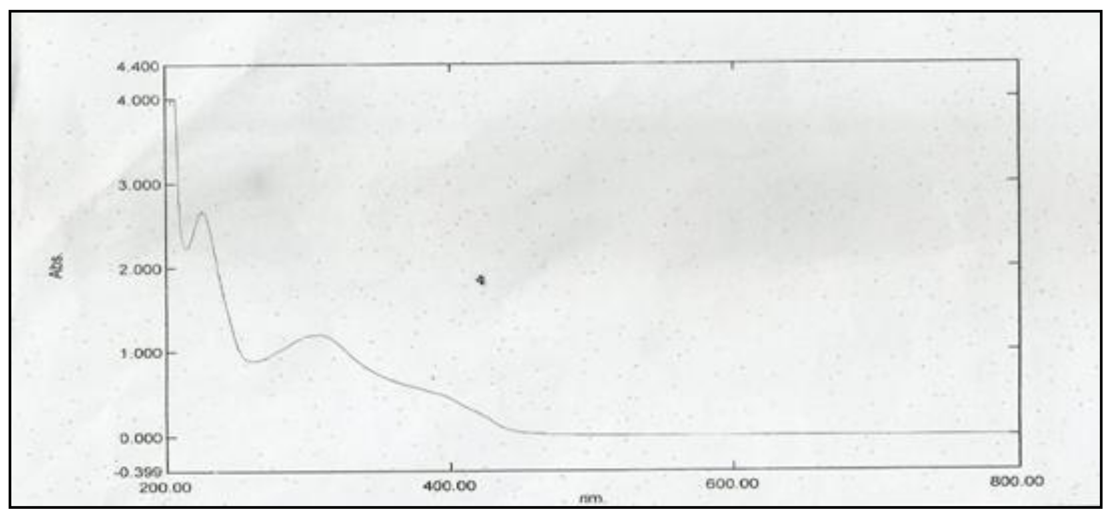


Fig 3.1.1. UV spectra of compound 3a

IR spectra

The IR spectra of the compounds show prominent bands at 1639 cm^{-1} ,1620 cm^{-1} and1628 cm^{-1} assignable respectively to the chelated ν C=O vibrations of ligands 3a,3b and3c.The position and intensity of the carbonyl stretching band depends on the molecular structure in its immediate vicinity and therefore very useful in characterizing the type of carbonyl function present in the compound.Compared with the frequency of the free carbonyl group here the decrease in frequency of vibration is due to extended conjugation and the presence of enolised conjugated 1,3-diketo group. Thus the observed position and intensity of these bands indicate that the compound exists in strong intramolecular hydrogen bonded form.The occurrence of an intense band in the region 3500-2500 cm^{-1} also supports the presence of hydrogen bonding.

The occurrence of bands in the region 1565 cm^{-1} -1595 cm^{-1} may be assigned due to ν C=C phenyl vibrations of the compounds. ν C-C alkenyl vibrations are observed in the range 1495-1515 cm^{-1} for the ligands.The bands in the range 1450-1000 cm^{-1} are due to C-C-C chelate ring and C-H chelate ring vibrations.A medium intensity band at nearly 970 cm^{-1} is characteristic of trans CH=CH vibrations.The important IR absorptions and their probable assignments are given in Table **3.1.3.**The IR spectra of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(**3a**) and 1,7-bis(2-chloro phenyl)hepta-1,6-diene-3,5-dione(**3b**) has been depicted in **Fig.3.1.2** and **Fig 3.1.3** respectively.

Table 3.1.3 IR spectral data of chloro substituted curcuminoid derivatives

Compounds			Probable IR assignments
3a	3b	3c	
1639	1620	1628	ν(C=O) chelated
1593	1568	1572	ν(C=C) phenyl
1513	1509	1498	ν(C-C) alkenyl
1489	1459	1461	ν_{as}(C-C-C) chelate ring
1412	1436	1411	ν_{s}(C-C-C) chelate ring
1091,1014	1128,1043	1101,1052	β(C-H) chelate ring
983	974	963	ν(CH=CH) trans)

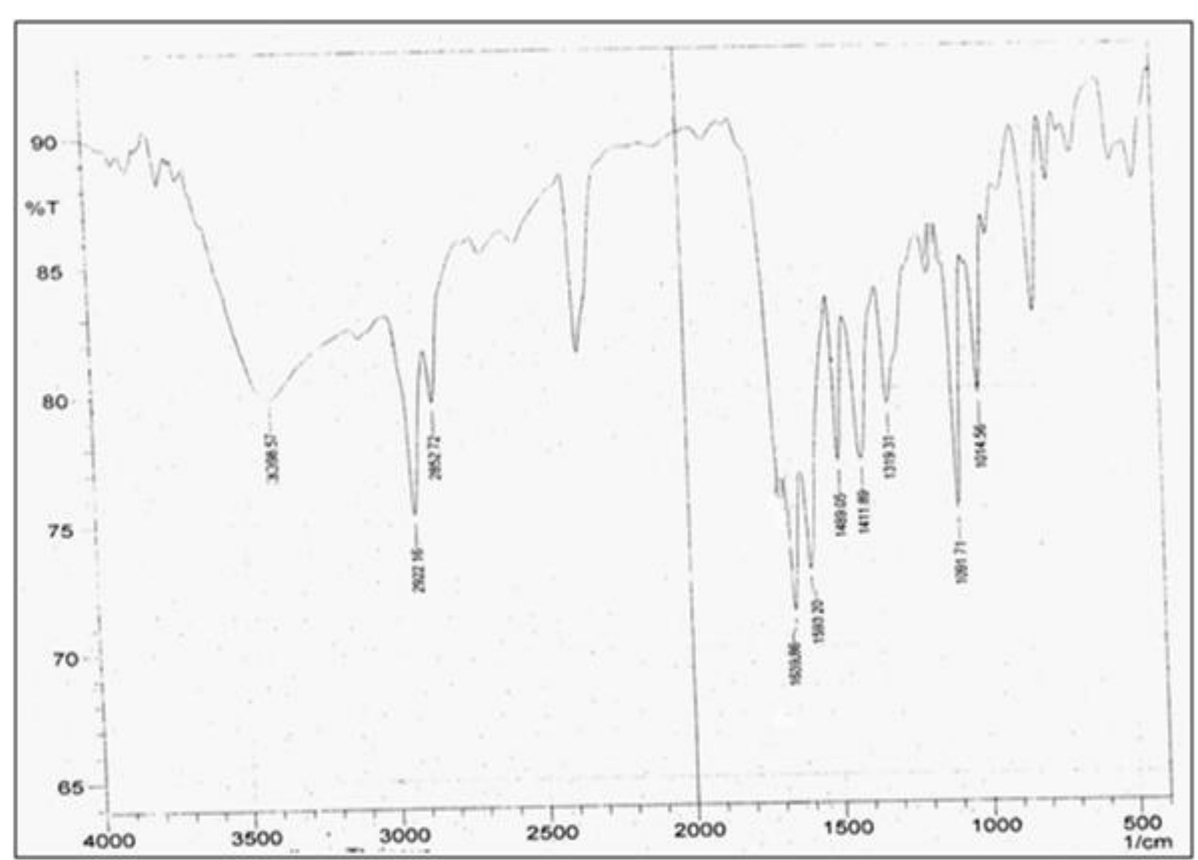


Fig.3.1.2 IR spectrum of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(3a)

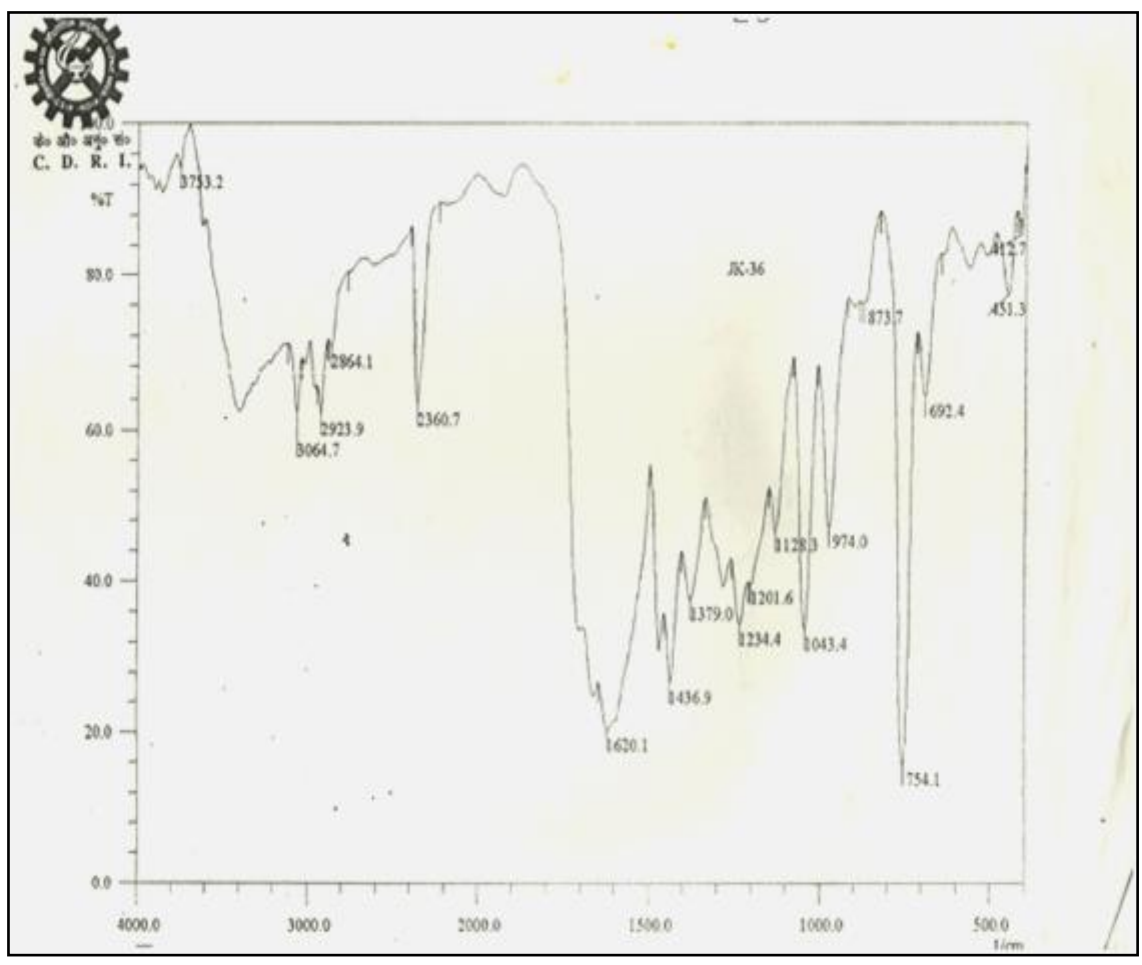


Fig.3.1.3 IR spectrum of 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione(3b)

^{1}H NMR spectra

The ^{1}HNMR spectra distinguishes the different types of protons present in the molecule.Each type of proton has a characteristic value of chemical shift.The protons of chloro analogues of 1,7-diphenyl heptanoids which can be identified from ^{1}H NMR spectra include enolic, methine, alkenyl and phenyl protons (Table 3.1.4) .All the compounds **3a, 3b** & **3c** displayed a one proton singlet downfield at ~ 16ppm assignable to strong intramolecularly hydrogen bonded enolic proton .This confirms the enolic structure of the molecule.All compounds also gave another one proton singlet at ~ 5.9ppm corresponding to the methine proton. Phenyl hydrogens appear in a range downfield from 7.1-7.9ppm.The hydrogens in an aromatic ring are deshielded by the large anisotropic field generated by electrons in the π ring system.The

alkenyl protons are observed in the range 6.4-8.2 ppm.The protons directly attached to the double bonded carbon are also deshielded. The ^{1}HNMR spectra of ligands 3a and 3b are brought out in **Fig 3.1.4** and **Fig 3.1.5** respectively. The^{1}HNMR spectral data of the compounds are given in **Table 3.1.4.**

Table 3.1.4 ^{1}H NMR spectral data of chloro analogues of curcuminoids

Compounds	Chemical shifts (δ ppm)			
	Enolic	**Methine**	**Alkenyl**	**Phenyl**
3a	16.024	5.82	6.57-8.04	7.3-7.63
3b	16.025	5.92	6.43-8.088	7.164-7.789
3c	16.02	5.9	6.4-8.1	7.35-7.75

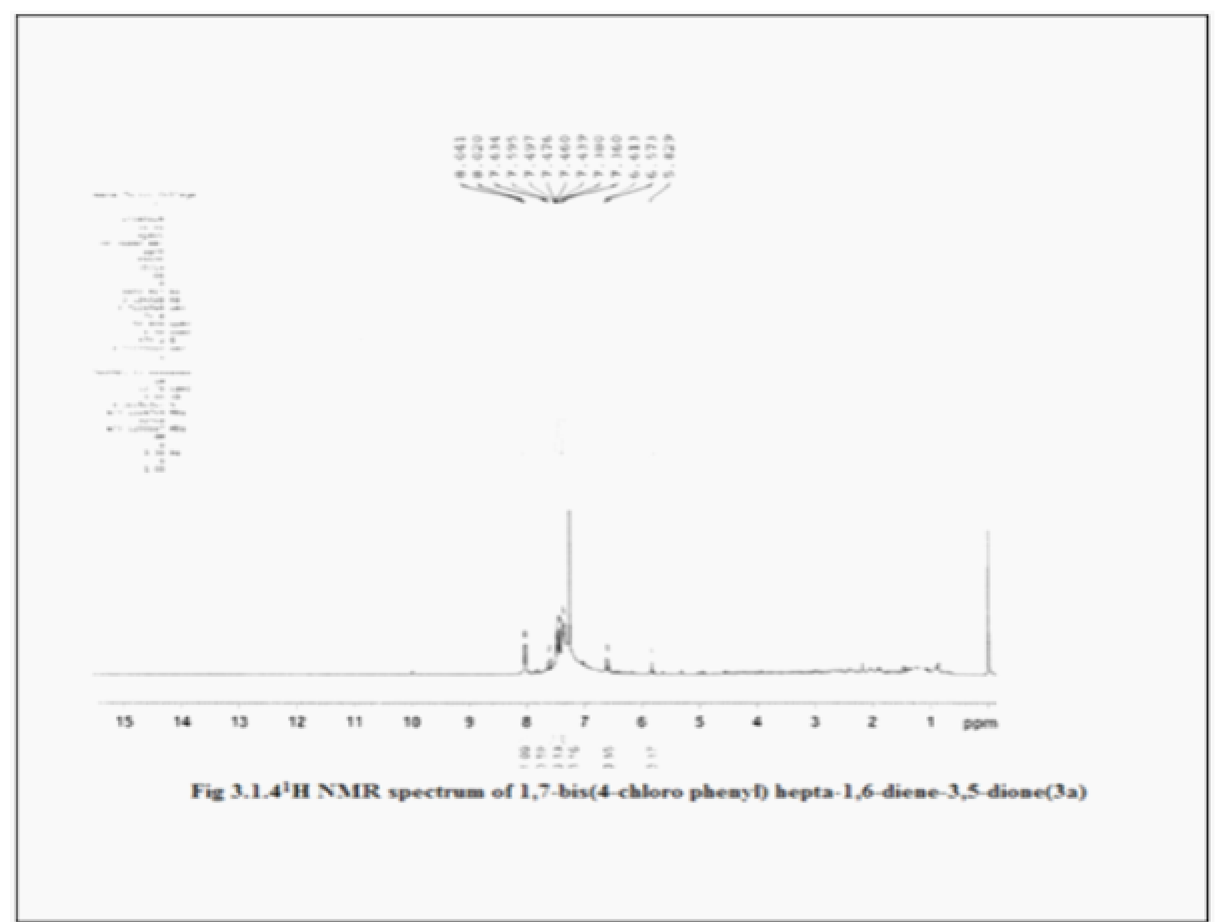


Fig 3.1.4 ^{1}H NMR spectrum of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(3a)

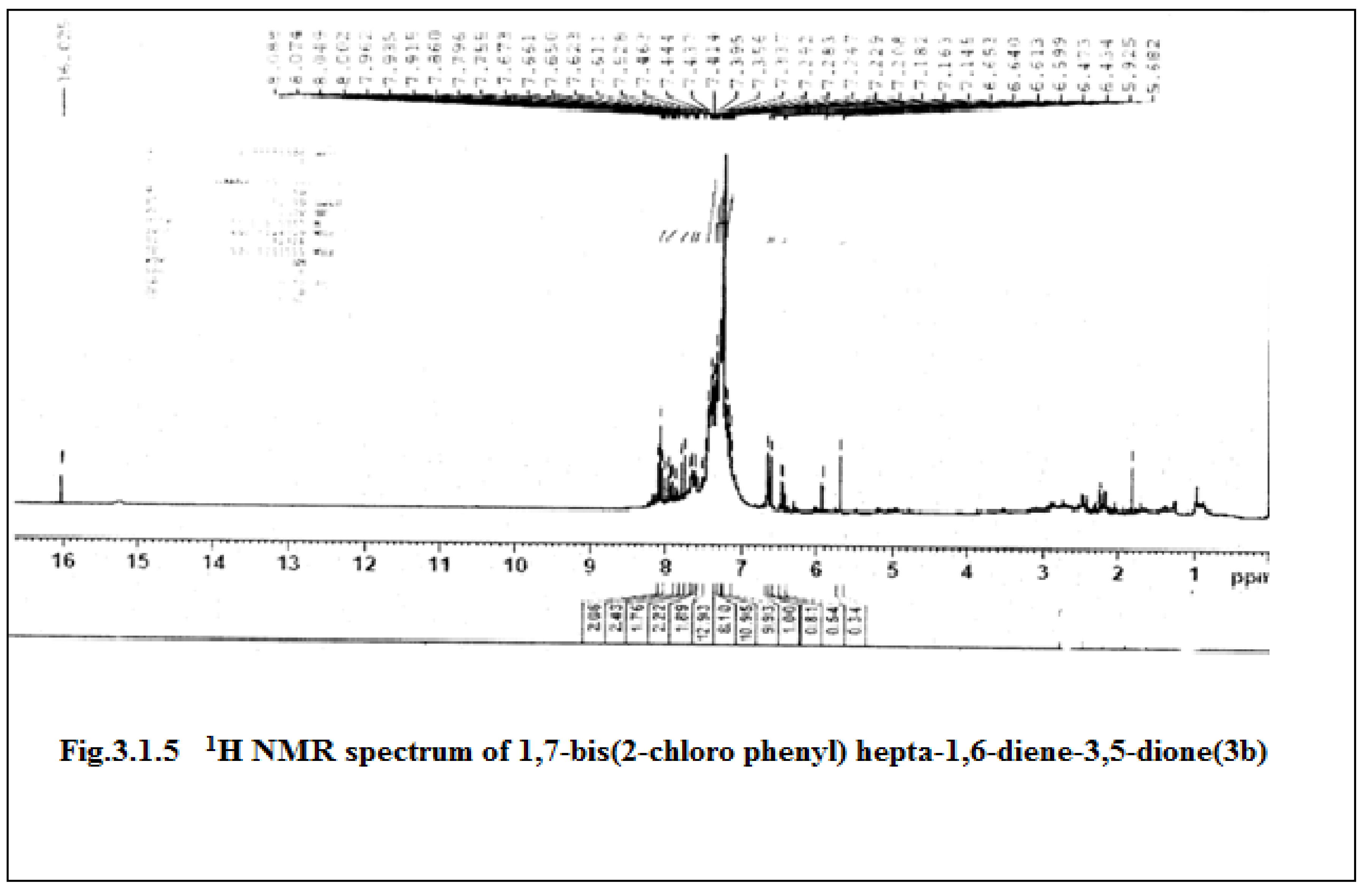


Fig.3.1.5 ^{1}H NMR spectrum of 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione(3b)

^{13}CNMR spectra

The ^{13}CNMR spectra helps to identify the non equivalent carbon atoms in a molecule..Each chemically and magnetically distinct carbon give rise to a single peak. The ^{13}CNMR spectral details of chloro analogues of 1,7- diaryl heptanoids representing ^{13}C chemical shifts are given in **Table 3.1.5, 3.1.6 & 3.1.7.**

Fig.3.1.6 Chemically distinct Carbons in 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione

Table 3.1.5 ^{13}CNMR spectral data of 3a

C1	**C2,C2'**	**C3,C3'**	**C4,C4'**	**C5,C5'**
103.4	191.09	139.6	132.4	134.1
C6,C6'	**C7,C7'**	**C8,C8'**	**C9,C9'**	**C10,C10'**
129.4	130.3	139.6	131.0	129.6

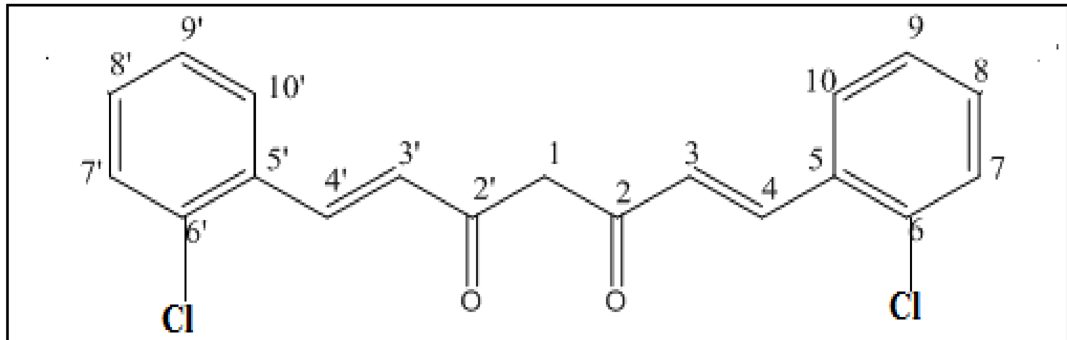


Fig. 3.1.7 Chemically distinct Carbons in 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione

Table 3.1.6 ^{13}CNMR spectral data of 3b

C1	**C2,C2’**	**C3,C3’**	**C4,C4’**	**C5,C5’**
105.6	193.4	137.5	131.4	135.4
C6,C6’	**C7,C7’**	**C8,C8’**	**C9,C9’**	**C10,C10’**
137.3	130.9	129.6	128.9	130

Fig.3.1.8 Chemically distinct Carbons in 1,7-bis(3,4-dichloro phenyl) hepta-1,6-diene-3,5-dione

Table 3.1.7 ^{13}C NMR spectral details of 3c

C1	**C2,C2'**	**C3,C3'**	**C4,C4'**	**C5,C5'**
109.5	193.6	138.6	133.1	137.5
C6,C6'	**C7,C7'**	**C8,C8'**	**C9,C9'**	**C10,C10'**
135.7	139.6	138.4	129.6	128.3

The chemical shift due to C1 (methine) is present at a position ~ at 107ppm. Here also there is a possibility of keto-enol tautomerism which makes the shift of C1 carbon to ~ at 107ppm. C2 carbon of carbonyl appears at a position at~ 190ppm and appears at the lowest field values. The C atoms to which the electronegative atom Chlorine is attached (C8 in 3a,C6 in 3b and C7,C8 in 3c) shows a large downfield shift because electronegativity produces deshielding effect. The alkenyl carbon C3 is present at a position at ~ 140ppm where as C4 is present at a position at ~ 135ppm. The carbon atoms in the phenyl ring are present between 130 – 143ppm. The carbon which is attached to the alkenyl carbon atom is down shielded.

Mass spectra

The mass spectral details of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(3a), 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione(3b), 1,7-bis(3,4-dichloro phenyl) hepta-1,6-diene-3,5-dione(3c) are given below in **Table 3.1.8.**

Table 3.1.8 Mass spectral fragments of 3a, 3b & 3c

Fragments*	**Ligands**	**M+/ M+1 ion**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Mass Pattern	3a	342	175	169	125	140	115	141	230	204
	3b	345	178	165	125	138	115	141	231	207
	3c	413.6	216	202	165	178	152	179	263	238

*The alphabets corresponds to the fragments given in **Scheme 3.1.2**

Mass spectrum of 3a shows distinct molecular ion, M+ ion peak at m/z=342. The base peak in the spectrum is observed at m/z=141 and is due to [Ar-CH=CH_2]+ where(Ar=4-chlorophenyl).The next intense peak is observed at m/z=169 and is due to [Ar-CH=CH-C=O]+.Elimination of important groups like C_2H_2,CH_2=C=O,C=O,C_2H_2O,CH_2-CH=C=O from the molecule gives different fragments and the values are depicted in Table 3.1.8.All other peaks are due to fragment ions which can be observed from the fragmentation pattern given in **Scheme 3.1.2.** The mass spectrum of **3a** is given in Fig.3.1.9.

The mass spectrum of **3b** is given in Fig.3.1.10.The mass spectrum shows an intense molecular ion peak at m/z=345.The other prominent peaks in the spectrum are due to fragment ions.Smaller fragments like O,OH,Cl, CH_2 etc. are removed from the molecular ion.The peaks at m/z=138 and 165 are very prominent in the spectra and are due to [Ar-CH=CH_2]+ and [Ar-CH=CH-C=O]+respectively where Ar=2-chlorophenyl.

The mass spectrum of **3c** is reproduced in Fig.3.1.11.The molecular ion peak of **3c** is observed at m/z= 413.6.The base peak in the spectrum is observed at m/z=178 and is assigned to the fragment ion [Ar-CH=CH_2]+,where Ar=3,4-dichlorophenyl. Important peaks appeared in the spectra of the compounds can be conveniently accounted by the fragmentation pattern given in **Scheme 3.1.2.**

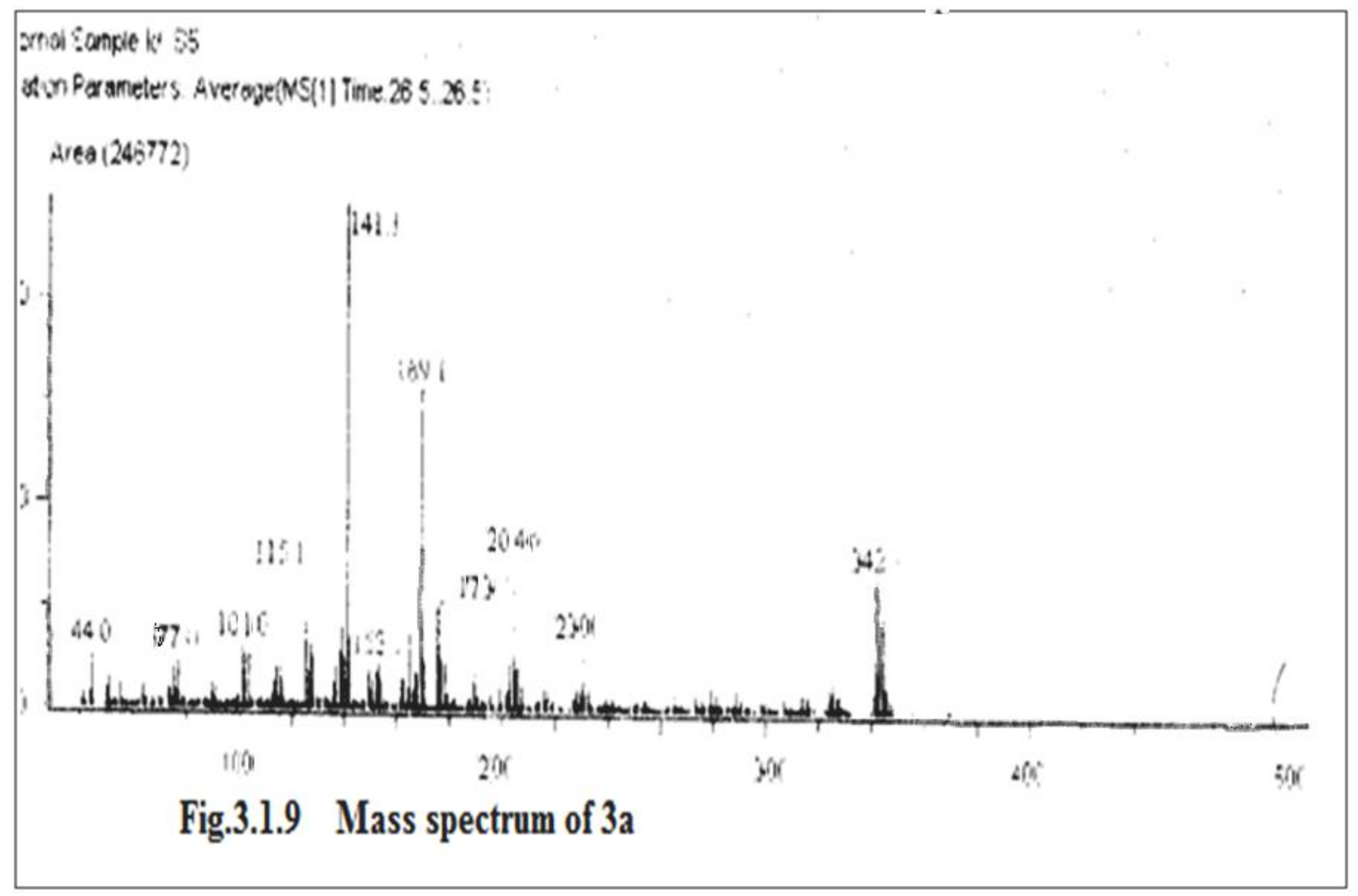


Fig.3.1.9 Mass spectrum of 3a

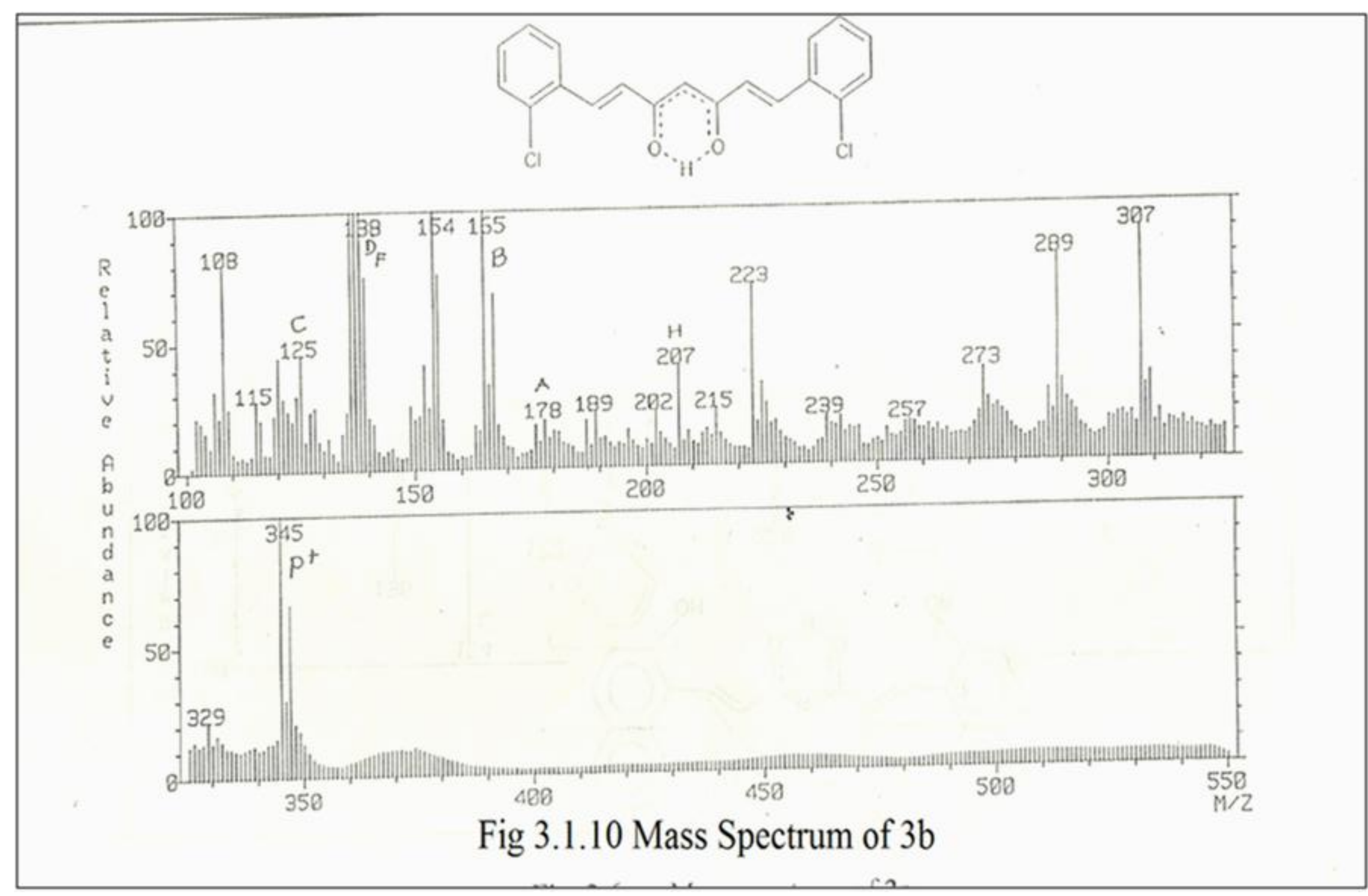


Fig 3.1.10 Mass Spectrum of 3b

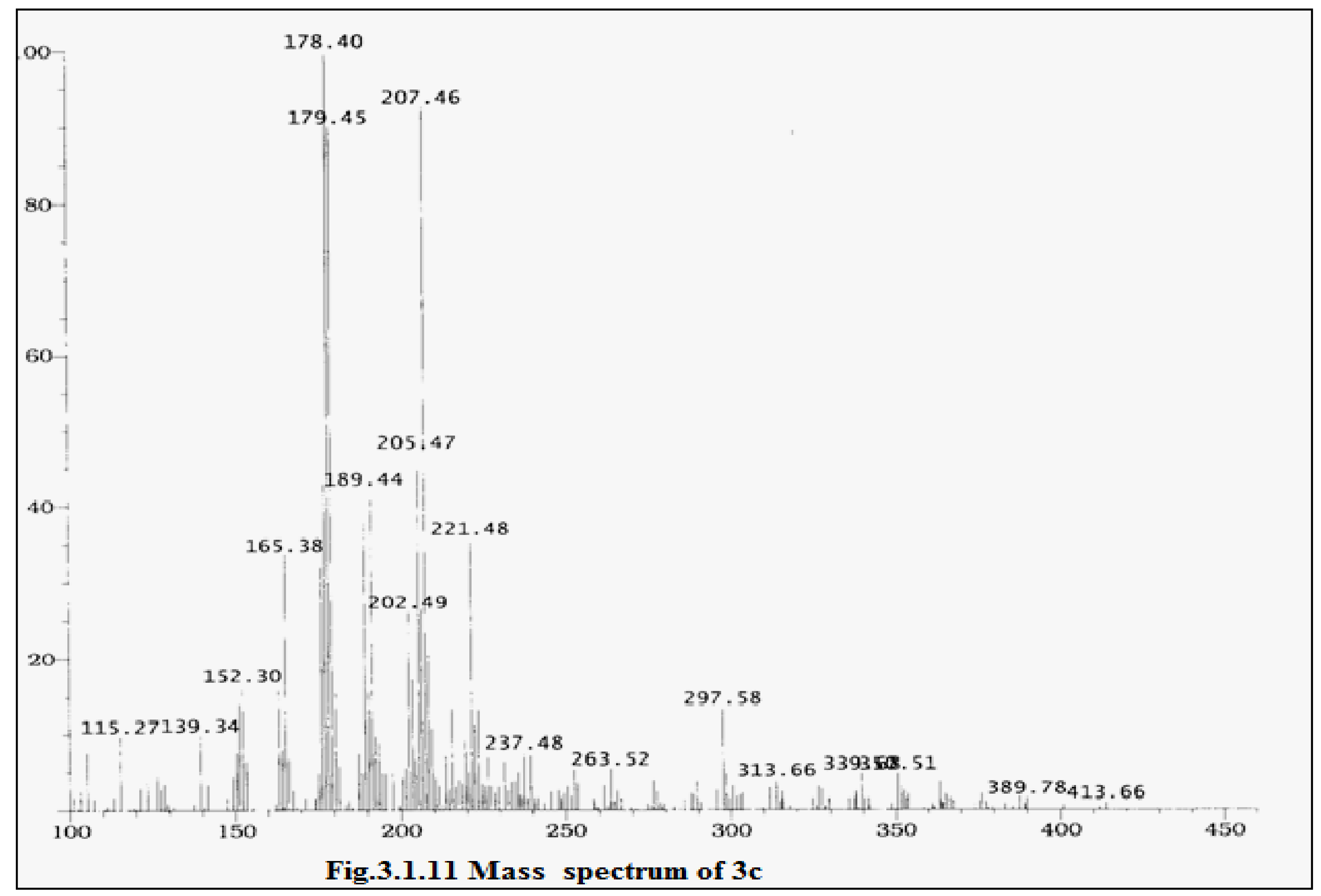
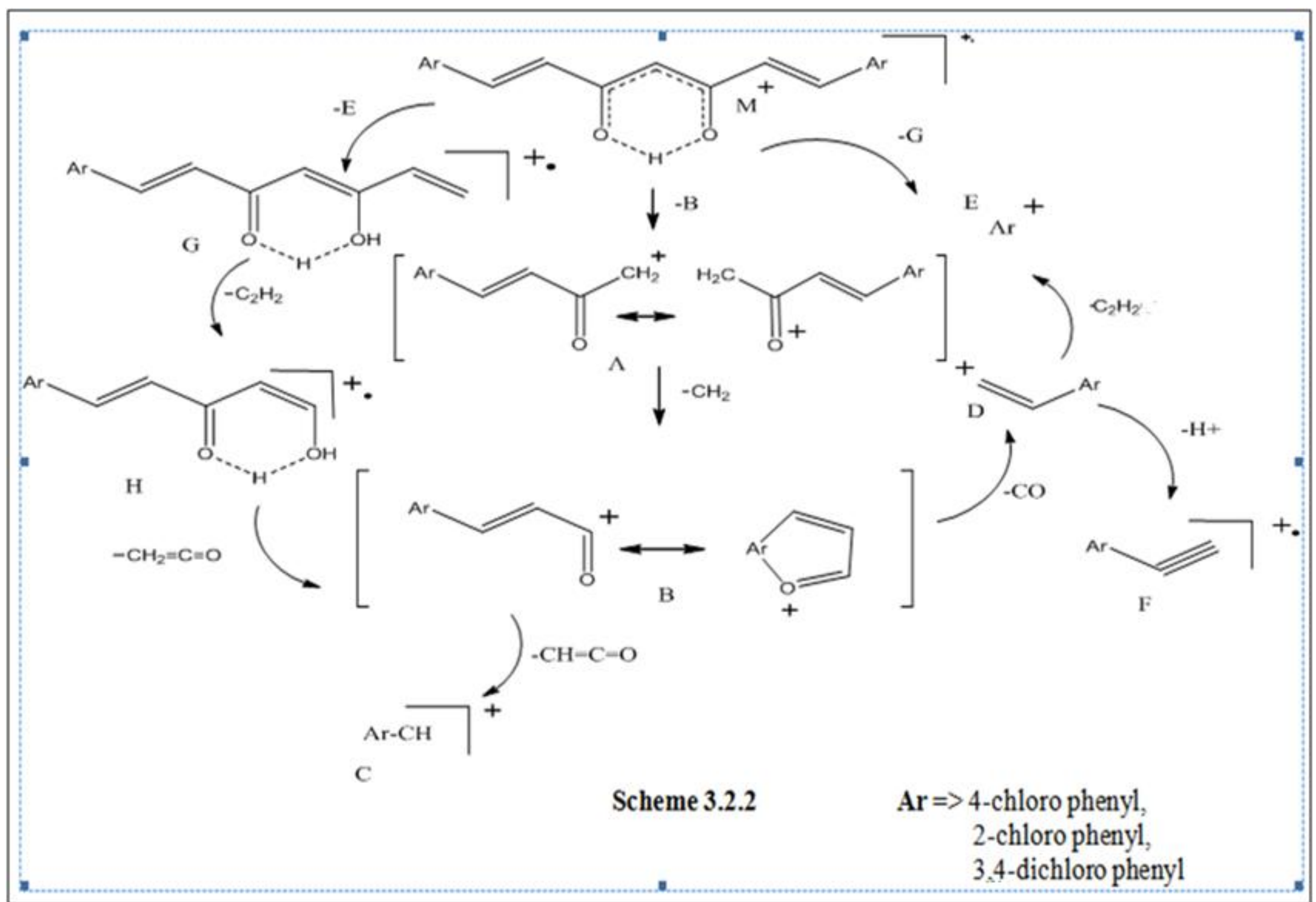


Fig.3.1.11 Mass spectrum of 3c



Scheme 3.2.2

SECTION-II

SYNTHESIS AND CHARACTERISATION OF TRANSITION METAL CHELATES OF CHLORO SUBSTITUTED 1,7-DIARYL HEPTANOIDS

3.2.1 Synthesis of metal complexes of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(3a), 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione(3b) & 1,7-bis(3,4-dichloro phenyl) hepta-1,6-diene-3,5-dione(3c)

Copper(II), Zinc(II), Nickel(II) and Oxovanadium(IV) complexes of chloro substituted curcuminoid analogues were synthesized by the following general method.

To a refluxing solution of the ligand(0.002 mol) in methanol(25ml),a methanolic solution of metal salt (0.001mol)was added and the reaction mixture was refluxed for nearly 2 hrs and cooled to room temperature. The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol.

Preparation of Cu(II) complex of the ligands

The Cu(II) complexes were prepared by adding a methanolic solution of copper(II)acetate (25 ml, 0.001mol) to a solution of **3a,3b and 3c** (25 ml, 0.002 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature. The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol.

Preparation of Zn(II) complex of the ligands

The Zn(II) complexes were prepared by adding a methanolic solution of zinc acetate (25 ml, 0.001mol) to a solution of **3a,3b and 3c** (25 ml, 0.002 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature. The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol.

Preparation of Ni(II) complex of the ligands

The Ni(II) complexes were prepared by adding a methanolic solution of nickel(II) acetate (25 ml, 0.001mol) to a solution of **3a,3b and 3c**(25 ml, 0.002 mol) in methanol and repeating the above procedure.

Preparation of Oxovanadium(IV) complex of the ligands

The VO (IV) complexes were prepared by adding a methanolic solution of V anadium(IV) Oxide sulphate (25 ml, 0.001mol) to a solution of **3a, 3b and 3c** (25 ml, 0.002 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature. The precipitated complex was filtered, washed with 1:1, methanol: water mixture and recrystallised from hot methanol.

The reaction involved in the formation of complexes is represented below in **Scheme 3.2.1.**

M (acetate)$_2$ M=(Cu,Zn,Ni)
Vanadium(IV) oxide sulphate M=(VO (IV))
+
2 Ar ... Ar → Ar ... Ar / M / Ar ... Ar

Ar = 4-chloro phenyl, 2-chlorophenyl, 3,4-dichlorophenyl

Scheme 3.2.1

3.2.2 Characterisation of transition metal complexes of chloro analogues of 1,7- diaryl heptanoids

Transition metal chelates (Cu, Zn,Ni,Vanadyl) of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(3a), 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione(3b), 1,7-bis(3,4-dichloro phenyl) hepta-1,6-diene-3,5-dione(3c) were characterized using physical,analytical and spectral datas. Elemental analysis (C, H and metal percentages), Melting point, UV and IR spectral datas of metal complexes of 3a, 3b and 3c are given in **Table3.2.1 , Table 3.2.2** and **Table 3.2.3** respectively.The datas given below suggest a ML_2 stoichiometry for all complexes prepared. Magnetic moment measurements show that Ni(II)complexes are diamagnetic ,Cu(II),VO(IV) chelates show paramagnetic moment.

Table 3.2.1 Physical, Analytical and spectral data of transition metal chelates of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione

Metal chelates	M.P (^oC)	Elemental analysis (%) Found/(calculated)			μ_{eff} BM	UV λmax (nm)	Characteristic IR stretching bands (cm^{-1})		
		C	H	Metal			(C=O)	(C-C-C)	(M-O)
Cu(II)	140	60.67 (61.24)	3.46 (3.79)	7.85 (8.10)	1.78	232, 312	1592	1495	465 420
Zn(II)	143	60.52 (61.05)	3.451 (3.76)	8.680 (9.10)		229, 311	1590	1502	454, 419
Ni(II)	145	60.96 (61.30)	3.41 (3.75)	7.82 (7.52)		233, 312	1594	1512	462, 425
VO(IV)	147	60.39 (61.52)	3.45 (3.56)	6.75 (7.10)	1.73	237, 315	1598	1511	463, 420

Table 3.2.2 Physical, Analytical and spectral data of transition metal chelates of 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione

Metal chelates	M.P. (^oC)	Elemental analysis (%) Found/(calculated)			UV λmax (nm)	Characteristic IR stretching bands (cm^{-1})		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu(II)	125	61.23 (60.67)	3.78 (3.46)	8.11 (7.85)	230, 310	1591	1494	465, 424
Zn(II)	128	61.03 (60.52)	3.75 (3.451)	9.02 (8.680)	227, 309	1589	1501	453, 419
Ni(II)	130	61.29 (60.96)	3.76 (3.41)	7.50 (7.82)	231, 310	1593	1512	460, 425
VO(IV)	132	61.51 (60.39)	3.55 (3.45)	7.01 (6.75)	235, 315	1598	1510	465, 419

Table 3.2.3 Physical, Analytical and spectral data of transition metal chelates of 1,7-bis(3,4-dichloro phenyl) hepta-1,6-diene-3,5-dione

Metal chelates	M.P. (^oC)	Elemental analysis (%) Found/(calculated)			UV λmax (nm)	Characteristic IR stretching bands (cm^{-1})		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu(II)	156	51.02 (51.26)	2.45 (2.473)	7.03 (7.138)	240, 340	1608	1520	475, 441
Zn(II)	160	50.99 (51.17)	2.449 (2.46)	7.25 (7.33)	243, 337	1589	1526	481, 427
Ni(II)	165	51.25 (51.44)	2.475 (2.486)	6.52 (6.63)	245, 339	1593	1521	485, 428
VO(IV)	168	50.98 (51.06)	2.39 (2.463)	5.67 (5.704)	248, 341	1595	1525	488, 442

UV spectra

The UV spectra of ligands and their corresponding complexes are quite similar.There is no much shift in the absorption values of $\pi \rightarrow \pi*$ and n→ $\pi*$ transitions.The complexes also show two absorption maxima corresponding to $\pi \rightarrow \pi*$ and n→ $\pi*$ transitions.There is a slight shift of absorption maxima to longer wavelength which indicate the involvement of the carbonyl oxygens in metal complexation.The UV spectra of ligand **3a** and its Zn(II) complex are shown in Fig.3.2.1.

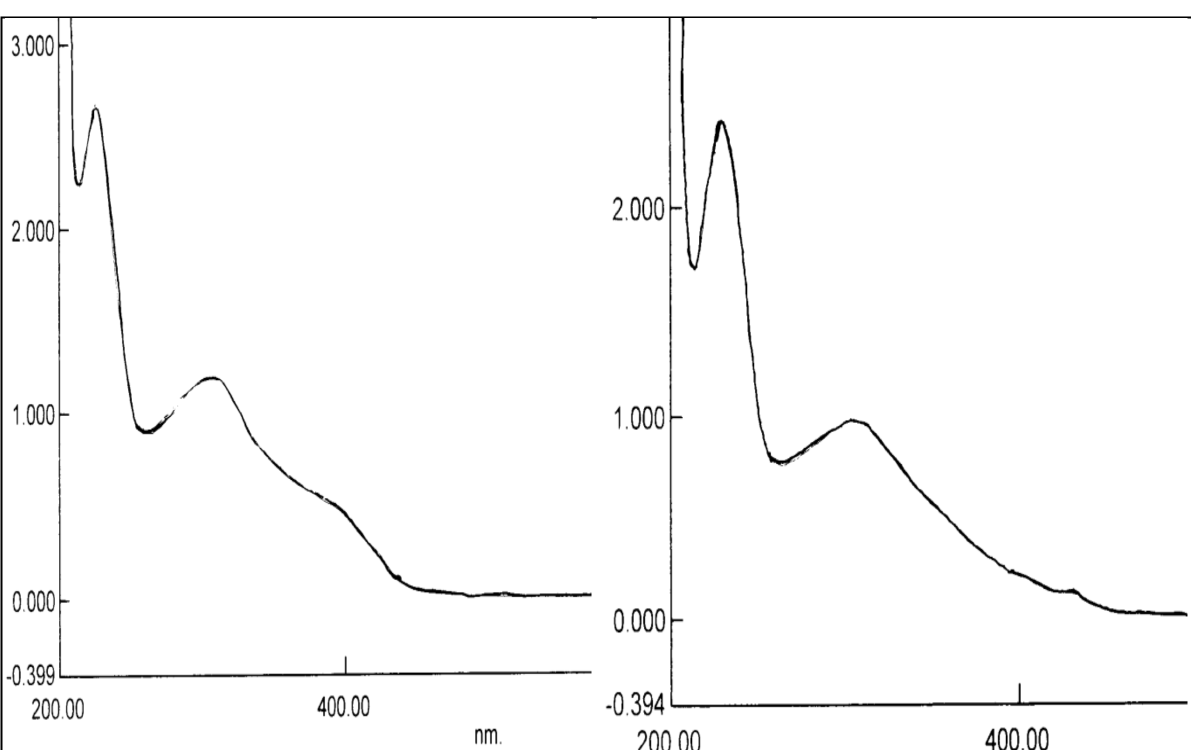


Fig.3.2.1 UV spectra of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione and its Zn(II) complex

IR spectra

The absence of a strong band in the region 1800-1650 cm^{-1} is one of the characteristic feature of metal complexes of 1,3-diketones. In the IR spectra of metal complexes, the peak of intramolecularly hydrogen bonded carbonyl moiety which is present at ~ 1630 cm^{-1} disappeared and a new band appeared at ~ 1595 cm^{-1} which is due to metal chelated carbonyl

group . The broad band in the region of 2600 -3500 cm^{-1} present in the ligand is reduced in the spectra of complexes. This indicates that the enolic proton in ligand is replaced by the metal ion during complexation. The involvement of carbonyl group in chelate formation is further evident from the appearance of two medium intensity bands in the region of 400 – 490 cm^{-1} due to νM-O vibrations. There is no change in the nature of alkenyl carbons due to complex formation. The IR spectra of Cu(II) complex of **3b** is depicted in **Fig.3.2.2.**

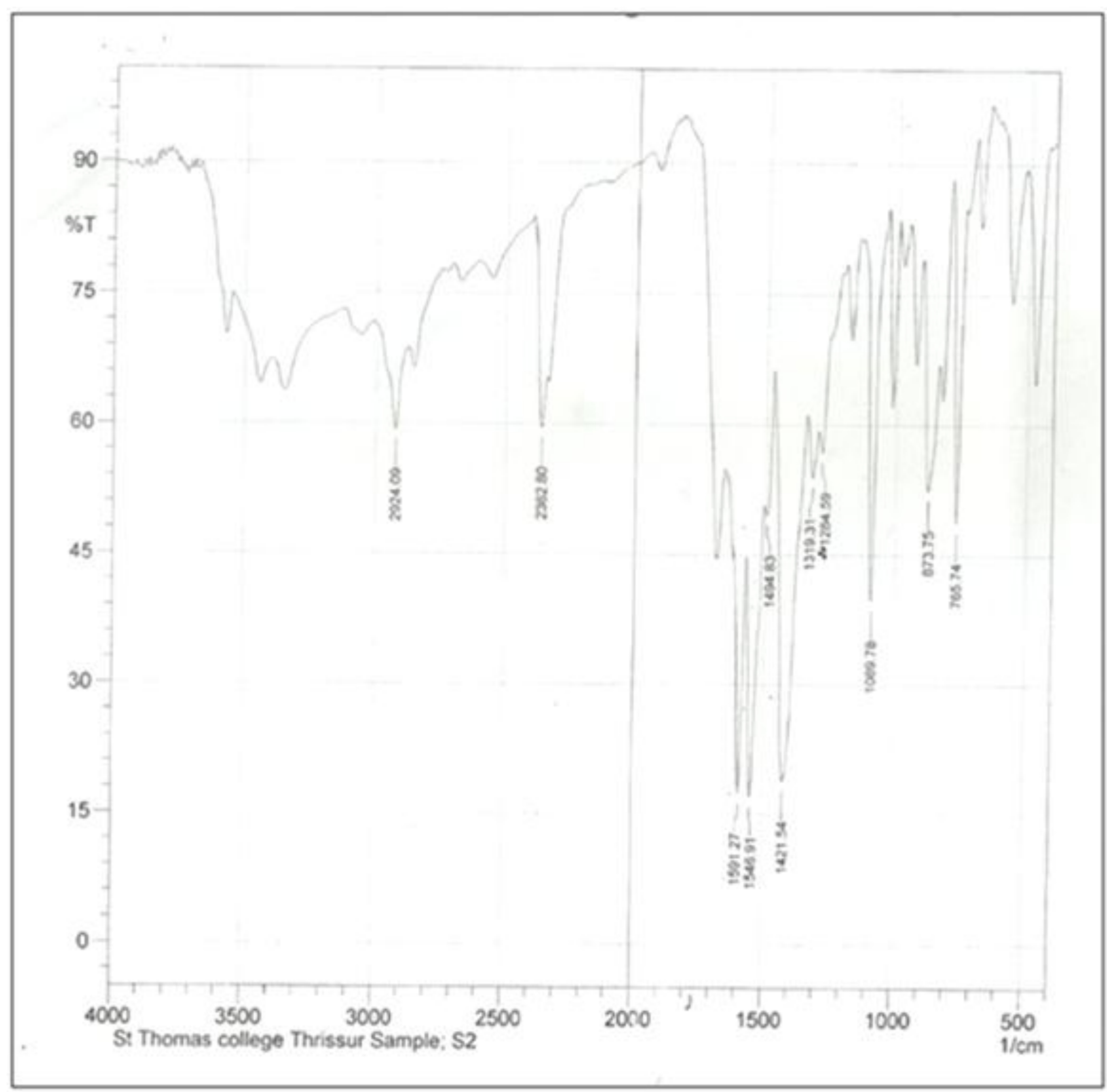


Fig.3.2.2 IR spectra of Cu(II) complex of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione

^{1}H NMR spectra

The ^{1}H NMR spectra of metal complexes are in conformity with structure,in which enolic proton present in the ligands is replaced by metal ion in metal complexes. This is evident by the disappearance of the signal at δ ~ 16ppm in metal complexes. The phenyl and alkenyl protons are not altered much since they are not involved in metal complexation. There is a slight shift of methine signals to the downfield of the spectra indicating the decreased electron density around the central carbon of the metal chelate ring system. Thus the spectra of ligand and complexes are much similar except those of enolic proton.

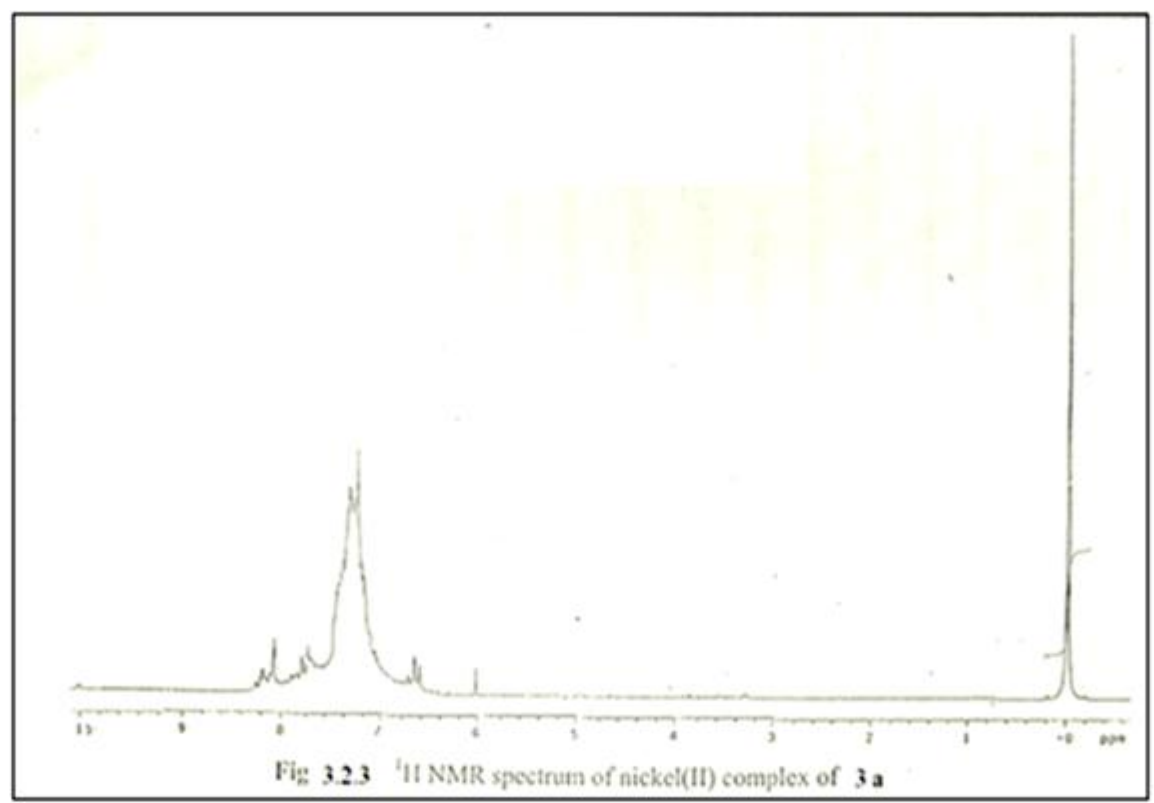


Fig 3.2.3 ^{1}H NMR spectrum of nickel(II) complex of **3a**

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 3.2.2 .**The fragment F in the table is that due to the ligand. Mass spectral details of the transition metal chelates of **3a, 3b** & **3c** are given in Table 3.2.4, 3.2.5 & 3.2.6. Mass spectrum of Zn(II) complex of 3a is given in Fig.3.2.4 and Cu(II) complex of **3b** is given in Fig.3.2.5.

Table 3.2.4 Mass spectral fragmental pattern of metal chelates of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione

Fragments	Metal Chelate	M+/ (M+1)/ (M+2) ion	A	B	C	D	E	F	G
Mass Pattern	Cu(II)	749	527	408	121	307	185	345	223
	Zn(II)	754	531	410	121	308	187	345	223
	Ni(II)	747	524	403	121	301	180	345	223
	VO(IV)	755	532	411	121	309	188	345	223

*The alphabets corresponds to the fragments given in **Scheme 3.2.2 .**

Table 3.2.5 Mass spectral fragmental pattern of metal chelates of 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione

Fragments	Metal chelates	M+/ (M+1)/ (M+2) ion	A	B	C	D	E	F	G
Mass Pattern	Cu(II)	749	527	408	121	307	185	345	223
	Zn(II)	754	531	410	121	308	187	345	223
	Ni(II)	747	524	403	121	301	180	345	223
	VO(IV)	755	532	411	121	309	188	345	223

*The alphabets corresponds to the fragments given in **Scheme 3.2.2 .**

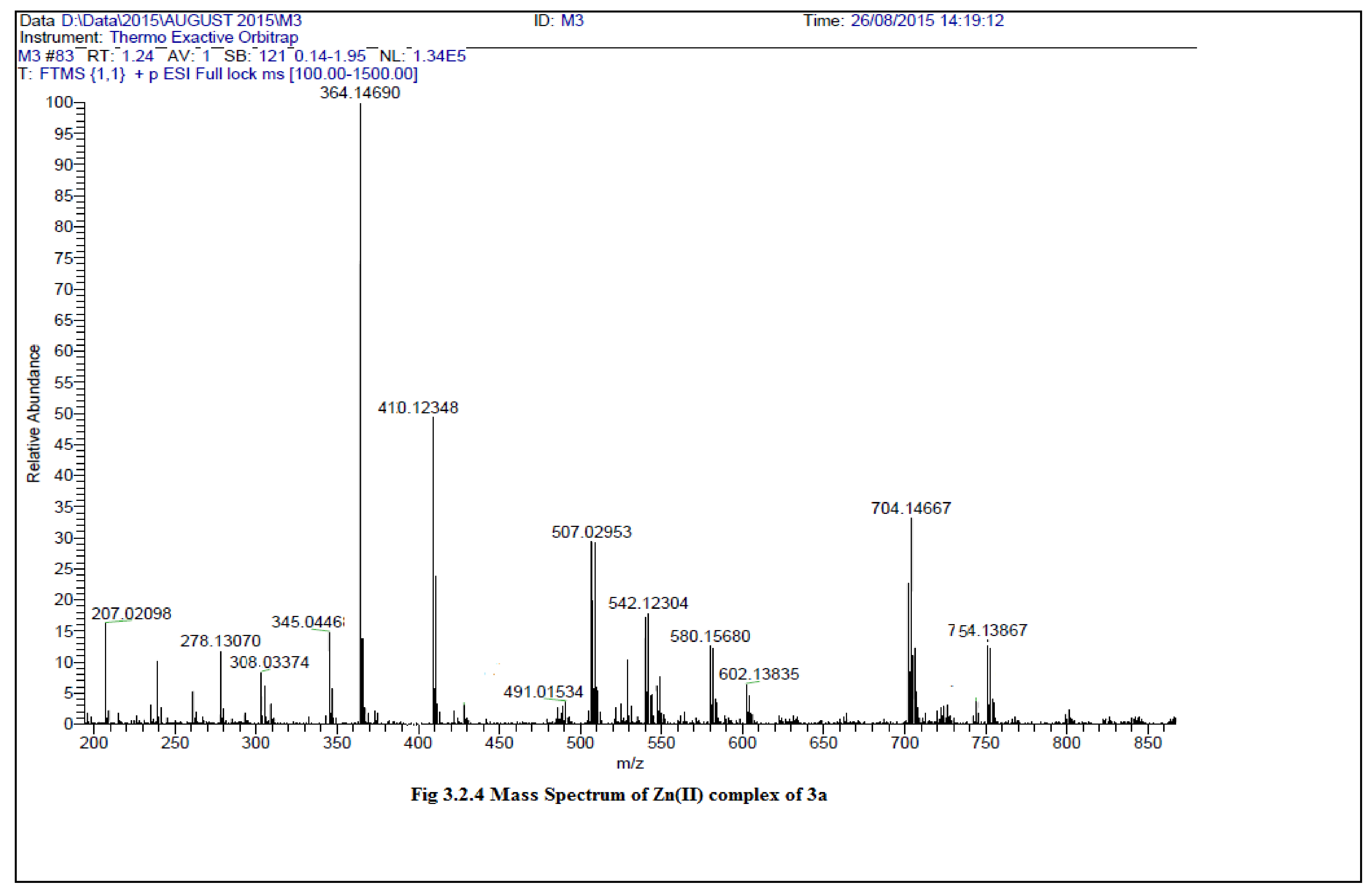


Fig 3.2.4 Mass Spectrum of Zn(II) complex of 3a

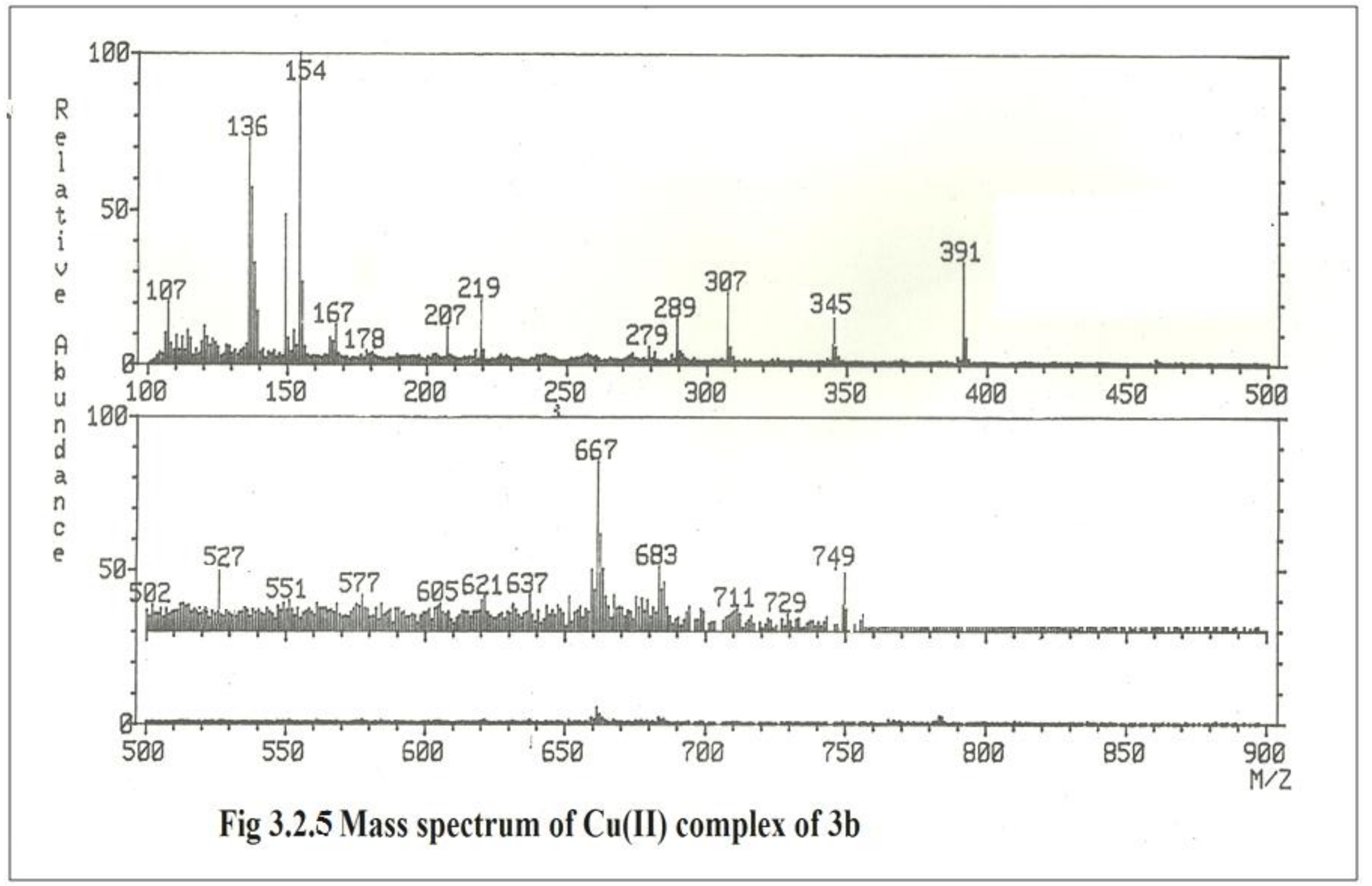


Fig 3.2.5 Mass spectrum of Cu(II) complex of 3b

Table 3.2.6 Mass spectral fragmental pattern of metal chelates of 1,7-bis(3,4-dichloro phenyl) hepta-1,6-diene-3,5-dione

Fragments	**Metal chelates**	**M+/ (M+1)/ (M+2) ion**	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Mass Pattern	Cu(II)	890	598	477	121	306	185	414	292
	Zn(II)	892	600	479	121	308	187	414	292
	Ni(II)	885	593	472	121	301	180	414	292
	VO(IV)	893	601	480	121	309	188	414	292

*The alphabets corresponds to the fragments given in **Scheme 3.2.2.**

Peaks corresponding to stepwise elimination of aryl groups is a characteristic feature of all the complexes. The molecular ion peak corresponding to $[ML_2]+$ ion is present in the mass spectra of complexes.The fragment ion peaks can be identified from the fragmentation pattern given in Scheme 3.2.2. 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 3.2.2 Fragmentation pattern of metal complexes

In the mass spectrum of Cu(II) complex of 3b ,the molecular ion peak is present at 749 which is not a very intense peak.The peak at 683 is due to the removal of Cu from molecular ion and the removal of Cu and Oxygen gives the peak at 667.The peak at 527 is due to the removal of 2Ar groups and the peak at 307 is due to the removal of 4Ar groups where Ar=2-chlorophenyl group.The peak at 391 is due to the removal of a ligand group and oxygen from the molecular ion.The peaks at 136,154 are also observed in the mass spectra of ligand and they are due to fragment ions of ligand.The peak at 121 is assigned to the rearranged cyclic species in the Scheme.

In the mass spectrum of Zn(II) complex of 3a ,the molecular ion peak is present at 754 which is not a very intense peak..The peak at 531 is due to the removal of 2Ar groups and the peak at 308 is due to the removal of 4Ar groups where Ar=4-chlorophenyl group.The peak at 345 is due to the ligand and the peaks at 278 and 208 are due to fragment ions of the ligand and are observed in the spectrum of the ligand.The peak at 410 is assigned to the fragment B in the Scheme.

The observed UV, IR, NMR and Massspectral details suggest that the metal complexes formed has a 1:2 metal ligand stoichiometry(ML_2) as given below.

n = 2 for Cu(II),Zn(II),Ni(II),VO(IV)

Ar = chloro substituted phenyl

SECTION-III

CYTOTOXIC AND ANTITUMOUR STUDIES OF CHLORO ANALOGUES OF 1,7–DIPHENYL HEPTANOIDS AND THEIR TRANSITION METAL CHELATES

This section deals with the cytotoxic and antitumour activities of chloro substituted 1,7-diaryl heptanoids namely 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(3a), 1,7-bis(2-chloro phenyl)hepta-1,6-diene-3,5-dione(3b)and1,7-bis(3,4-dichloro phenyl) hepta-1,6-diene-3,5-dione(3c) and their metal chelates Cu(II),Zn(II),Ni(II) & VO(IV). Invitro cytotoxic activity against DLA and EAC cell lines were studied.The Invivo antitumour activity was determined by using EAC cell line induced ascites tumour model in mice and its comparison with a std.anticancer drug cyclophosphamide.

3.3.1. Invitro Cytotoxic activity of ligands and metal chelates

Short term cytotoxic activity of compounds 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(3a),1,7-bis(2-chlorophenyl)hepta-1,6-diene-3,5-dione(3b), 1,7-bis(3,4-dichloro phenyl) hepta-1,6-diene-3,5-dione(3c) and their metal chelates Cu(II),Zn(II),Ni(II) &VO(IV) were assayed by determining the percentage viability of DLA and EAC cells using Trypan blue dye exclusion technique (Moldeus *et al*,1978).

3.3.2. *In vitro* Cytotoxic studies of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(3a), and their metal complexes [Cu(II),Zn(II),Ni(II) &VO(IV)] towards EAC cells

The curcuminoid analogue 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione and their metal complexes were used for *in vitro* cytotoxicity study towards EAC cells. All the test compounds were prepared in different concentrations namely 10,20,50,100,200 µg/ml.The cytotoxic nature of the compounds were determined in terms of % cell death produced by

them.The results of the study is given in Table **3.3.1** and represented diagrammatically in Fig.**3.3.1.**

Table 3.3.1. ***In vitro* Cytotoxic studies of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione (L_1) and their metal complexes towards EAC**

Drug Con. µg/ml	% Cell death				
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$	$VO(L_1)_2$
200	25	76	62	47	26
100	21	65	49	35	22
50	11	45	35	23	12
20	5	26	20	18	6
10	3	16	11	9	4

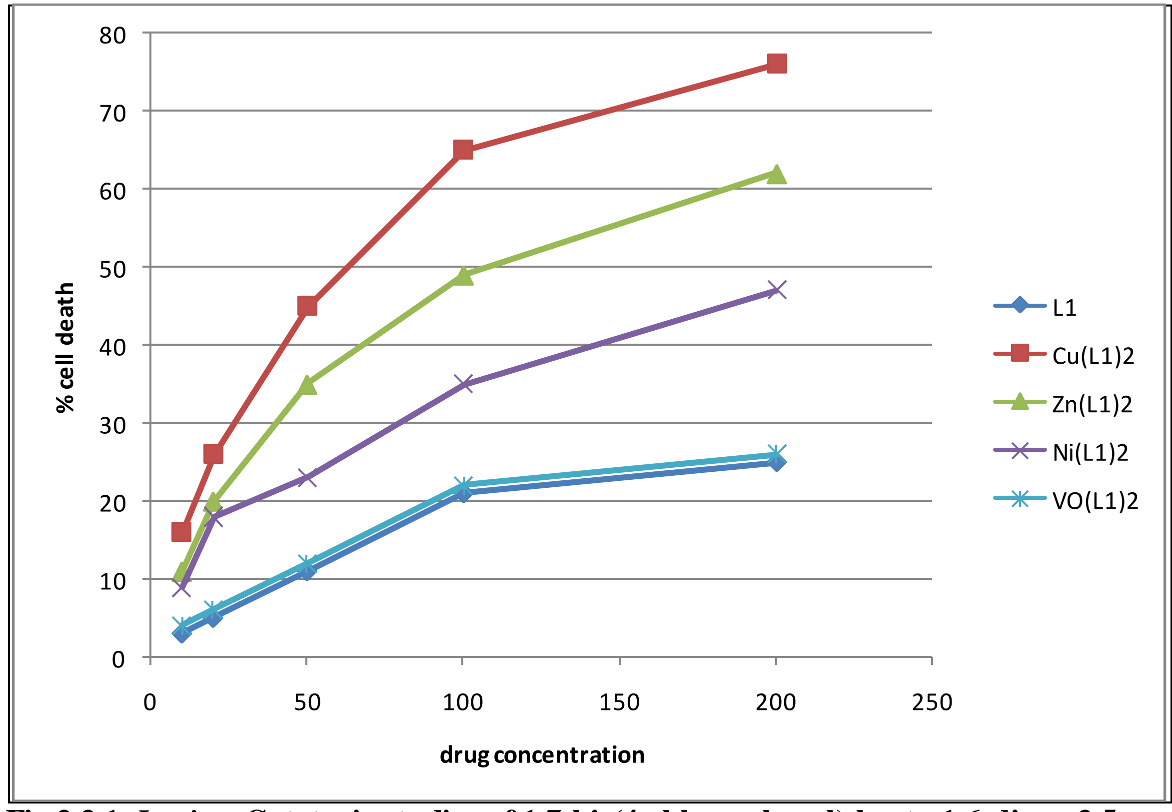


Fig.3.3.1. ***In vitro* Cytotoxic studies of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione (L_1) and their metal complexes towards EAC**

The ligand 3a produced only 25% cell death towards EAC cell lines at a concentration of 200µg/ml.At lower concentration the activity of the compound is quite negligible.All the metal complexes produced greater % of cell death compared with the ligands.They possessed less activity at lower concentrations.As concentration increases the % of cell death increases.The % cell death produced by Cu(II),Zn(II),Ni(II) and VO(IV) complexes at 200µg/ml are 76,62,47 & 26 % respectively.Among the metal complexes ,the Cu(II) complex of ligand possessed maximum activity and VO(IV) complex exhibited minimum activity.The vanadyl complex only showed a very slight increase in activity compared with ligand.The cytotoxic nature of compounds follow the sequence Cu(II)> Zn(II) > Ni(II) >VO(IV) >ligand.

3.3.3 *In vitro* Cytotoxic studies of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione (L_1) and their metal complexes [Cu(II),Zn(II),Ni(II) &VO(IV)] towards DLA cells

The ligand and the metal complexes were studied for their activity against DLA cells.

The % cell death were also calculated with DLA cell lines.The results of the study in terms of % cell death is represented in Table **3.3.2** and diagrammatically in Fig. **3.3.2** .

Table.3.3.2 *In vitro* Cytotoxic studies of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione (L_1) and their metal complexes towards DLA

Drug Con. µg/ml	% Cell death				
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$	$VO(L_1)_2$
200	23	74	60	45	24
100	20	63	47	33	21
50	10	41	31	20	11
20	6	22	19	17	7
10	3	14	10	8	3

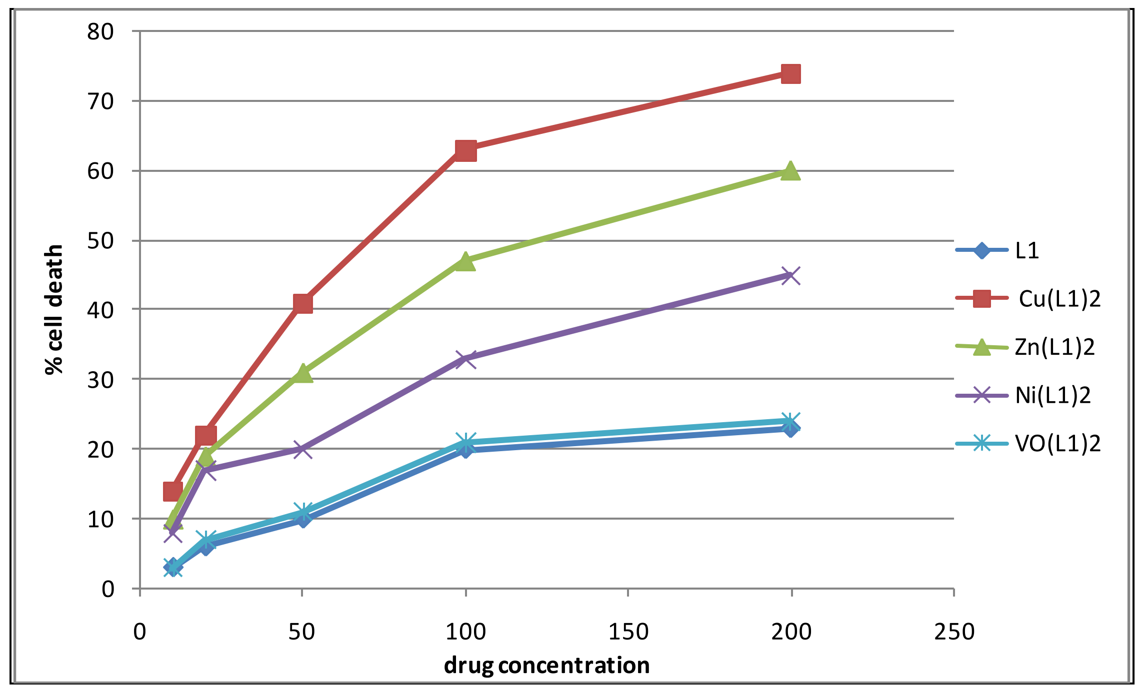


Fig.3.3.2 ***In vitro*** **Cytotoxic studies of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione (L_1) and their metal complexes towards DLA**

The results of the study indicate that the activity of compound 3a and metal complexes towards DLA cells follows a similar pattern as that with EAC cells. The ligand as well as the metal complexes showed a slight decrease in activity when compared with their activities towards EAC cells. The compound 3a produced 23% cell death where as its Cu(II) complex produced 74% cell death, which is thrice the activity of ligand. The activity of metal complexes followed the order Cu(II)>Zn(II)>Ni(II)> VO(IV) and the % cell death produced by them were 74,60,45 and 24% respectively.

3.3.4. *In vitro* Cytotoxic studies of 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione and their metal complexes [Cu(II),Zn(II),Ni(II) &VO(IV)] towards EAC & DLA cells

In vitro Cytotoxic studies were done using 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione(3b) and their metal complexes towards EAC & DLA cell lines. The % cell death

towards EAC & DLA cells are given in Table **3.3.3** and Table **3.3.4** respectively. The results are also depicted in Fig. **3.3.3** &**3.3.4** respectively.

Table 3.3.3. ***In vitro*** **Cytotoxic studies of of 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione (L_2) and their metal complexes towards EAC**

Drug Con. µg/ml	% Cell death				
	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$	$VO(L_2)_2$
200	22	74	60	45	25
100	20	63	45	33	21
50	9	43	32	22	10
20	8	24	18	17	9
10	4	14	9	8	5

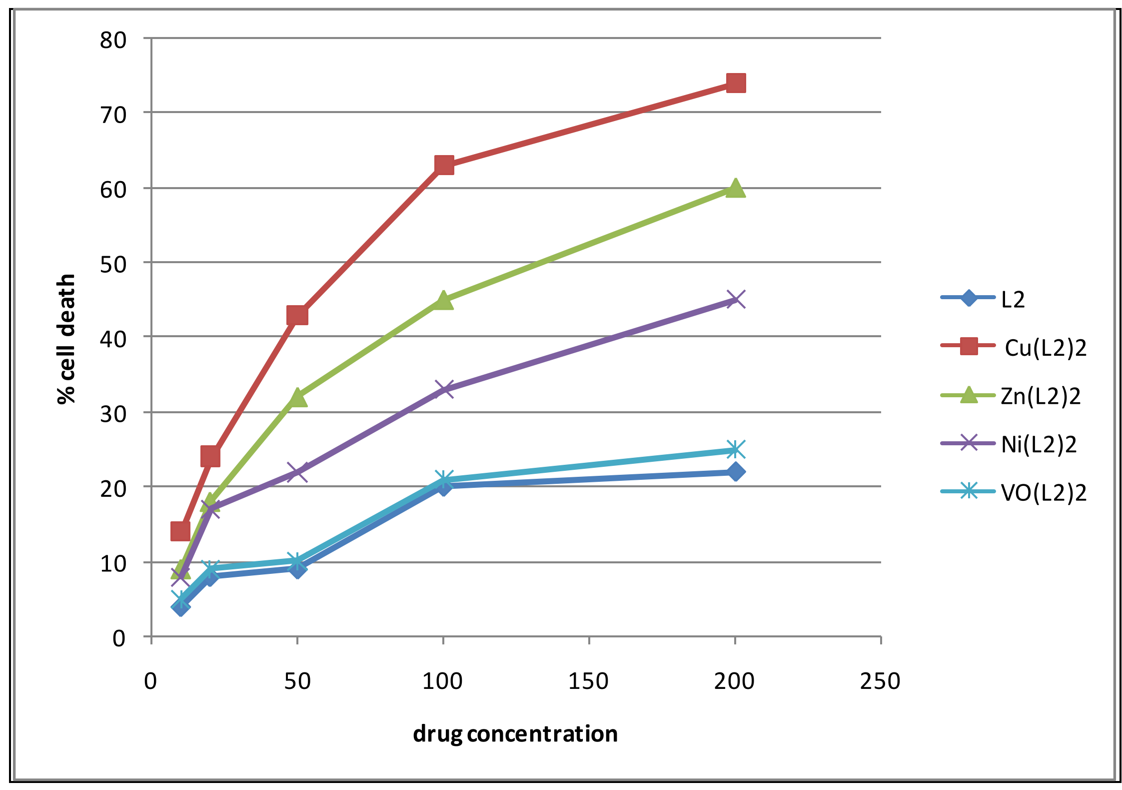


Fig.3.3.3. ***In vitro*** **Cytotoxic studies of 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione (L_2) and their metal complexes towards EAC**

Table 3.3.4. ***In vitro*** **Cytotoxic studies of 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione (L_2) and their metal complexes towards DLA**

Drug Con. µg/ml	% Cell death				
	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$	$VO(L_2)_2$
200	20	72	58	43	22
100	19	60	44	31	20
50	8	39	30	19	9
20	4	21	18	16	5
10	3	13	9	7	4

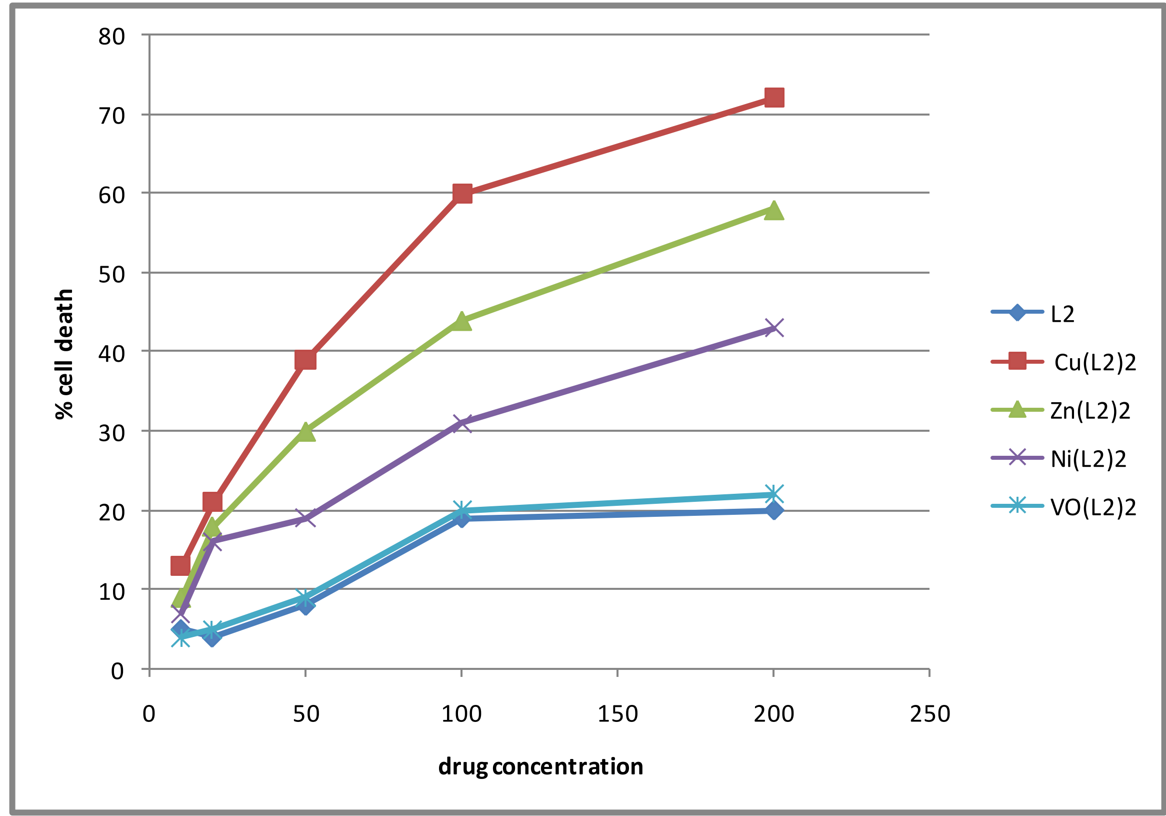


Fig.3.3.4. ***In vitro*** **Cytotoxic studies of 1,7-bis(2-chloro phenyl) hepta-1,6-diene-3,5-dione (L_2) and their metal complexes towards DLA**

The ligand 3b with chloro substituent in ortho position of the phenyl ring was less effective than ligand 3a with chlorine in para position.The ligand and metal complexes possessed very little activity at lower concentrations.The ligand 3b could not produce much cell death even at higher concentration with DLA and EAC cells.But the Cu(II) and Zn(II) complexes of the ligand exhibited activity against both cell lines,nearly 70 and 60% respectively.The Vanadyl complexes have the lowest value among the metal complexes.They produced only 25% cell death.

3.3.5 *In vitro* Cytotoxic studies of 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione(3c) and their metal complexes towards EAC & DLA cells

In vitro Cytotoxic studies were done using 1,7-bis(3,4-dichloro phenyl) hepta-1,6-diene-3,5-dione(**3c**) and their metal complexes towards tumour bearing cells DLA & EAC. Ligand and complexes were given as drug with concentrations 200, 100, 50, 20 & 10µg/ml. The observed % cell death towards EAC cells are given in **Table 3.3.5** & **Fig.3.3.5** and towards DLA cells are given in **Table 3.3.6** & **Fig.3.3.6.**

Table 3.3.5 ***In vitro*** **Cytotoxic studies of 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione (L_3) and their metal complexes towards EAC**

Drug Con. µg/ml	% Cell death				
	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$Ni(L_3)_2$	$VO(L_3)_2$
200	20	49	35	32	24
100	15	34	23	19	16
50	11	20	18	15	12
20	6	11	8	7	5
10	0	2	1	0	0

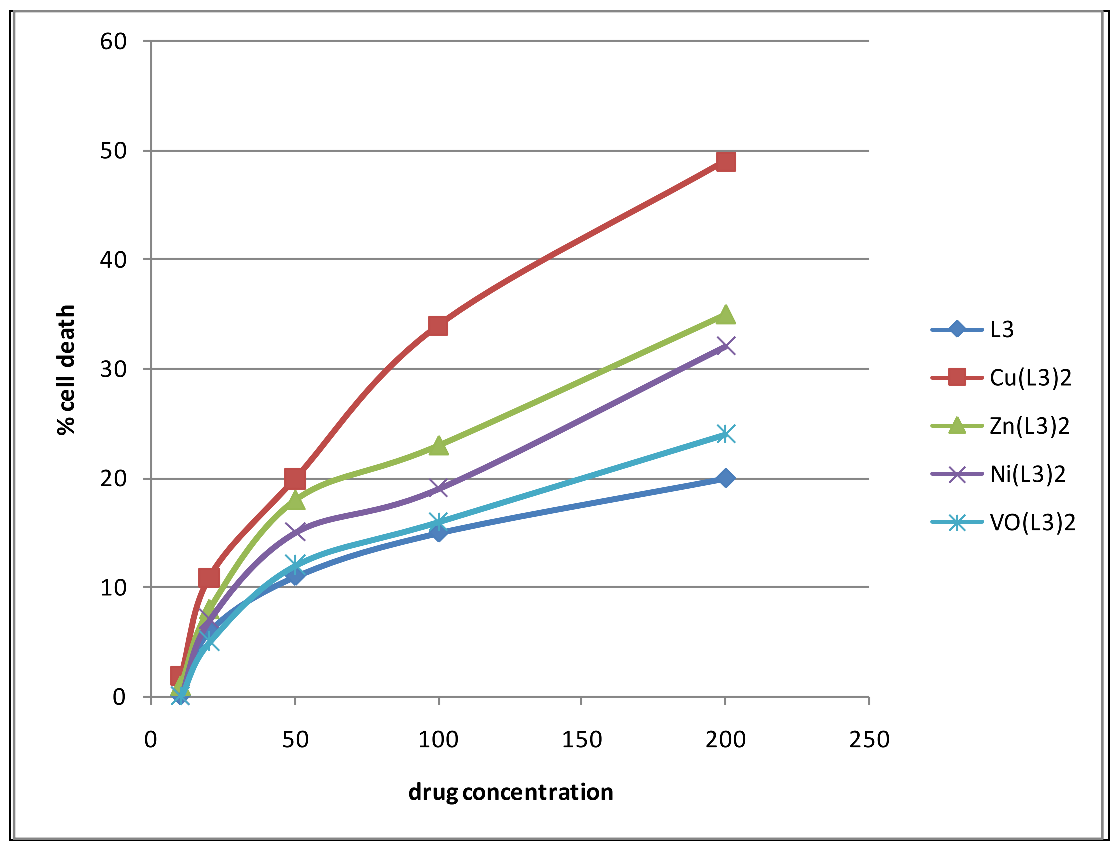


Fig.3.3.5. ***In vitro*** **Cytotoxic studies of 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione(L_3) and their metal complexes towards EAC**

The results show that the ligand 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione(L_3) and their metal complexes could not significantly inhibit the growth of EAC cells.They were quite inactive at lower concentrations.Cu(II) complex with a concentration of 200 µg/ml produced maximum activity (49%). Even with a concentration of 200 µg/ml the ligand gave only 20% cell death.However the metal complexes were a little more active than the ligand against EAC cells.

Table3.3.6. ***In vitro*** **Cytotoxic studies of 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione(L_3) and their metal complexes towards DLA**

Drug Con. µg/ml	% Cell death				
	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$Ni(L_3)_2$	$VO(L_3)_2$
200	15	45	30	29	20
100	8	28	18	16	12
50	5	18	13	12	10
20	3	9	6	4	3
10	0	2	1	0	0

The % cell death produced by the ligand with dichloro substituted phenyl ring and Ni(II) and Vanadyl complexes is zero with con. 10µg/ml.The activity of the ligand against DLA cells is negligible even at all concentrations. Out of the metal complexes, Cu complexes have the maximum value (45%) where as VO(IV) complexes have the least(20%). The ligand as well as the metal complexes were not effective in bringing cell death with DLA cells.

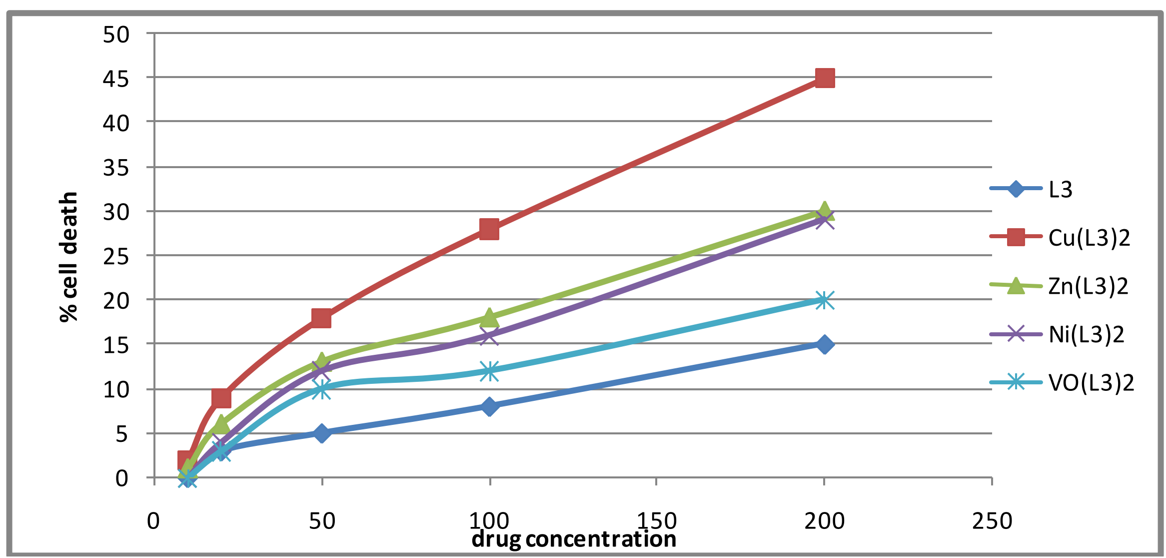


Fig.3.3.6. In vitro Cytotoxic studies of 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione(L_3) and their metal complexes towards DLA

Conclusion

A comparative study of *in vitro* cytotoxicity of chloro analogues of curcuminoids, i.e, 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(**3a**), 1,7-bis(2-chloro phenyl)hepta-1,6-diene-3,5-dione(**3b**) & 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione(**3c**)were done. It was observed that **3a** has the maximum activity both towards EAC & DLA. The order of activity among the chloro compounds are **3a** > **3b** > **3c.** Among the metal complexes Cu(II) possessed maximum activity.This may be due to the steric factor of chloro group. In **3a** (4-chloro) the steric factor is minimum where as in di chloro (**3c**) it is maximum.

IN VIVO ANTITUMOUR STUDIES OF 1,7-BIS(4-CHLORO PHENYL) HEPTA-1,6-DIENE-3,5-DIONE (L_2) AND THEIR Cu(II) AND Zn(II) METAL COMPLEXES

The effect of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione (L_2) and their Cu(II) and Zn(II) metal complexes on the survival rate of ascites tumour bearing animals were studied.Swiss Albino mice (male,6-8 weeks old)weighing 28-30g were divided into 11 groups of five animals each.Viable EAC cells in 0.1ml of PBS were injected into the peritoneal cavity so as to develop tumour in animals.The animals were then injected with test compounds as drug in different concentrations. **Group1** was kept as control without drug treatment. **Group 2** was treated with Standard drug Cyclophosphamide(25mg/kg body weight).Ligand 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione with concentrations 20µg/ml, 10µg/ml and 5µg/ml were administered as drug in **Group 3-5** respectively. **Group 6-8 :**Cu(II) complex of ligand as drug with concentrations 20µg/ml, 10µg/ml & 5µg/ml respectively is given as i.p(intraperitoneal injection). **Group 9-11**: Zn(II) complex of ligand as drug with concentrations 20µg/ml, 10µg/ml & 5µg/ml respectively is given as intraperitoneal injection..

3.3.6.Effect of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione and the Cu(II) &Zn(II) complexes on ascites tumour

All the test compounds were injected intraperitoneally and were given as drug and their effect in reducing ascites tumour development in mice were studied.The no. of days survived by the control group,the animals given standard drug,and the animals treated with test compounds and their % increase in life span is found and the results are presented in Table **3.3.7.**

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Table 3.3.7 Effect of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(L2) and the Cu(II) &Zn(II) 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±1.10	
2.Standard drug		5/5	30.6±0.489	76.87
3.L_2	20	5/5	20.0±1.095	15.60
4. L_2	10	5/5	18.8±0.74	8.67
5. L_2	5	5/5	17.5±1.16	1.15
6.$Cu(L_2)_2$	20	5/5	24.6±1.04	42.19
7. $Cu(L_2)_2$	10	5/5	20±1.095	15.60
8. $Cu(L_2)_2$	5	5/5	19.8±1.469	14.45
9.$Zn(L_2)_2$	20	5/5	20.5±1.20	18.49
10.Z n$(L_2)_2$	10	5/5	18.9±0.45	9.24
11.$Zn(L_2)_2$	5	5/5	17.9±0.50	3.46

The test compounds namely 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(L_2) and their Cu(II) and Zn(II) metal complexes were given as drug in different concentrations namely 5,10,20 µg/ml.The no. of days survived by control group is 17.3±1.1 where as for standard drug cyclophosphamide it is 30.6±0.489.The animals which were given the drug 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione survived for 20.0±1.095 days with the concentration 20µg/ml and produced an increase in life span of 15% compared with the untreated animals.The ligand in lower concentrations is not very effective in increasing the life span of animals.The treatment with Cu(II) complex at concentration 20µg/ml increased the life span of tumour bearing animals by 42% where as the Zn(II) complex increased the life span of tumour bearing animals only by 18%.Compared with the increase in life span produced by the std.drug (76%) ,the ligand and Zn complexes possessed little antitumour ctivity.But the Cu(II) complex has shown significant activity and the animals treated with the compound survived for 24.6±1.04 days.

SECTION-IV

ANTIBACTERIAL STUDIES OF CHLORO SUBSTITUTED 1,7-DIARYL HEPTANOIDS AND THEIR Cu(II),Zn(II)&VO(IV)METAL COMPLEXES

3.4.1 Antibacterial studies of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(3a), 1,7-bis(2-chlorophenyl)hepta-1,6-diene-3,5-dione(3b) and their Cu(II) ,Zn(II) and VO(IV) complexes

The compounds and their complexes were evaluated for their antibacterial activities.The invitro antibacterial studies were performed on three types of bacterial strains namely Escherichia Coli, Klebsiella Pneumoniae & Bacillus Subtilis using Agar well diffusion method.The test compounds showed varying degree of inhibition against different bacterial strains. The results of the antibacterial activity of chloro substituted 1,7-diaryl heptanoids and their complexes revealed that the ligands and their complexes possess antibacterial activity (Table 3.4.1).The activity is expressed in terms of diameter of zone of inhibition in mm.

Table 3.4.1 Antibacterial studies of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(L_1),1,7-bis(2-chloro phenyl)hepta-1,6-diene-3,5-dione(L_2) and their Cu(II) ,Zn(II) and VO(IV) complexes

Bacteria	Diameter zone of inhibition in mm							
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$VO(L_1)_2$	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$VO(L_2)_2$
E Coli	13.5	17.5	13	15	13	18	14	17
Klebsiella	11	15	12	13	12	16	13	15
Bacillus	4.5	8.5	6	7	6	11.5	7	10.5
Standard	20	20	20	20	20	20	20	20

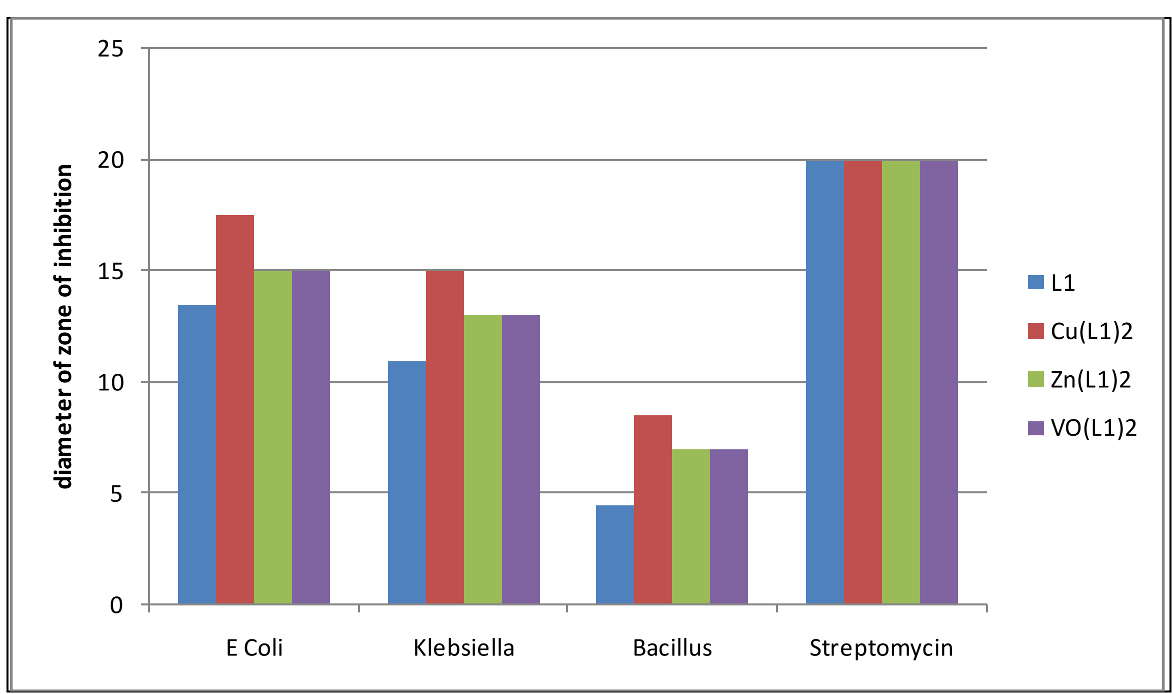


Fig.3.4.1. Antibacterial studies of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione (L_1) and their complexes

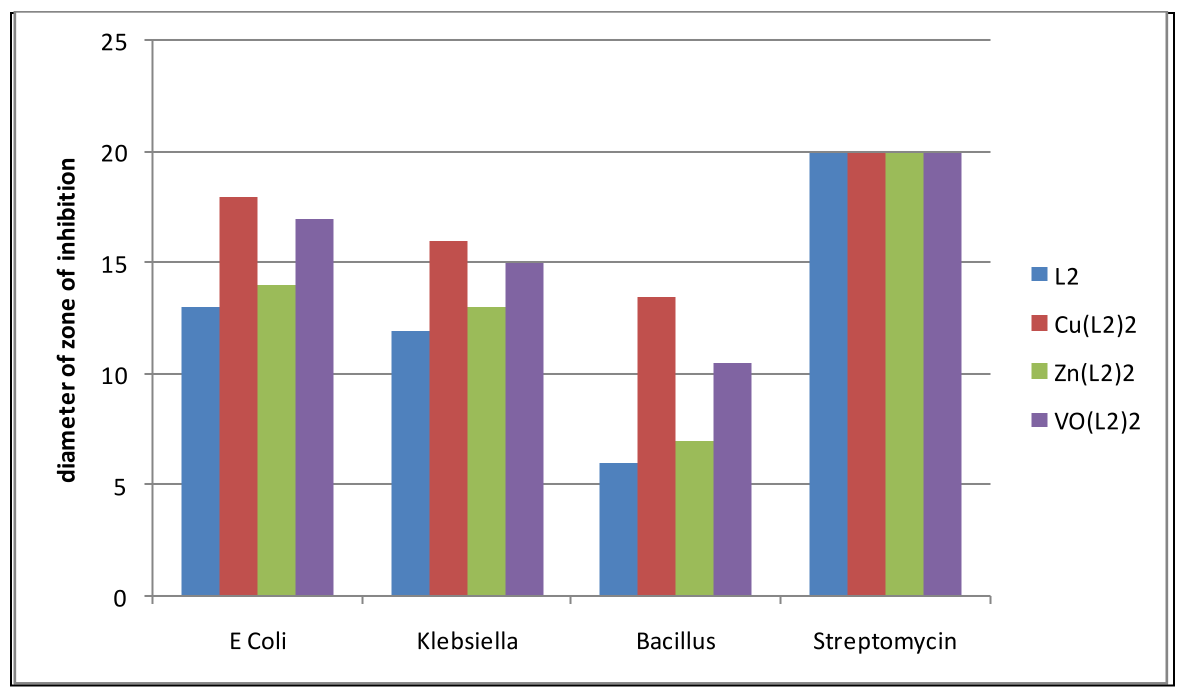


Fig.3.4.2 Antibacterial studies of 1,7-bis(2-chloro phenyl)hepta-1,6-diene-3,5-dione (L_2) and their complexes

The results given in Table 3.4.1 reveals that the curcuminoid analogues with chloro substituted phenyl ring was more active against E.Coli species producing a zone of inhibition of 13.5mm.Both the ligands were less active against Bacillus species.All the metal complexes possessed better antibacterial activity than that of ligands.Out of the three metal complexes, Cu(II) complexes show maximum activity against all bacterial strains.The Cu(II) complexes of both the ligands exhibited significant antibacterial effect to E.Coli &Klebsiella species producing a zone of inhibition of 18 mm and 16 mm respectively.This is comparable with the activity of the std.drug.The VO(IV) complexes also could significantly inhibit the growth of E.Coli &Klebsiella strains.The Zn(II) complexes had minimum activity.All the compounds were less active against Bacillus species.

3.4.2 Antibacterial studies of 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione and their Cu(II) ,Zn(II) and VO(IV) complexes

The antibacterial screening of dichloro substituted 1,7-diaryl heptanoids and their complexes were done with three types of bacterial strains namely Escherichia Coli,Klebsiella Pneumoniae & Bacillus Subtilis using Agar well diffusion method.The antibacterial activity was measured in terms of diameter of zone of inhibition in mm.The curcuminoid analogue and their metal chelates in the concentration 5mg/ml in DMSO were used for the study.The solvent acts as negative control and streptomycin served as positive control.The results of the study are presented in **Table.3.4.2.**

Table 3.4.2 Antibacterial studies of 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione(L3) and the metal complexes.

Bacteria	Diameter of zone of inhibition in mm			
	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$VO(L_3)_2$
E Coli	11	14	11.5	12.5
Klebsiella	7.5	12	9	10.5
Bacillus	4	7	4.5	6
Standard	20	20	20	20

The dichloro substituted 1,7-diaryl heptanoids exhibits less antibacterial activity when compared with mono chloro substituted ligands.The ligand L3 and metal complexes were most active against E.Coli species.Their antibacterial effect with Bacillus is negligible.All metal complexes were more active than ligands.Zn(II) complex showed almost comparable activity with the ligand.The VO(IV) complexes were slightly more effective than the ligand in inhibiting bacterial growth and the Cu(II) complexes exhibited maximum activity.

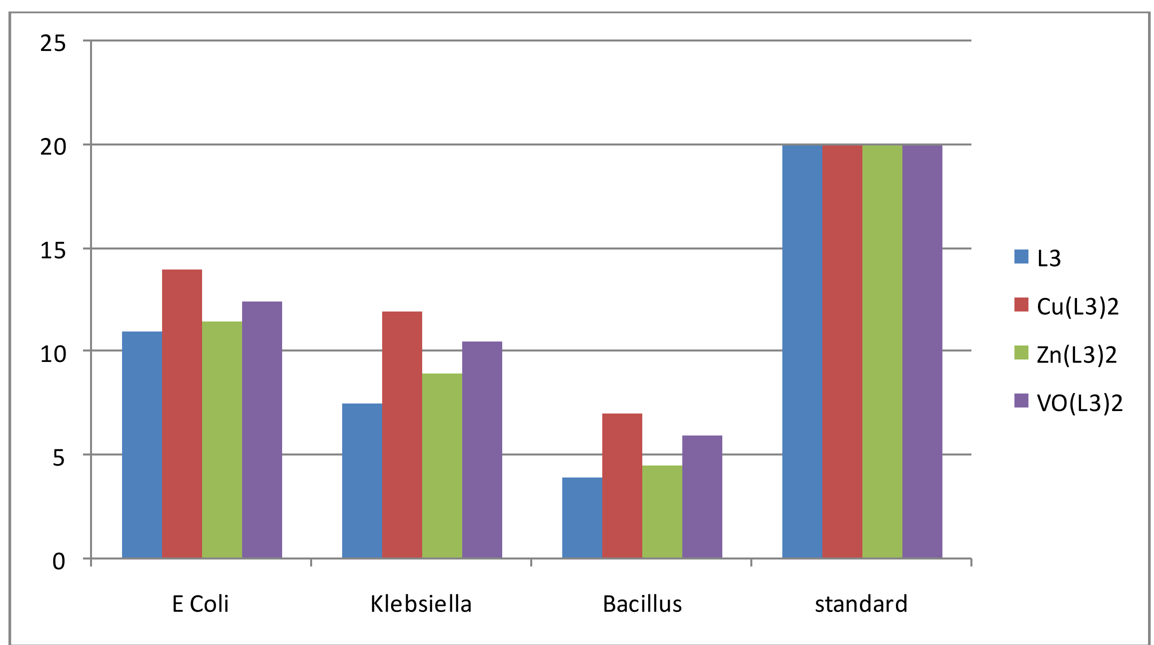


Fig.3.4.3 Antibacterial studies of 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione (L_3) and their complexes.

SECTION-V

ANTIFUNGAL STUDIES OF CHLORO SUBSTITUTED 1,7-DIARYL HEPTANOIDS AND THEIR VO(IV)METAL COMPLEXES

3.5.1 Antifungal studies of chloro substituted curcuminoid analogues and their VO(IV) complexes

The Chloro derivatives of curcuminoid analogues namely 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(3a), 1,7-bis(2-chloro phenyl)hepta-1,6-diene-3,5-dione(3b), and 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione(3c) and their VO(IV) complexes were studied for their antifungal nature.The activity was determined with the fungi species Aspergillus Niger, Penicillium Chrysogenum and Alternaria Alternate by Disc diffusion method. Ligands and their Vanadyl metal complexes with different concentrations [100, 250, 500µg/ml] were used as drugs. The results of the antifungal studies are represented in **Table 3.5.1, 3.5.2 and 3.5.3.**

Table3.5.1 Antifungal studies of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione (L_1) and its VO(IV) complexes

Fungi	Diameter of zone of inhibition in mm					
	L_1			$VO(L_1)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	9.5	11	14	12	15	17.5
Penicillium	10	12	15.5	12.5	15	18
Alternaria	10	13	16	13	16	18.5

Table 3.5.2 Antifungal studies of 1,7-bis(2-chloro phenyl)hepta-1,6-diene-3,5-dione (L_2) and its VO(IV) complexes

Fungi	Diameter of zone of inhibition in mm					
	L_2			$VO(L_2)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	10	12	13.5	12.5	16	18
Penicillium	10.5	12	15	13	15	19
Alternaria	11	13	15.5	13.5	16	19.5

It is observed from the results that as concentration of the test compound increases ,the antifungal nature increases.All the compounds show maximum results with a drug concentration of 500µg/ml.For the ligand with para chloro substituted phenyl ring maximum activity was seen with Alternaria species,with a zone of inhibition of 16mm.But the ligand was also quite active against Aspergillus and Penicillium at higher concentrations.The VO(IV) complexes were highly active against all fungi species.The zone of inhibition produced is 17.5mm,18mm,18.5mm with Aspergillus, Penicillium and Alternaria respectively.

The ligand with para chloro substituted phenyl ring was slightly more active than the ortho substituted derivative against all species.The VO(IV) complexes exhibited higher potency against all fungi species.The results are comparable with those of reference which showed remarkable activity with zone of inhibition 21 mm.

Table3.5.3 Antifungal studies of 1,7-bis(3,4-dichloro phenyl)hepta-1,6-diene-3,5-dione (L_3) and its VO(IV) complexes

Fungi	Diameter of zone of inhibition in mm					
	L_3			$VO(L_3)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	11.5	14	17	17.0	20	21
Penicillium	11	13	16	13.5	16	19
Alternaria	12	15	17	14.5	18	18.5

The ligand with dichloro substituted phenyl ring as well as its Vanadyl complex showed remarkable antifungal activity and is more active than the monochloro substituted derivative.The ligand as well as the complex possess significant activity at all concentrations. The ligand gave 73% activity and the complex showed 91% activity against Aspergillus species as compared with the activity of standard drug flucanazole.The VO(IV) complex exhibits promising antifungal nature.A comparative study of their activity is represented graphically in Fig.3.5.1.

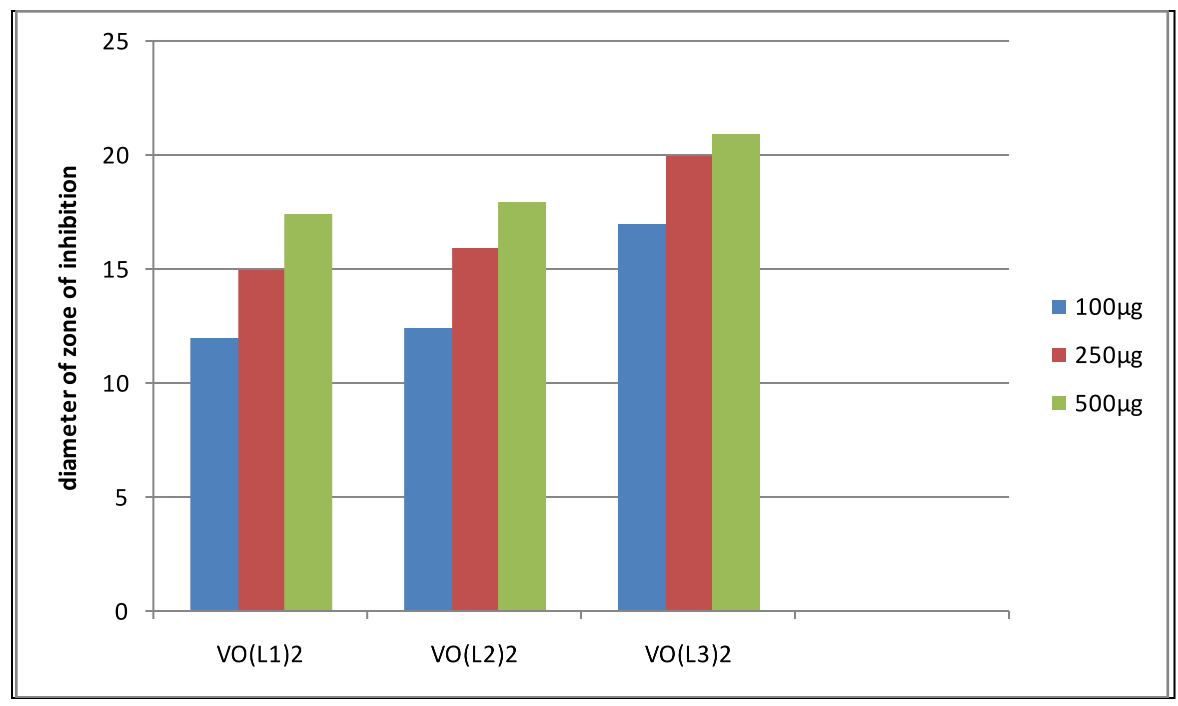


Fig.3.5.1 VO(IV)complexes of chloro analogues of 1,7-diaryl heptanoids against Aspergillus

The VO(IV) complex of dichloro compound gave a maximum of 21mm zone of inhibition against fungi Aspergillus with a con. 500µg. Thus among the chloro analogues the maximum activity was found with VO(IV) complex of 3c against Aspergillus with a con. 500µg. The values of VO(IV) complexes of chloro analogues with varying concentrations against Aspergillus are depicted in Fig.3.5.1. The results are in comparison with control disc.

CHAPTER-IV

SYNTHESIS, CHARACTERISATION AND BIOCHEMICAL ACTIVITIES OF DI &TRI SUBSTITUTED 1,7-DIARYLHEPTA-1,6-DIENE-3,5-DIONES AND THEIR TRANSITION METAL CHELATES WITH Cu(II), Zn(II), Ni(II) & OXOVANADIUM(IV)

SECTION I

SYNTHESIS AND CHARACTERIZATION OF 1,7-BIS(ARYL)HEPTA-1,6-DIENE-3,5-DIONES WITH DI &TRISUBSTITUTED PHENYL RING

This chapter deals with the synthesis of curcuminoid analogues with a disubstituted and trisubstituted phenyl ring in them.These compounds are quite similar to natural curcumin in structure which is 1,7-bis(3-methoxy-4-hydroxy phenyl)-hepta-1,6-diene-3,5-dione.It is a symmetrical disubstituted compound.Here three compounds namely 1,7-bis(3-ethoxy-4-hydroxyphenyl) hepta-1,6-diene-3,5-dione(4a),1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione(4b) and 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione(4c)are synthesized.The ligand 4a contains an ethoxy and hydroxyl group on the phenyl ring. The ligand 4b contains a disubstituted phenyl ring with hydroxyl group in 2,4 positions.The ligand 4c has been prepared with a trisubstituted phenyl ring with methoxy groups in 3,4,5 positions.

4.1.1. Synthesis of di and tri substituted 1,7–diphenyl heptanoids

The curcuminoid analogues with di and trisubstituted phenyl rings in them were prepared by the condensation of disubstituted benzaldehydes, (3-ethoxy-4-hydroxy benzaldehyde, 2,4-dihydroxy benzaldehyde) and trisubstituted benzaldehyde(3,4,5-trimethoxy benzaldehyde) with acetyl acetone-boric oxide complex in ethyl acetate medium in presence of tri(secbutyl) borate and n-butyl amine. The product formation can be represented in a schematic way in **Scheme 4.1.1.**

4.a- R_2 = $-OCH_2-CH_3$, R_3 = -OH, R_1, R_4 = H

4.b - R_1, R_3 = -OH, R_2, R_4 = H

4.c - R_1 = H, R_2, R_3 & R_4 = $-OCH_3$

Scheme 4.1.1

Here 2 moles of aldehyde reacts with 1 mole of 1,3-diketone to form the 1,7-diphenyl heptanoids.The above reaction produces two products namely 1,7-diaryl heptanoids as major product and 6-aryl hexanoids as minor product.The major products were purified by column chromatography over silica gel (60 – 120 mesh) using 4:1 (v/v) chloroform:acetone mixture as the eluent and recrystallised twice from hot benzene to get pure crystalline material. The aldehyde used for synthesis, structures of the compounds prepared, its systematic name, and yield are given in Table 4.1.1.

Table 4.1.1 Synthetic details of Di and Trisubstituted 1,7-(diaryl)hepta-1,6-diene-3,5-diones

Compounds	Aldehyde used for Synthesis	Structure of Ligands	Systematic name	Yield %
4a	3-ethoxy-4-hydroxy benzaldehye		1,7-bis(3-ethoxy-4-hydroxyphenyl) hepta-1,6-diene-3,5-dione	72
4b	2,4-dihydroxy benzaldehyde		1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione	65
4c	3,4,5-trimethoxy benzaldehyde		1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione	80

The compounds 4a, 4b & 4c are crystalline in nature,brownish black in colour and show sharp melting points.They are soluble in organic solvents like acetone, ethyl acetate,chloroform,methanol etc.The observed C,H percentage and molecular weight determination as given in Table 4.1.2 together with mass spectral data of the compounds suggest the structure of the compound is as given in Scheme 4.1.1. above.

Table 4.1.2 Physical, Analytical & UV spectral data of di & trisubstituted 1,7-diaryl heptanoids

Compounds	M.P. (^{o}C)	Elemental analysis (%) C	H	Molecular weight	UV λmax (nm)
		Found/(Calculated)			
4a	176	68.81(69.69)	5.71(6.06)	394(396)	337, 441
4b	179	66.81(67.05)	4.52(4.70)	339(340)	265, 431
4c	193	64.083(65.78)	5.96(6.14)	453(456)	269, 445

4.1.2 Characterisation of the compounds 4a,4b,4c.

The compounds1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione(4a),1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione(4b)and 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione(4c) synthesized were characterized by UV, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral techniques.

UV spectra

The electronic spectra of the compounds was recorded in methanol and the data are given in Table 4.1.2.All the compounds in methanolic solution showed a broad characteristic UV – visible absorption at around 300-500nm with maximum absorption band at wavelength in the range 260-340 nm and a weak absorption band in the range 420-445 nm.The maximum absorption is due to the electronic dipole allowed $\pi \rightarrow \pi *$ excitations of its extended conjugation system.The weak absorption band corresponds to dipole forbidden n$\rightarrow \pi *$ excitations.

IR spectra

The IR spectrum of all the compounds 4a, 4b & 4c showed a broad band at a range from 3200-3600 cm^{-1} which is due to ν OH group in enol form.The low intensity bands observed in the region 2500-3000 cm^{-1} are assigned to the aromatic ν C-H vibrations and methyl group motions.The important absorption band at nearly 1625 cm^{-1} corresponds to the stretching vibrations of C=O in the compounds.This is the most useful functional group available for IR elucidation.The value of carbonyl absorption is less than the value of the free ,unbound C=O group .This is a proof for C=O in conjugation with C=C and the existence of intra molecular hydrogen bonding.The probable IR assignments due to V(C=C) phenyl, V(C-C) alkenyl etc are given in Table 4.1.3.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.All compounds showed peaks due to V(CH=CH) trans vibrations .The IR spectra of compounds 4a, 4b and 4c are present in Fig.4.1.1 , Fig.4.1.2 & Fig.4.1.3 respectively.

Table 4.1.3 IR spectral data of di and tri substituted 1,7-diaryl heptanoids

Compounds			Probable IR assignments
4a	4b	4c	
1634	1624	1630	V(C=O) chelated
1578	1547	1580	V(C=C) phenyl
1508	1512	1539	V(C-C) alkenyl
1477	1480	1503	V_{as}(C-C-C) chelate ring
1438	1462	1454	V_{s}(C-C-C) chelate ring
1116,1038	1130,1064	1120,1039	β(C-H) chelate ring
969	966	994	V(CH=CH) trans

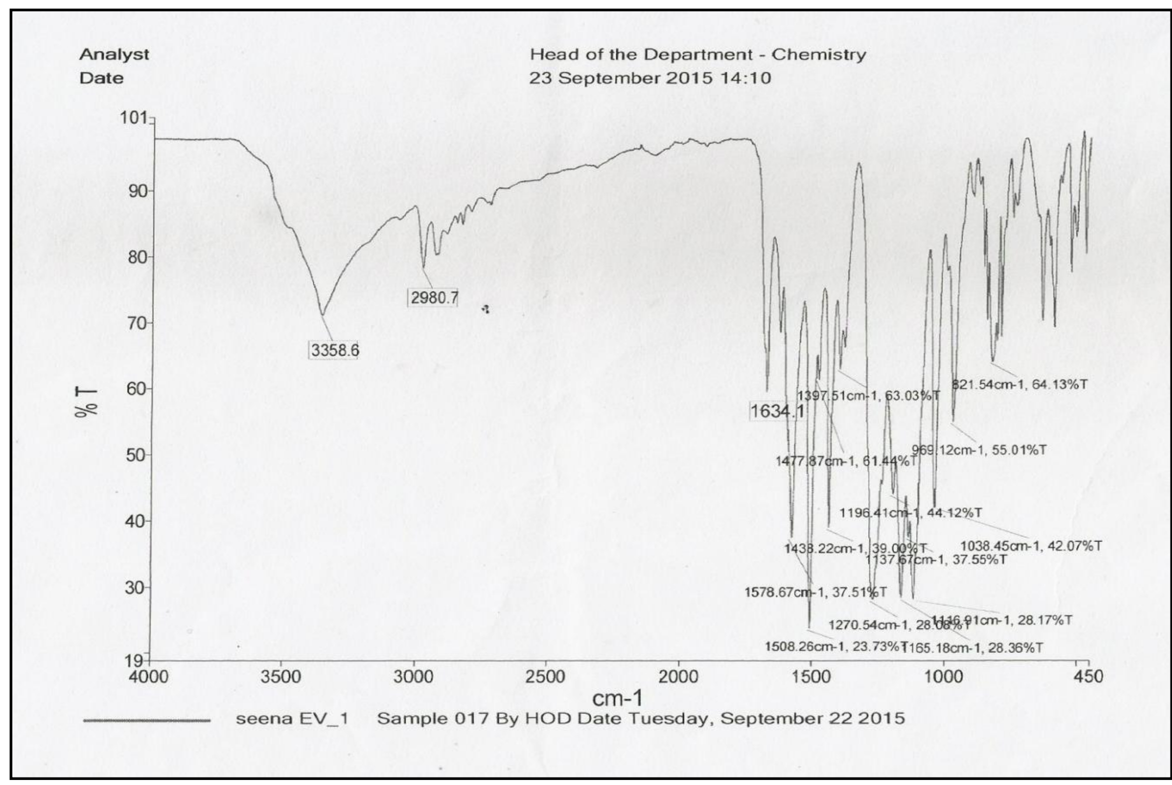


Fig.4.1.1 IR spectrum of 1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione

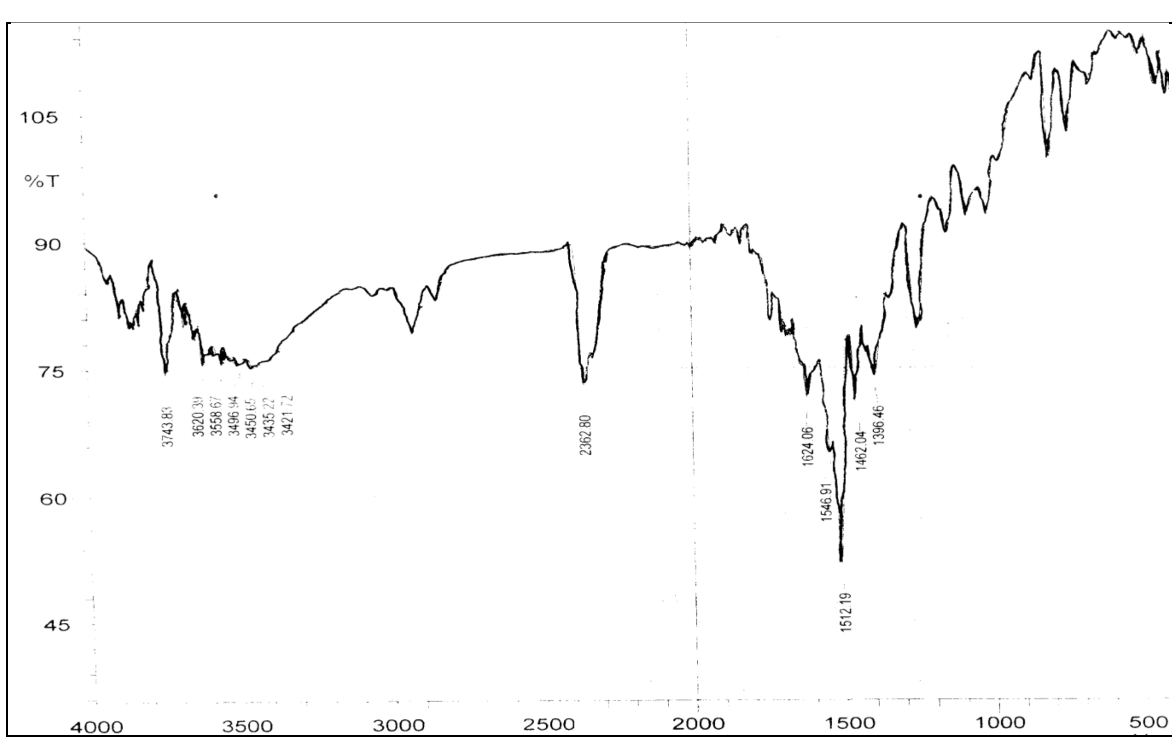


Fig.4.1.2 IR spectrum of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione

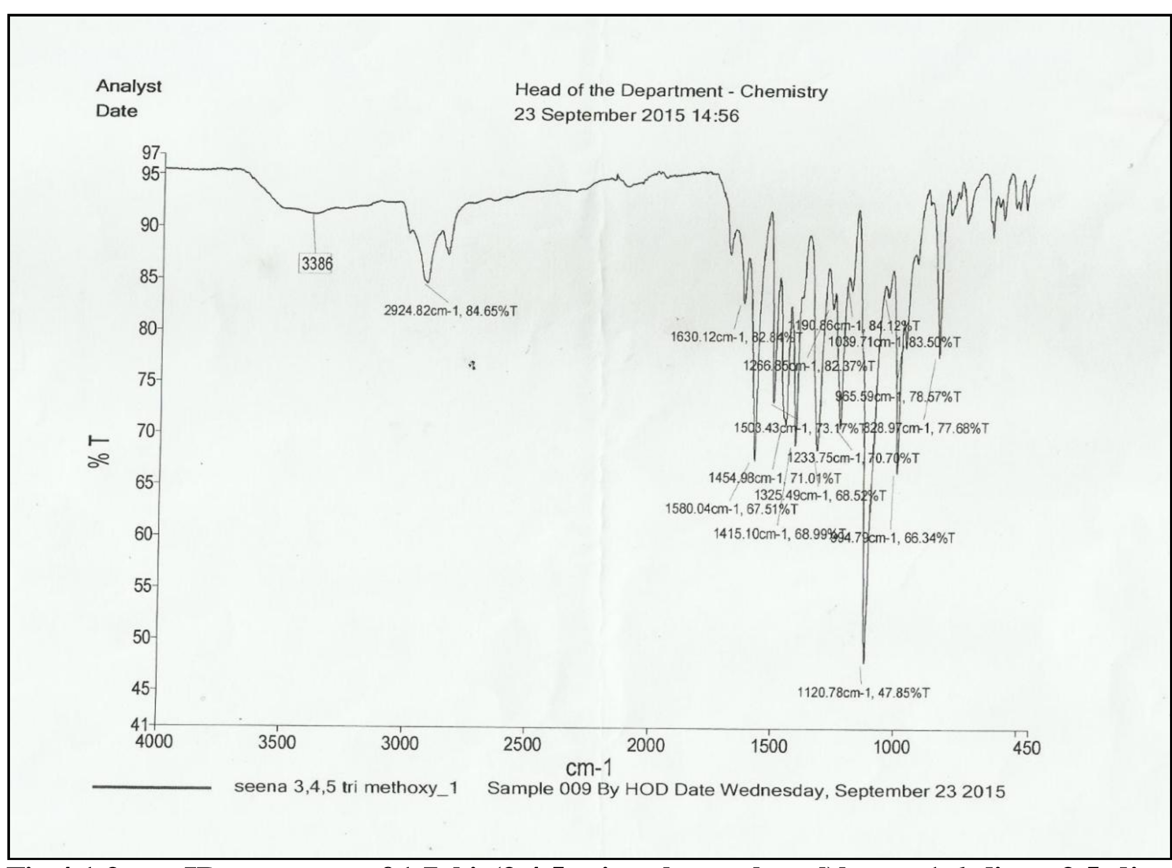


Fig.4.1.3 IR spectrum of 1,7-bis(3,4,5-trimethoxy phenyl)hepta-1,6-diene-3,5-dione

^{1}H NMR spectra

The ^{1}H NMR spectra of di & tri substituted 1,7-diaryl heptanoids show specific peaks corresponding to enolic, methine, alkenyl, methyl, phenyl and phenolic groups (**Table 4.1.4**).

Table 4.1.4 ^{1}H NMR spectral data of di and tri substituted 1,7-diphenyl heptanoids

Compound	Chemical shifts (δ ppm)							
	Enolic	Methine	Alkenyl	Phenyl	Substituent			
					OCH_2	OCH_3	Phenolic	CH_3
4a	16.02	5.89	6.042-7.611	7.051-7.135	4.17-4.22	-	9.832	1.480-1.514
4b	16.08	6.09	6.9 – 8.35	7.1 – 7.9			10.04	
4c	16.10	5.913	6.52-7.623	7.15-7.289	-	3.91-3.96	-	-

Ligands displayed a one proton singlet at ~ 16ppm assignable to strong intra molecularly hydrogen bonded enolic proton . Another one proton singlet at ~ 6ppm corresponds to the strong intra molecularly hydrogen bonded methine proton.The enolic proton and methine proton can be identified from the structure given below. Phenyl protons are present at a very specific region of 7.0 – 7.9 ppm whereas alkenyl protons are distributed over a region of 6.4 – 8.35ppm. The ^{1}H NMR spectra of 4a & 4c are depicted in Fig.4.1.5. & Fig.4.1.6 respectively.

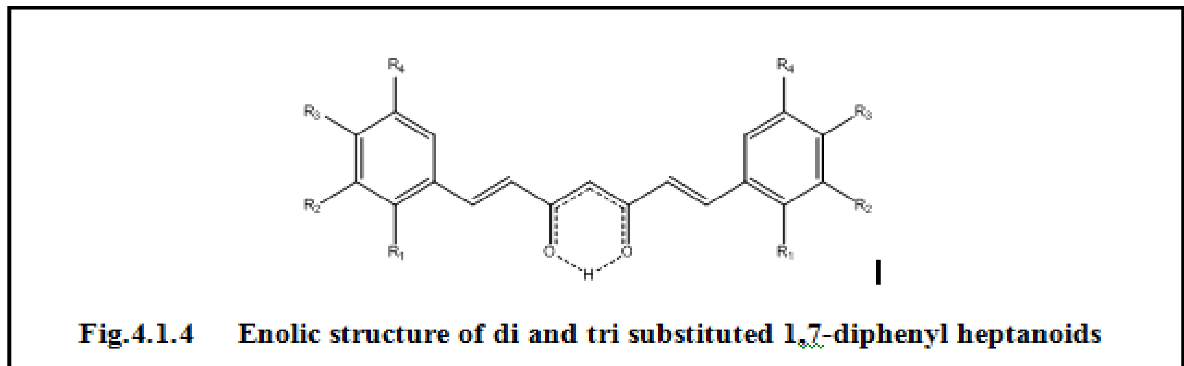


Fig.4.1.4 Enolic structure of di and tri substituted 1,7-diphenyl heptanoids

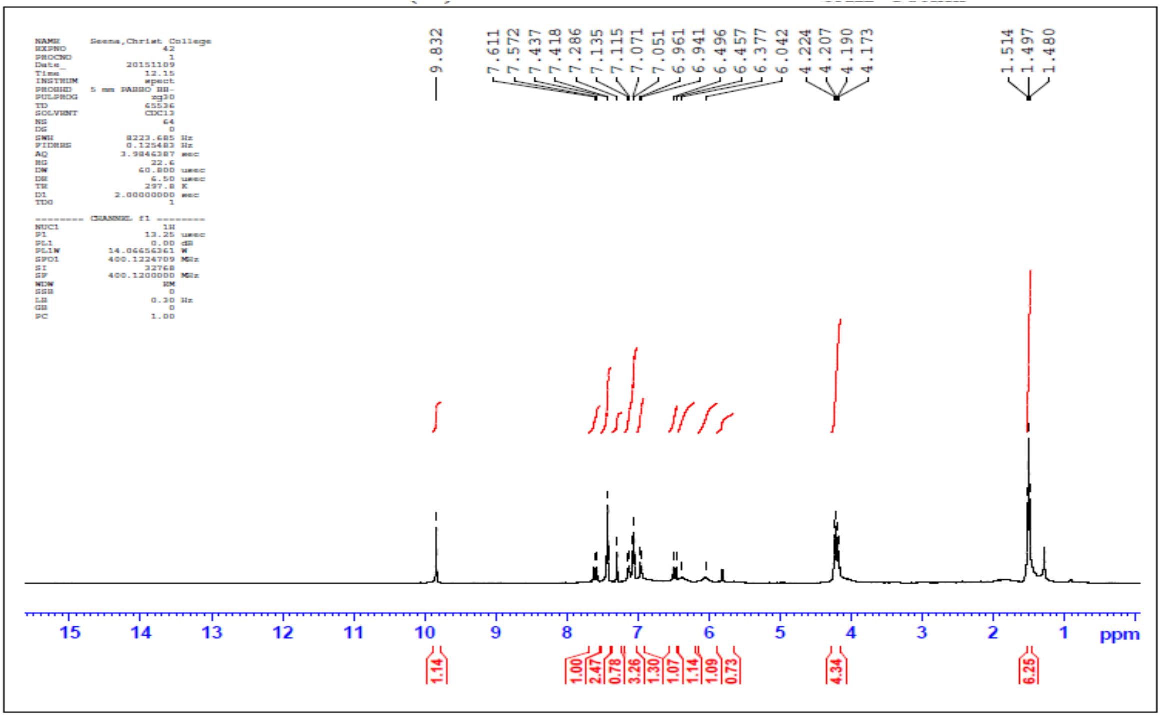


Fig.4.1.5 ^{1}H NMR spectrum of 1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione

The methyl protons of 4a are observed at a region of 1.480-1.514 whereas that of $–OCH_2$-protons are present at a region of 4.17-4.22 ppm. Methoxy protons of 4c are observed at 3.96ppm. The phenolic proton of 4a is seen at 9.832 ppm whereas that of 4b is observed at 10.04ppm.

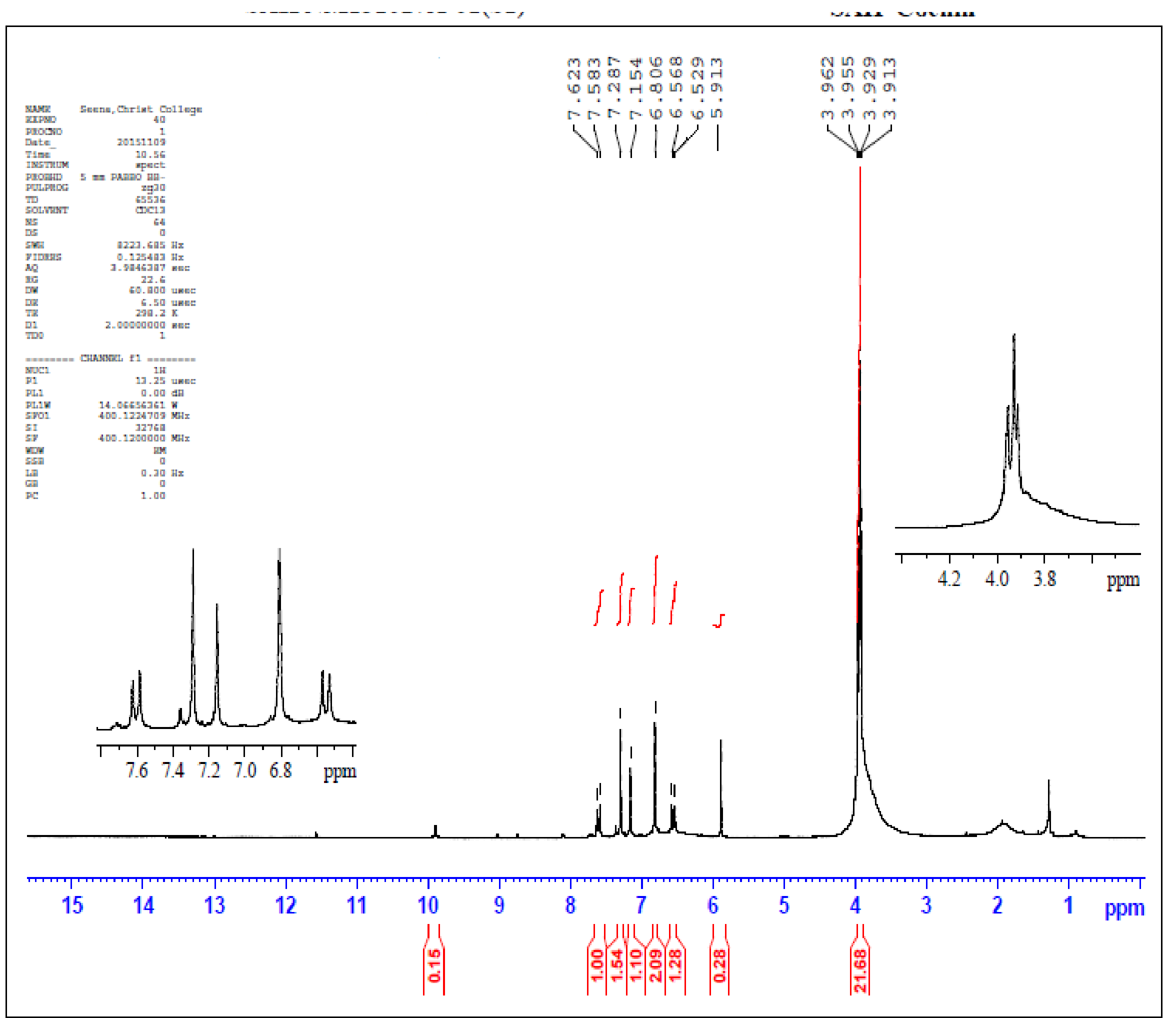


Fig.4.1.6 ^{1}H NMR spectrum of 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione)

^{13}C NMR spectra

The ^{13}C NMR spectral data of 4a, 4b & 4c are given in Table 4.1.5, 4.1.6 & 4.1.7. **^{13}C NMR spectra of 4a & 4c** are depicted in Fig.4.1.7 & 4.1.8. The peak corresponding to methine (C1) carbon is present ~ at 105ppm. Here also there is a possibility of keto-enol tautomerism which makes the shift of C1 carbon to ~ at 100ppm. C2 carbon of carbonyl appears at a position at~ 185 ppm. The alkenyl carbon are present at a position nearer to the phenyl ring system. The aromatic carbon atoms are present between 115 – 160 ppm. In 4a C7 carbon which is attached to the ethoxy group is seen at 160.927ppm whereas that of C8 attached to hydroxyl group is seen at 148.018 ppm. The ethoxy carbon atoms of 4a are observed at 64.63 & 29.63 ppm.

Table 4.1.5 ^{13}C NMR spectral data of 4a (chemical shift in ppm)

C1	**C2,C2’**	**C3,C3’**	**C4,C4’**	**C5,C5’**	**C6,C6’**
110.64	183.29	140.631	122.71	146.108	127.263
C7,C7’	**C8,C8’**	**C9,C9’**	**C10,C10’**	**C11,C11’**	**C12,C12’**
160.927	148.018	121.707	114.826	64.638	29.69

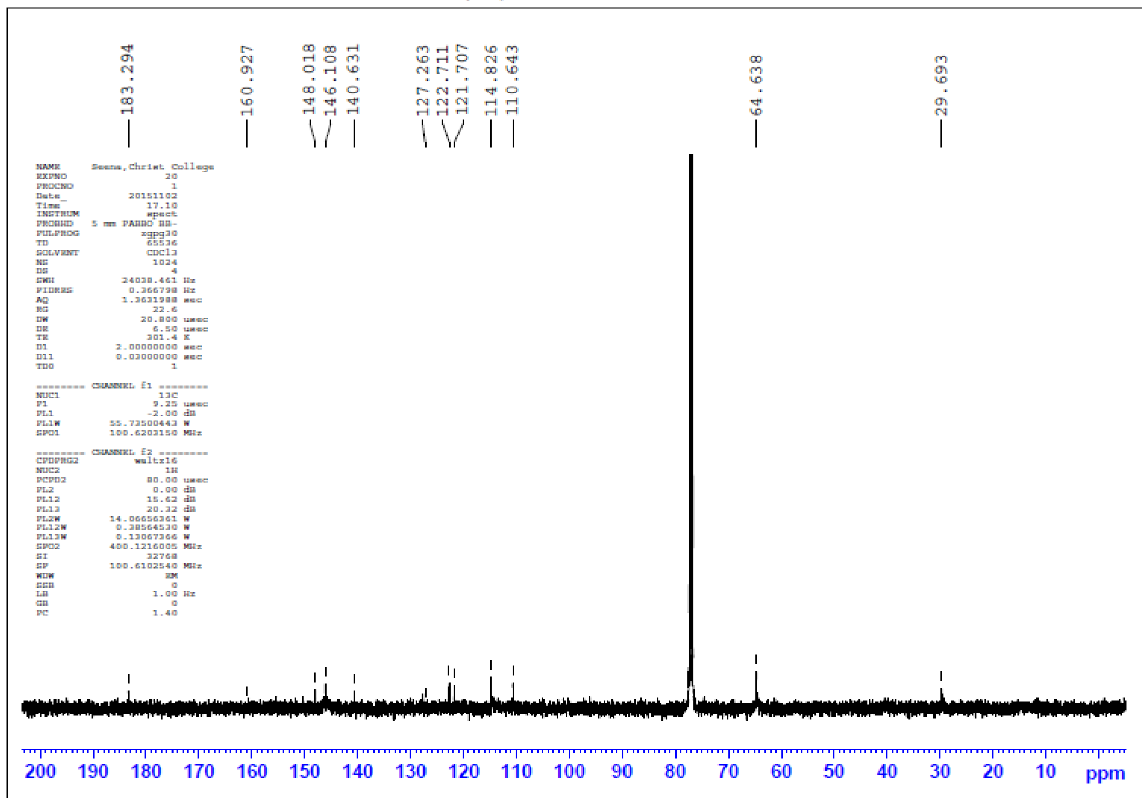


Fig.4.1.7 ^{13}C NMR spectrum of 1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione

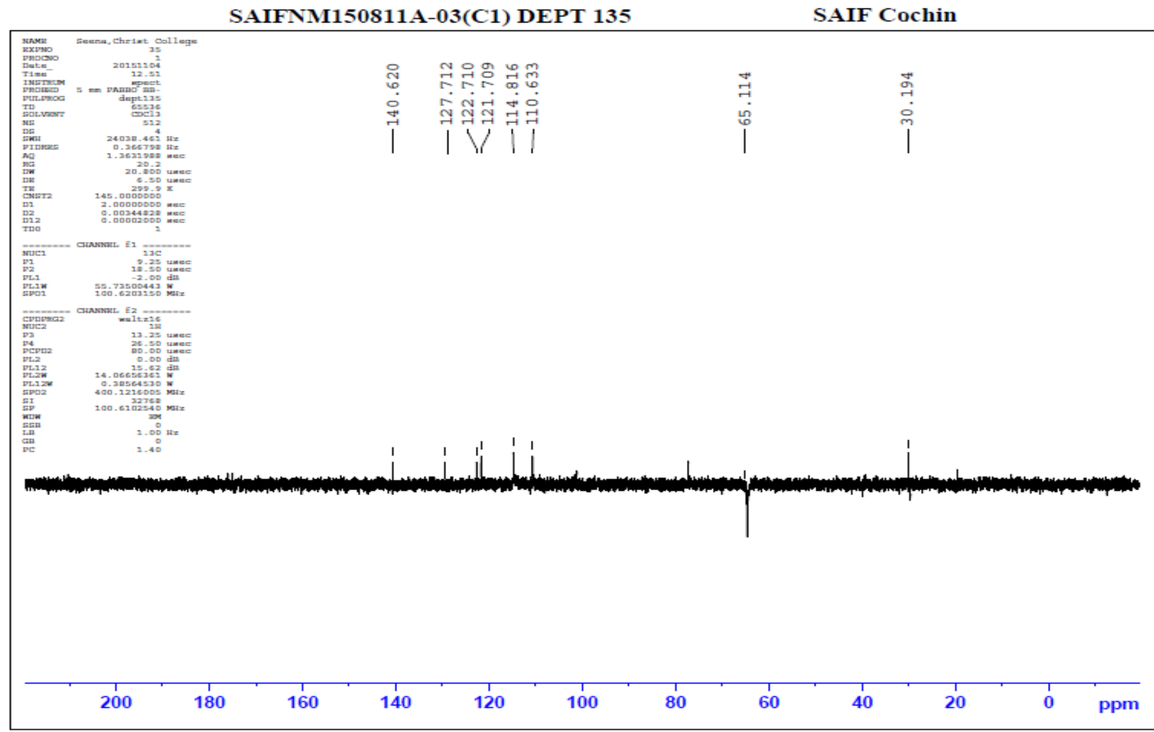


Fig.4.1.8 DEPT-135 spectrum of 1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione

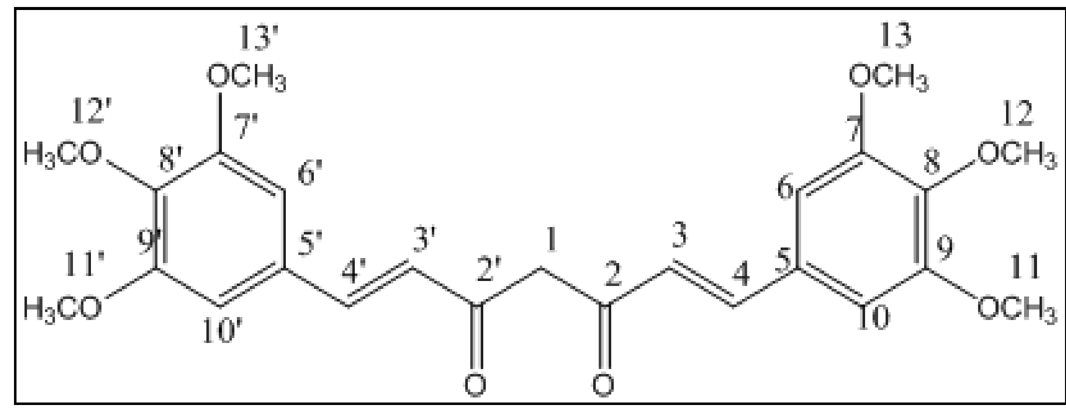
The **DEPT-135** spectrum helps to identify the carbon atoms which bear no hydrogen atoms by comparison with the ^{13}C NMR Spectrum.It also gives idea about the –CH2 groups present in the compound since the signals arising from the group forms a negative peak.Here the peak at 65 is a negative peak and so it is confirmed that C11 is a methylene carbon.Comparing with ^{13}C NMR spectrum,the peaks absent in DEPT spectrum are due to C2,C5,C7 and C8 which are carbons with no hydrogens on it.

Table 4.1.6 ^{13}C NMR spectral data of 4b (chemical shift in ppm)

C1	**C2,C2'**	**C3,C3'**	**C4,C4'**	**C5,C5'**
102.43	185.5	139.54	121.46	134.82
C6,C6'	**C7,C7'**	**C8,C8'**	**C9,C9'**	**C10,C10'**
134.35	130.32	135.65	122.45	130.82

In 4b, the C1 carbon seen between two carbonyl groups gave a peak at 102 ppm. The C2 carbon of the carbonyl group produced a peak at 185.5 ppm.The hydroxyl groups are attached to carbons C6 & C8 and the peaks are observed at positions 134.35 & 135.65 respectively. The carbons in the aromatic ring are observed in the range 120-135 ppm.

Table 4.1.7 ^{13}C NMR spectral data of 4c (chemical shift in ppm)



C1	C2,C2’	C3,C3’	C4,C4’	C5,C5’	C6,C6’	C7,C7’
105.34	191.09	140.620	106.770	131.72	140.15	153.67
C8,C8’	**C9,C9’**	**C10,C10’**	**C11,C11’**	**C12,C12’**	**C13,C13’**	
163.67	153.494	123.407	56.197	61.016	56.298	

In 4c methoxy groups are attached to carbon atoms C7, C8 & C9. The peaks are down shielded and are observed at position 163.67 ppm for C8 and 153.4 for C7 & C9. Methoxy carbon atoms are present at positions 56.29ppm & 61.01ppm.

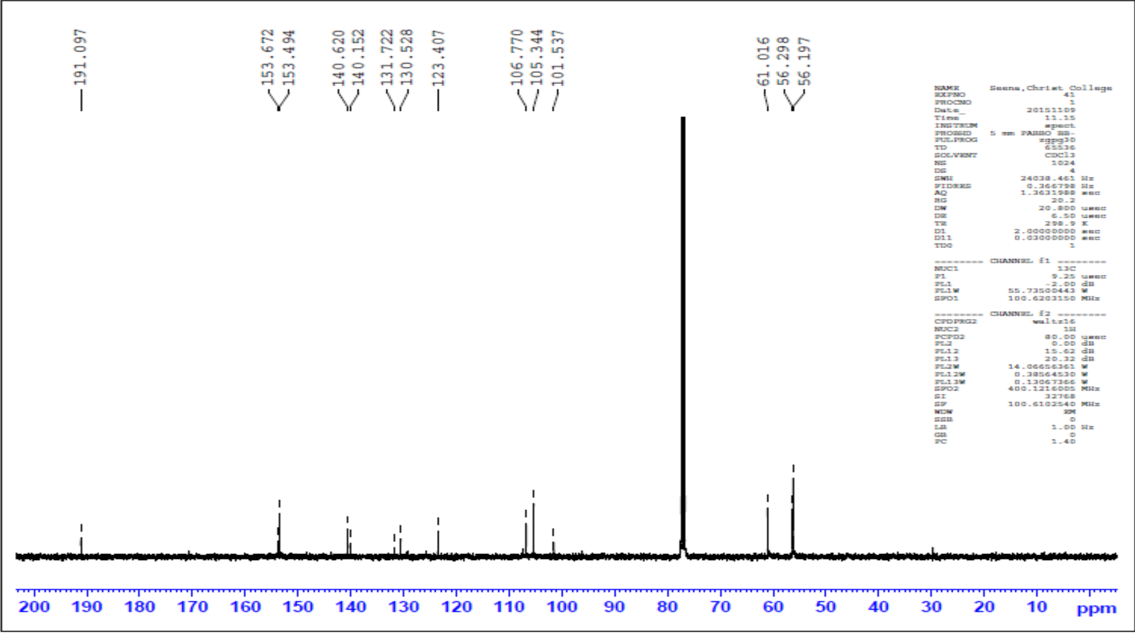


Fig.4.1.9 ^{13}C NMR spectrum of 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione)

2 D COSY NMR

The proton NMR Spectrum of the compound being studied is plotted along both the horizontal and vertical axes.The important peaks in the spectrum are the off diagonal spots.The presence of off diagonal spots in the spectrum correlates the different proton spots and confirms the different protons coupled with each other.The 2D H-H COSY NMR Spectrum of 4a is given below in Fig.4.1.10.The protons that are not coupled to other protons in the molecule do not show off diagonal peaks.The different protons in the compound and their couplings can be identified from the spectrum.The 2D C-H HETCOR Spectrum of 4a is given below in Fig.4.1.11.This spectrum gives the correlations between proton peaks and carbon peaks.The spots in the spectrum helps to identify the different hydrogens attached to the different carbons.Here the spots are observed corresponding to their peaks in the ^{13}C NMR Spectrum.

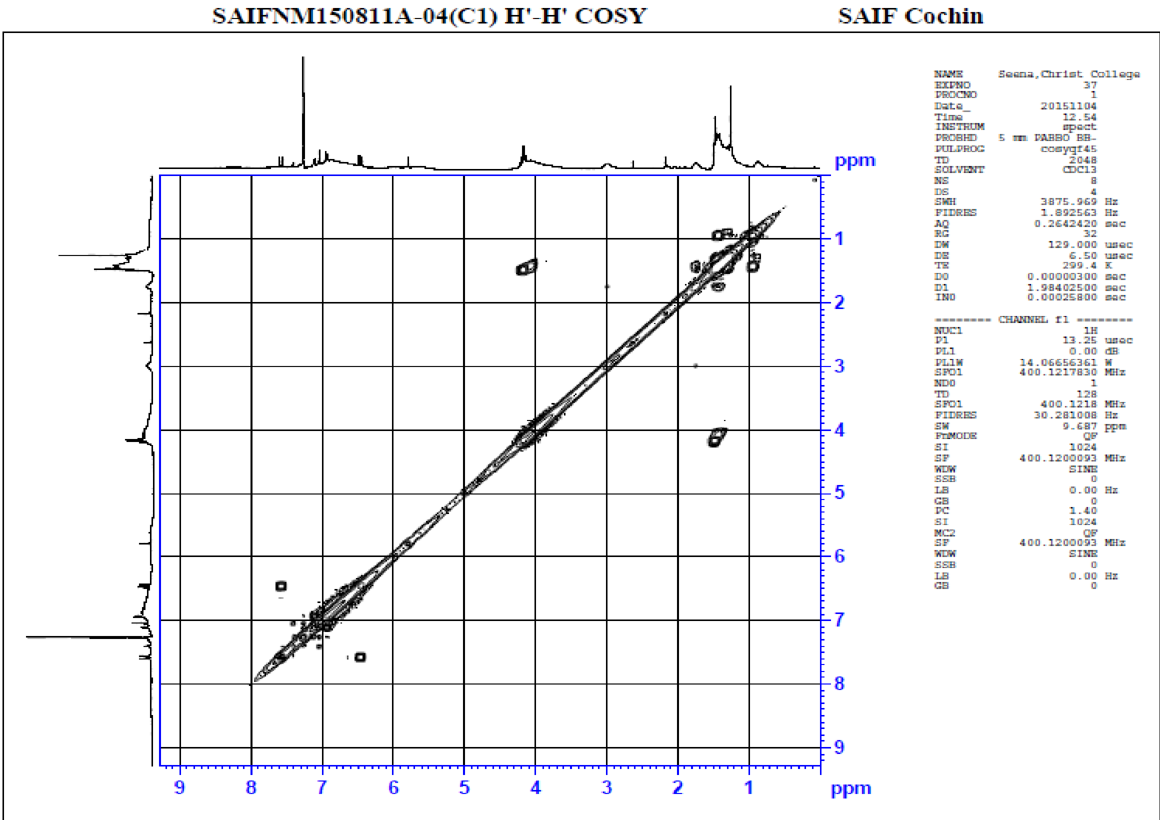


Fig.4.1.10 2 D H-H COSY NMR Spectrum of 1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione

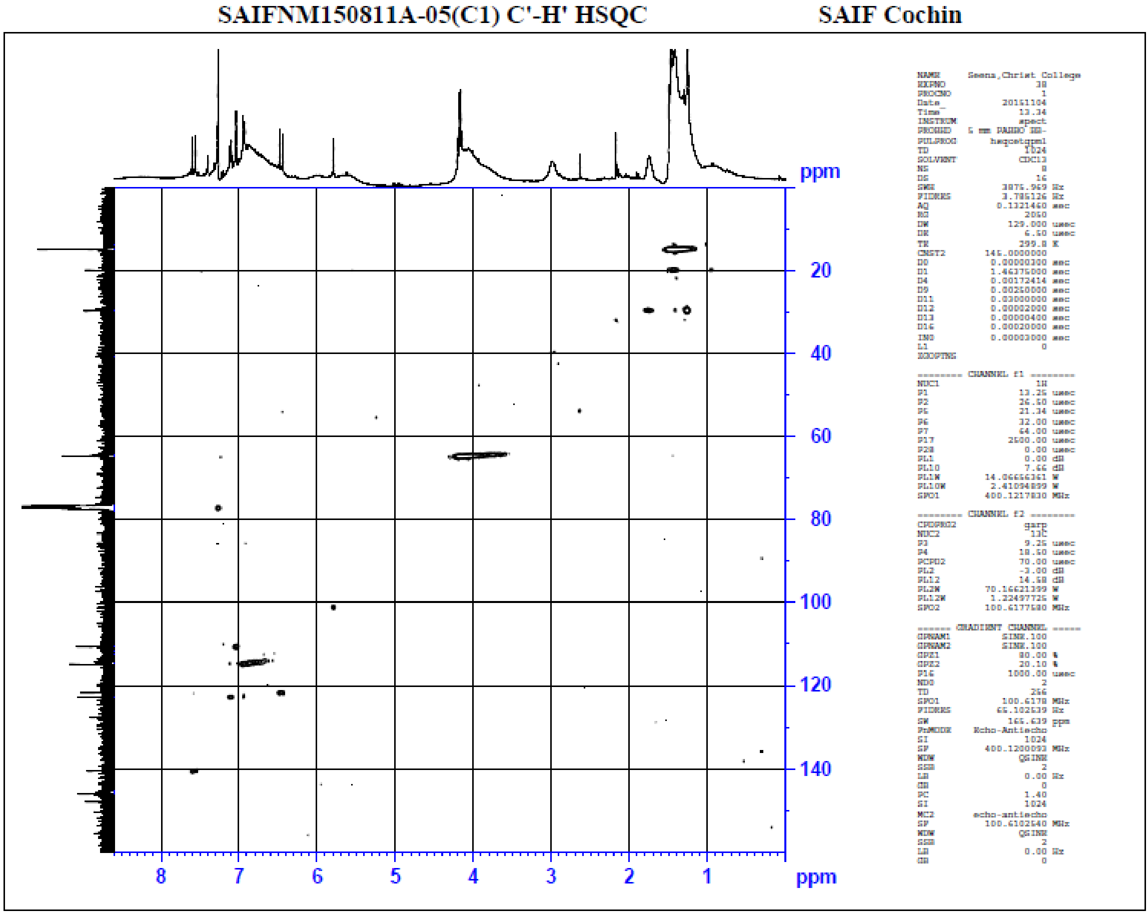


Fig.4.1.11 2 D C-H COSY NMR Spectrum of 1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione

Mass spectra

The mass spectra of 4a shows distinct M+1 ion peak at 397. Elimination of important groups from the molecule gives different fragments (Scheme 4.1.2) and the values are depicted in Table 4.1.8. The mass spectrum of 4a is given in Fig.4.1.12. Smaller fragments like O, OH, CH_2 etc. are removed from the molecular ion and give the corresponding peaks in the Spectrum.

Table 4.1.8 Mass spectral data of 4a, 4b & 4c

Fragments*	Ligands	M+/ M+1 Ion	A	B	C	D	E	F	G	H
Mass pattern	4a	397.6	205	191	151	163	139	162	257	233
	4b	341	176	162	121	134	110	133	230	205
	4c	456	234	221	180	207	168	206	288	263

*The alphabets corresponds to the fragments given in **Scheme 4.1.2**

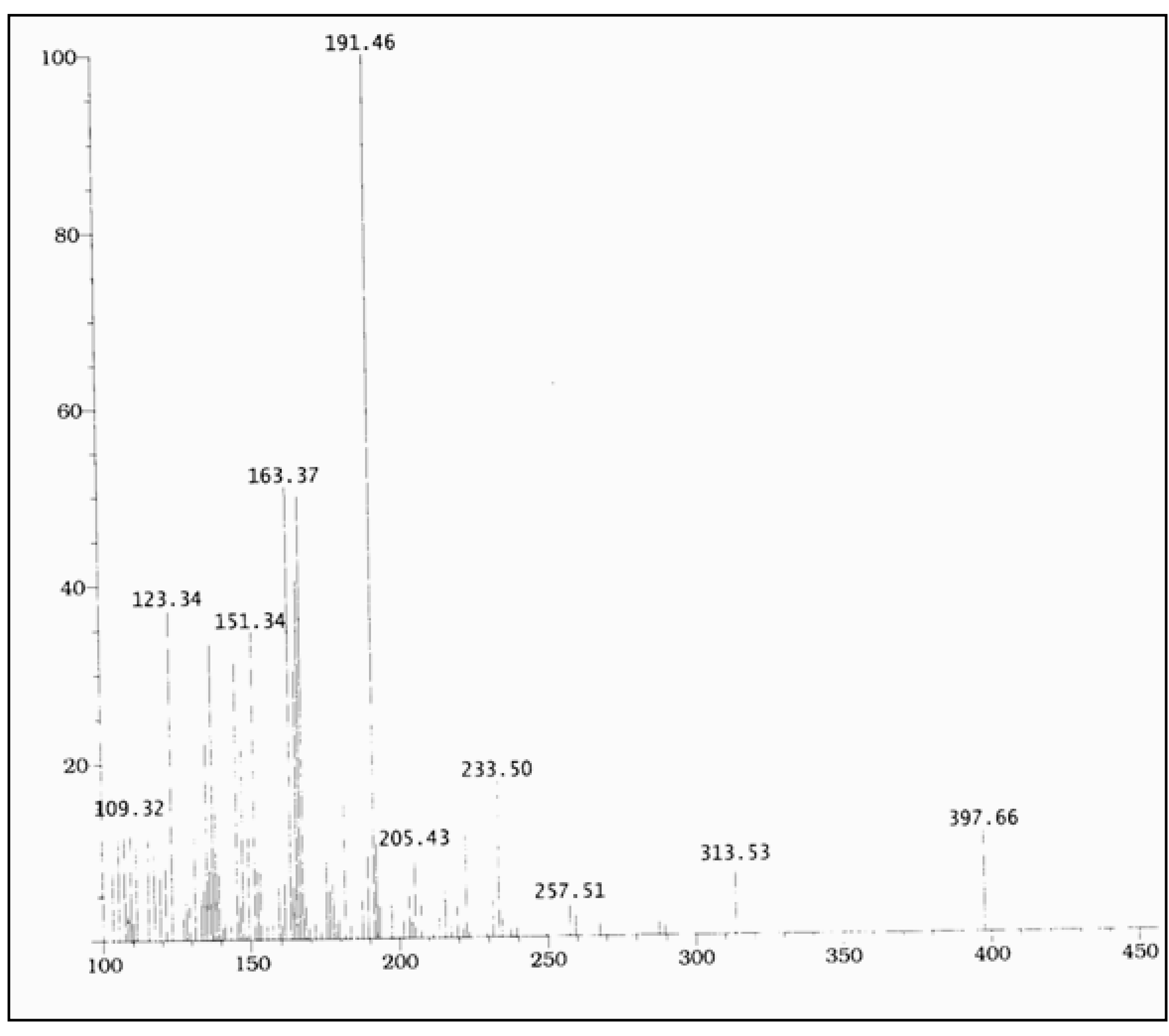


Fig.4.1.12 Mass Spectrum of 1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione

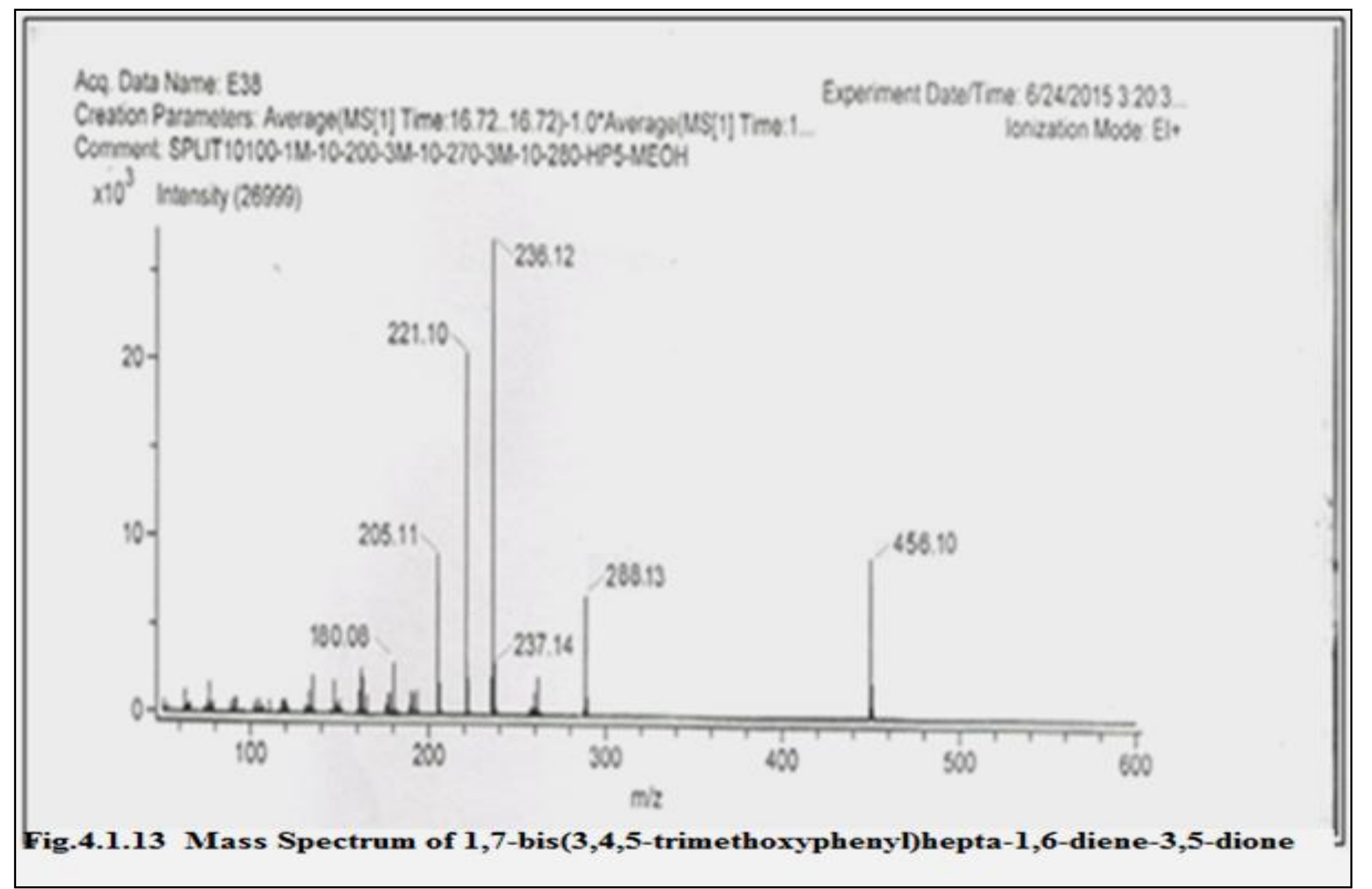
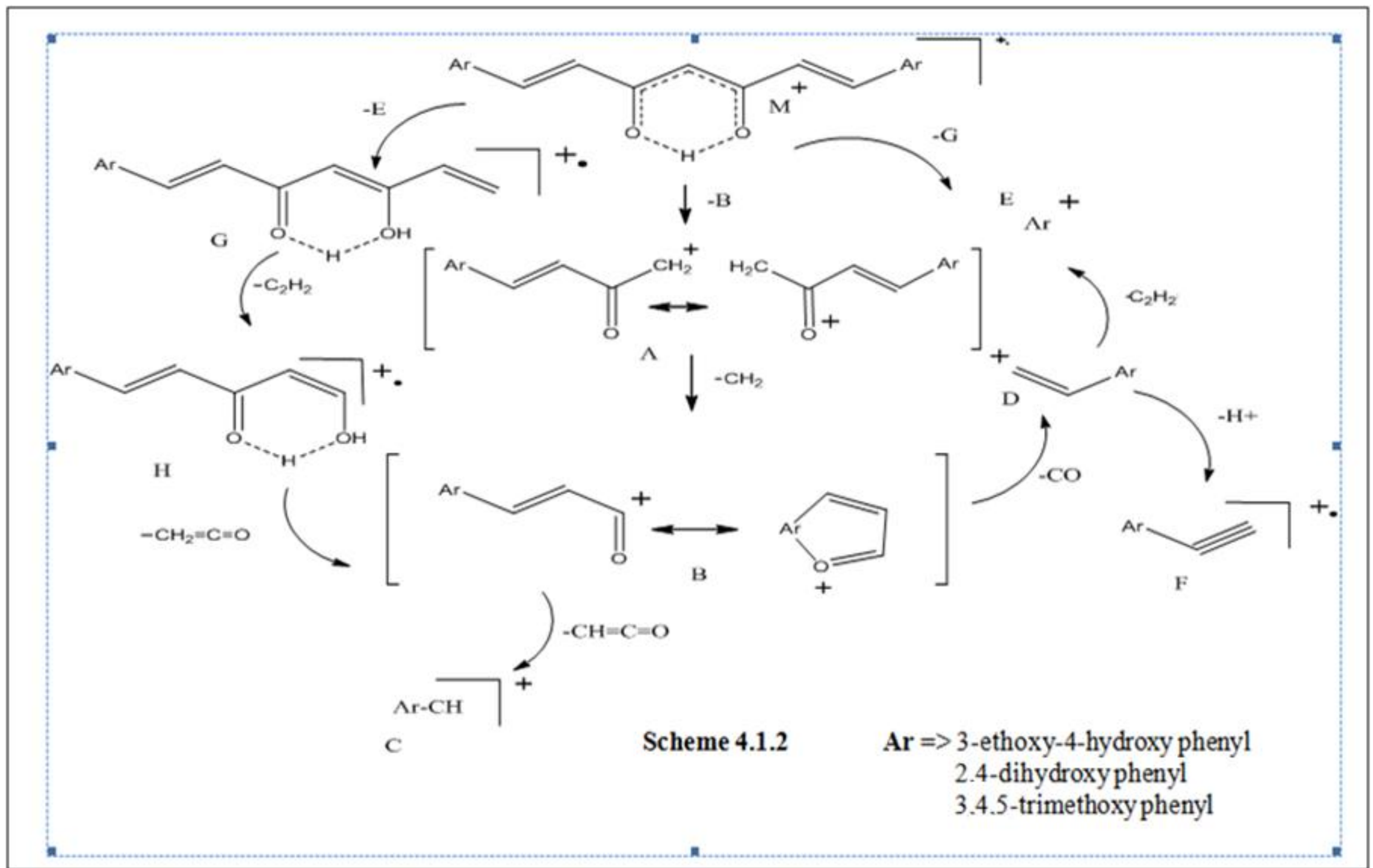


Fig.4.1.13 Mass Spectrum of 1,7-bis(3,4,5-trimethoxyphenyl)hepta-1,6-diene-3,5-dione



Ar
Ar
M
-E
-G
G
-B
E
Ar
-C2H2
CH2
H2C
Ar
Ar
A
-C2H2
Ar
-CH2
D
Ar
H
-H+
-CO
-CH2=C=O
Ar
B
Ar
F
-CH=C=O
Ar-CH
C
Scheme 4.1.2
Ar => 3-ethoxy-4-hydroxy phenyl
2.4-dihydroxy phenyl
3.4.5-trimethoxy phenyl

SECTION-II

SYNTHESIS AND CHARACTERISATION OF TRANSITION METAL CHELATES OF 1,7-DIARYL HEPTANOIDS WITH DI &TRI SUBSTITUTED PHENYL RING

4.2.1 Synthesis of metal complexes of 1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione(4a),1,7-bis(2,4-dihydroxyphenyl)hepta-1,6-diene-3,5dione(4b)and1,7bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione(4c)

Copper(II), Zinc(II), Nickel(II) and Oxovanadium(IV) complexes of curcuminoid analogues with dihydroxy,ethoxy hydroxy and trimethoxy substituted phenyl rings were synthesized by the following general method. To a refluxing solution of the ligand(0.002mol) in methanol (25ml),a methanolic solution of metal salt (0.001mol)was added and the reaction mixture was refluxed for nearly 2 hrs and cooled to room temperature.The metal salts used were Copper acetate, Zinc acetate, Nickel acetate and Vanadium (IV)oxide sulphate for the preparation of Cu(II), Zn(II), Ni(II) & VO(IV) complexes respectively.The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol.The general reaction involved in the formation of complexes is given below in Scheme.4.2.1.

Scheme 4.2.1

4.2.2 Characterisation of transition metal complexes of curcuminoid analogues with di & tri substituted phenyl rings

Metal chelates (Cu, Zn, Ni, Vanadyl) of ligands 4a, 4b & 4c were characterized using analytical and various spectral techniques like UV, IR, NMR and mass data. Elemental analysis (C, H and metal percentages), physical data, UV and IR spectral data are given in Table 4.2.1, 4.2.2 & 4.2.3. The analytical data together with mass spectral data suggest a ML_2 stoichiometry for all the synthesized complexes.

Table 4.2.1 Analytical and spectral data of metal complexes of 1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione(4a)

Metal chelates	M.P. (^{o}C)	Elemental analysis (%) Found/(calculated)			UV λmax (nm)	Characteristic IR stretching bands (cm^{-1})		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu(II)	210	63.65 (64.675)	5.37 (5.39)	7.15 (7.44)	345, 449	1586	1505	457, 425
Zn(II)	208	64.11 (64.531)	5.31 (5.378)	7.501 (7.646)	347, 450	1581	1508	450, 426
Ni(II)	205	64.73 (65.041)	5.13 (5.42)	6.75 (6.915)	349, 451	1582	1509	455, 423
VO(IV)	216	64.31 (64.41)	5.07 (5.368)	5.87 (5.944)	352, 453	1596	1510	475, 428

Table 4.2.2 Analytical and spectral data of metal complexes of 1,7-bis(2,4-dihydroxyphenyl)hepta-1,6-diene-3,5dione

Metal chelates	M.P. (°C)	Elemental analysis (%) Found/(calculated)			UV λmax nm	Characteristic IR stretching bands (cm^{-1})		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu(II)	189	61.02 (61.49)	3.837 (4.046)	8.31 (8.564)	273, 439	1615	1530	460, 428
Zn(II)	164	61.47 (61.89)	3.95 (4.072)	7.05 (7.967)	275, 440	1604	1527	474, 423
Ni(II)	166	61.05 (61.34)	4.03 (4.036)	8.85 (8.79)	278, 442	1564	1517	464, 428
VO(IV)	168	61.13 (61.21)	4.08 (4.627)	6.358 (6.838)	283, 446	1587	1515	480, 419

Table 4.2.3 Analytical and spectral data of metal complexes of 1,7bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione

Metal chelates	M.P. (°C)	Elemental analysis (%) Found/(calculated)			UV λmax nm	Characteristic IR stretching bands (cm^{-1})		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu(II)	254	60.87 (61.63)	5.24 (5.547)	6.354 (6.523)	446, 271	1594	1525	461, 432
Zn(II)	239	61.19 (61.51)	5.44 (5.536)	6.61 (6.705)	449, 273	1593	1516	468, 429
Ni(II)	241	61.02 (61.93)	5.28 (5.575)	5.98 (6.059)	446, 274	1592	1525	469, 424
VO(IV)	250	61.04 (61.41)	5.27 (5.527)	5.02 (5.214)	448, 274	1589	1517	478, 417

UV spectra

The UV spectra of complexes are quite similar to that of the spectra of respective ligands with two absorption maxima corresponding to n→π* transition and π→π* transition.The complexes showed maximum absorption shifted by 1-8nm which indicate the involvement of the carbonyl oxygens of compounds in metal complexation.The variation of absorption peak depends on the nature of the metal ion. For comparison, the UV spectra of ligand 4a and its Cu(II) complex are shown in Fig.4.2.1.

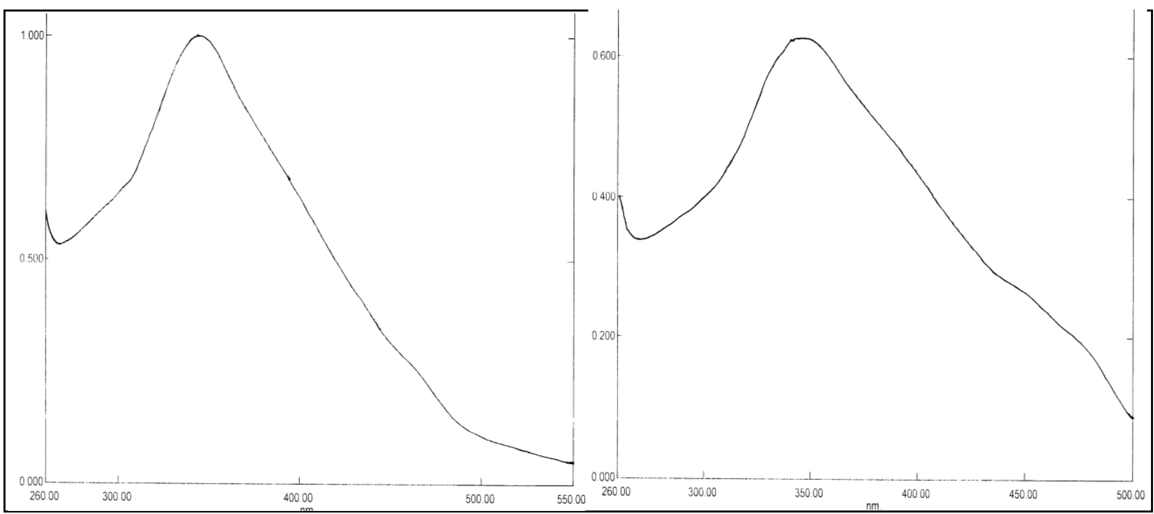


Fig.4.2.1 UV spectra of 4a and its Cu(II) complex

IR spectra

The IR spectra of all metal complexes are similar but different from that of the ligands.A representative IR spectra of the complex has been depicted in Fig.4.2.2.The spectral details are summarized in Tables 4.2.1, 4.2.2 & 4.2.3.In complexes the bands due to C=O stretching is shifted to lower frequencies during complexation.The peak of carbonyl moiety which is present at ~ 1630 cm^{-1} disappeared and a new band appeared at ~ 1590 cm^{-1}. This suggests that the ligand coordinates with the central metal ions through the C=O group.The broad band in the region of 2600 -3500 cm^{-1} present in the ligand also reduced in the spectra of

complexes which indicate replacement of the chelated proton by the metal ion during complexation.The new bands occurred in the range 460 and 420 cm $^{-1}$ further supports the formation of M-O bond.(metal-oxygen). The IR spectrum of Cu(II) complex of 4a is depicted in Fig.4.2.2. The IR spectrum of Ni(II) complex of 4b is depicted in Fig.4.2.3.

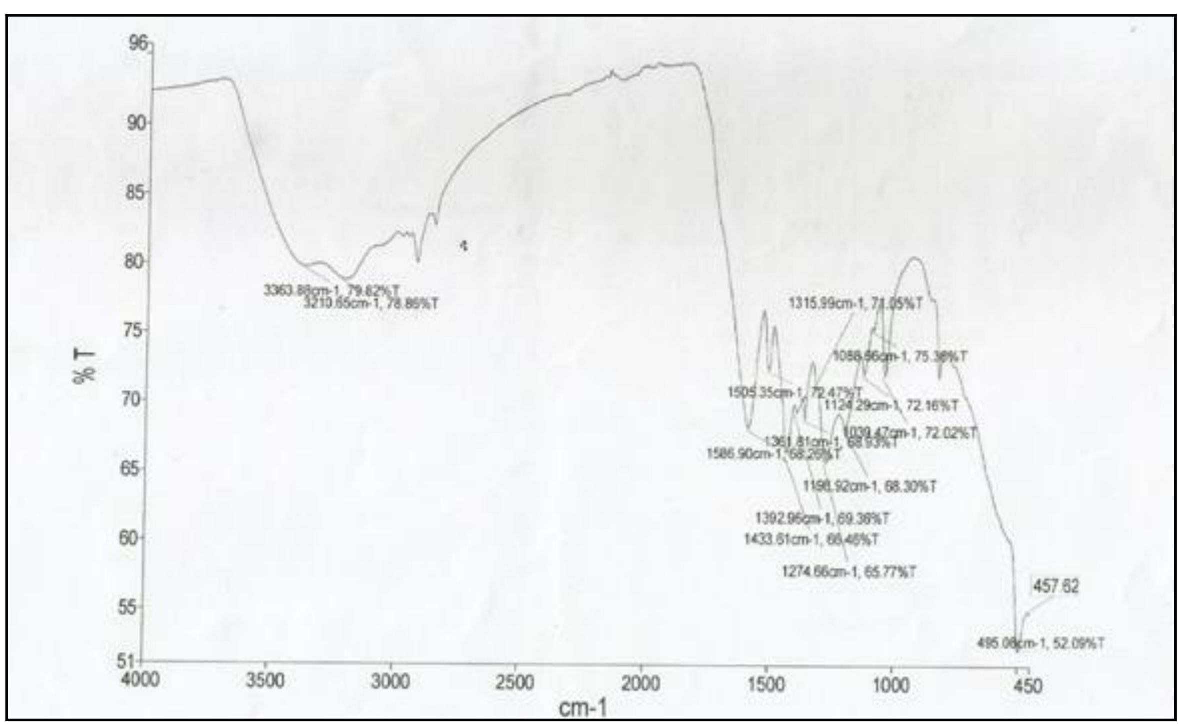


Fig.4.2.2. IR spectrum of Cu(II) complex of 4a

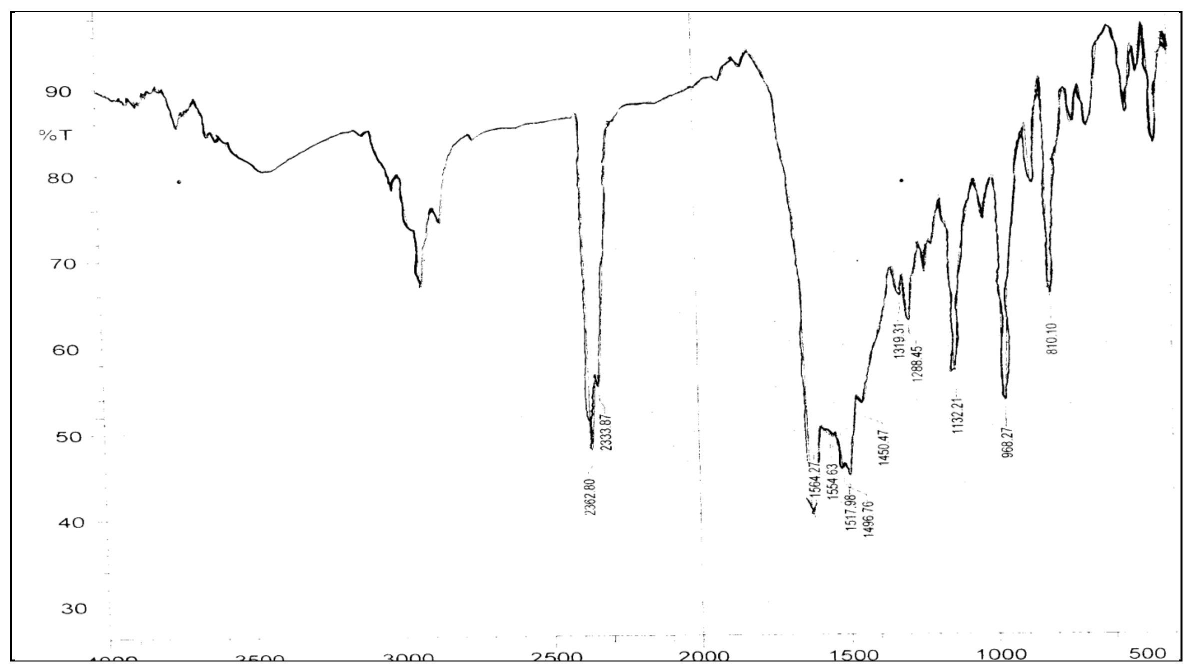


Fig.4.2.3 IR spectrum of Ni(II) complex of 4b

^{1}H NMR spectra

The enolic proton present in the ligands is replaced by metal atom in metal complexes. This is evident by the disappearance of the signal at δ ~ 16ppm in metal complexes. The phenyl and alkenyl protons are not altered much since they are not involved in metal complexation. There is a slight shift of methine signals to the downfield of the spectra. Thus the spectra of ligand and complexes are much similar except those of enolic proton.

Mass spectra

Mass spectral fragments are another important tool in elucidating the structure of metal complexes. The fragmental patterns of the metal chelates of **4a, 4b** & **4c** follow the **Scheme 4.2.2.** Mass spectral pattern of metal chelates of **4a, 4b** & **4c** are given in Table 4.2.4,4.2.5 & 4.2.6. respectively. The mass spectrum of VO(IV) complex of 4a is given below.

It was found that some fragments rearrange to form stable cyclic species as shown in the Scheme. Peaks corresponding to stepwise elimination of aryl groups are present in the mass spectra of metal complexes. In all the cases [ML_2]+ ion is the most intense peak.

Table 4.2.4 Mass spectral fragmental pattern of metal chelates of 4a

Fragments	Metal Chelates	M+/ (M+1)/ (M+2) ion	A	B	C	D	E	F	G
Mass Pattern	Cu(II)	853.5	579.5	458.5	121	305.5	184.5	397	274
	Zn(II)	855.4	581.4	460.4	121	307.4	186.4	397	274
	Ni(II)	848.69	574.6	454	121	300.6	179.6	397	274
	VO(IV)	856.94	582.9	461.9	121	308.9	187.9	397	274

*The alphabets corresponds to the fragments given in **Scheme 4.2.2.**

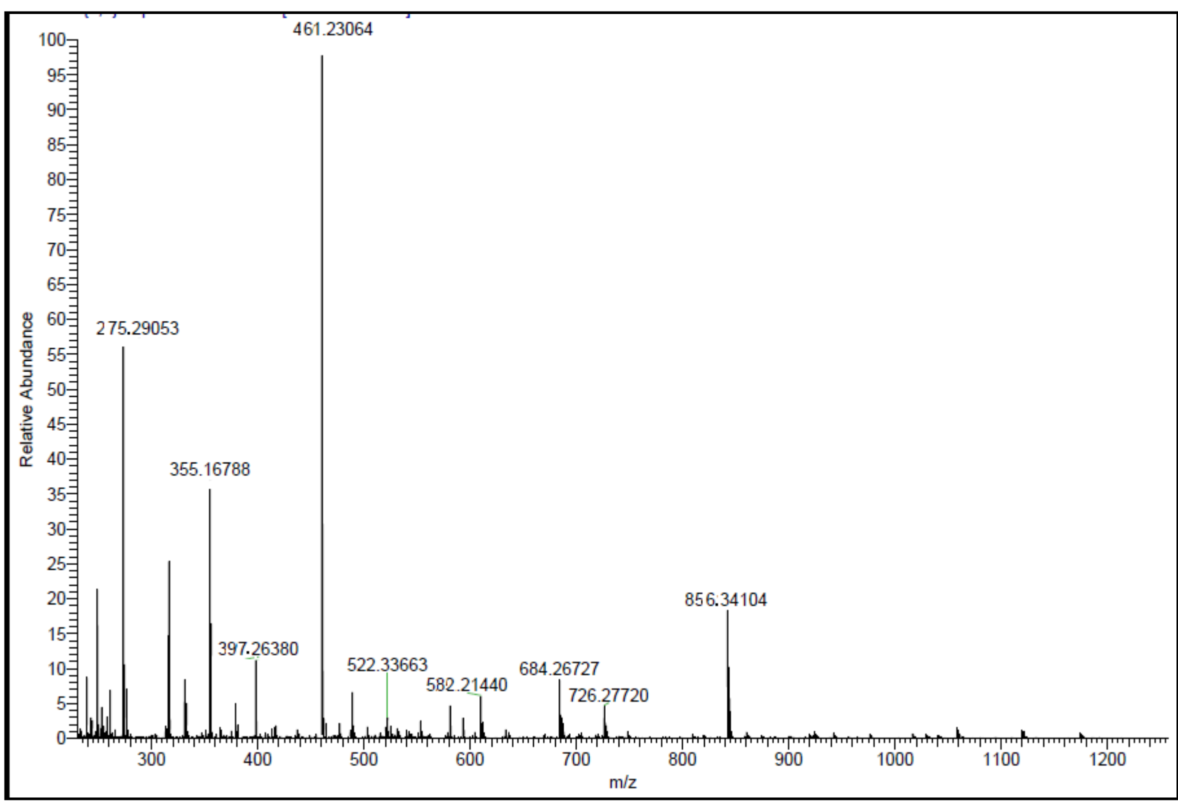


Fig.4.2.4 Mass Spectrum of VO(IV) complex of 4a.

Table 4.2.5 Mass spectral fragmental pattern of metal chelates of 4b

Fragments	Metal chelates	M+/ (M+1)/ (M+2) ion	A	B	C	D	E	F	G
Mass Pattern	Cu(II)	741.5	524	402.5	121	305.5	184.5	341	218
	Zn(II)	743.4	525.4	404.4	121	307.4	186.4	341	218
	Ni(II)	736.6	519	398	121	300.6	179.6	341	218
	VO(IV)	744.94	526.9	405.9	121	308.9	187.9	341	218

*The alphabets corresponds to the fragments given in **Scheme 4.2.2**

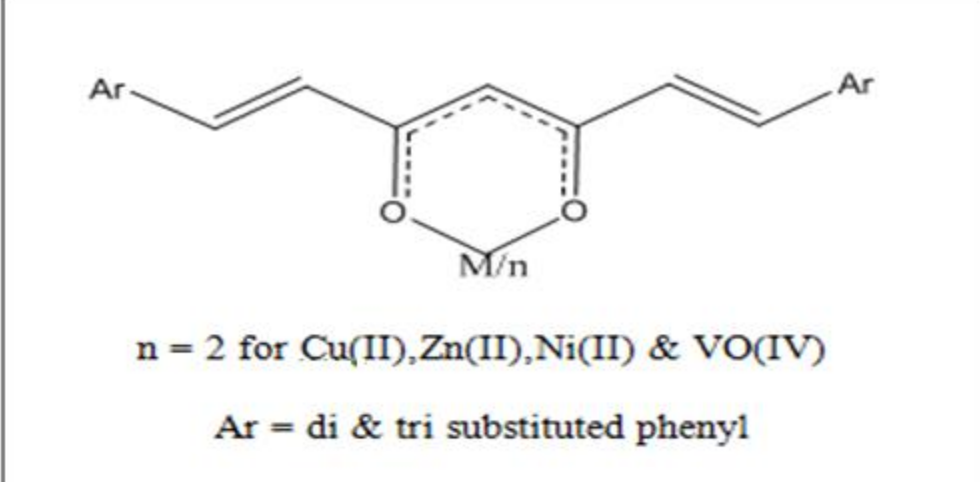
Table 4.2.6 Mass spectral fragmental pattern of metal chelates of 4c

Fragments	Metal chelates	M+/ (M+1)/ (M+2) ion	A	B	C	D	E	F	G
Mass Pattern	Cu(II)	974	640	518.5	121	305.5	184.5	456	334
	Zn(II)	976	642	521	121	308	187	456	334
	Ni(II)	969	635	513	121	301	180	456	334
	VO(IV)	977	643	523	121	309	188	456	334

*The alphabets corresponds to the fragments given in **Scheme 4.2.2**

Scheme 4.2.2

From the observed UV, IR, ^{1}H NMR and Mass spectral data, the metal complexes of the compounds have a ML_2 stoichiometry as given below.



SECTION-III

IN VITRO ANTITUMOUR STUDIES OF CURCUMINOID ANALOGUES WITH DI AND TRI SUBSTITUTED ARYL RINGS AND THEIR TRANSITION METAL COMPLEXES

Short term cytotoxic activity of the compounds 1,7-bis(3-ethoxy-4-hydroxy phenyl)hepta-1,6-diene-3,5-dione(**4a**), 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione(**4b**) & 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione(**4c**) and their Cu,Zn,Ni & Vanadyl metal complexes are described in this chapter. The activity was assayed by determining the percentage viability of DLA and EAC cells using the Trypan blue dye exclusion technique.The results of the study are given below.

4.3.1.In vitro Cytotoxic studies of 1,7-bis(3-ethoxy-4-hydroxy phenyl)hepta-1,6-diene-3,5-dione(4a) and their metal complexes

In vitro Cytotoxic studies are carried out with 1,7-bis(3-ethoxy-4-hydroxy phenyl)hepta-1,6-diene-3,5-dione(**4a**) and their metal complexes.The effect of these compounds against cells EAC & DLA is found in terms of % cell death.The results with EAC cells are described in **Table 4.3.1** and **Fig.4.3.1** and the results with DLA cells are described in **Table 4.3.2** and **Fig.4.3.2.**

Table 4.3.1. *In vitro* Cytotoxic studies of 1,7-bis(3-ethoxy-4-hydroxy phenyl)hepta-1,6-diene-3,5-dione(L_1) and their metal complexes towards EAC

Drug Con. µg/ml	% Cell death				
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$	$VO(L_1)_2$
200	26	85	70	40	80
100	14	73	58	30	68
50	8	58	42	21	52
20	2	41	26	10	36
10	0	20	5	1	15

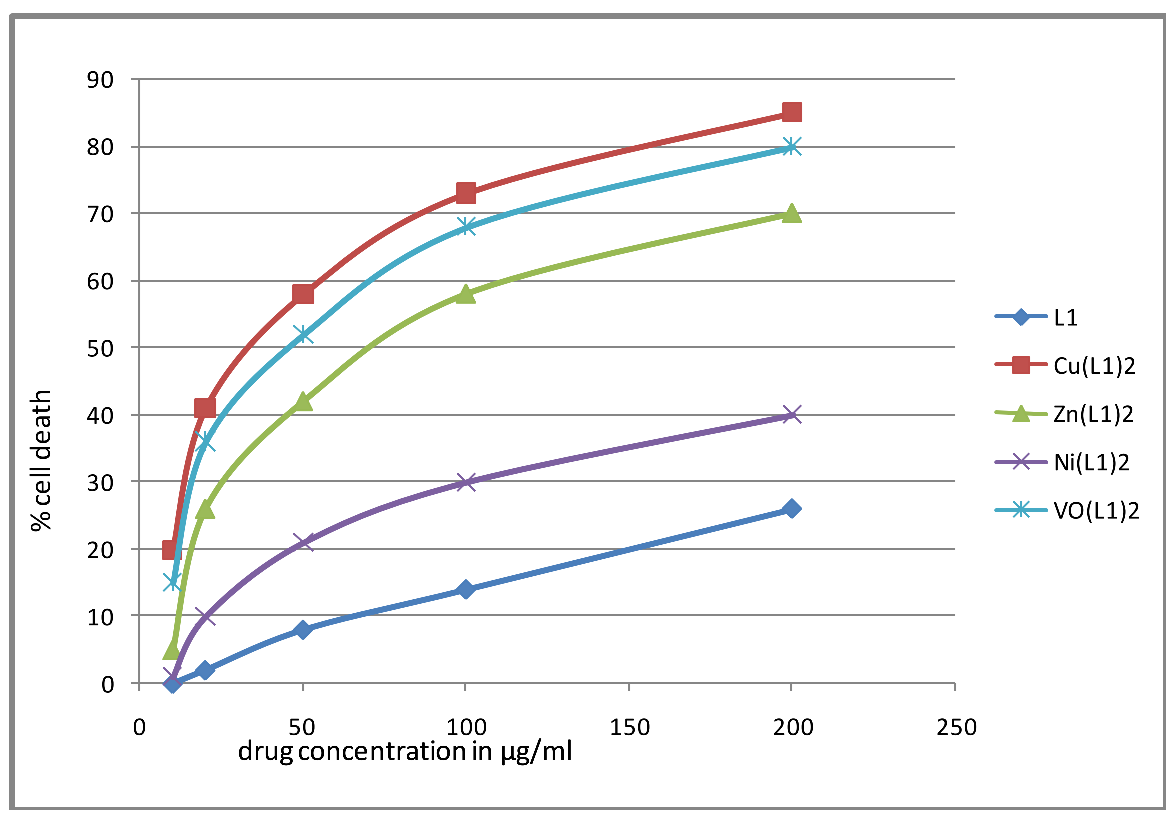


Fig.4.3.1. ***In vitro*** **Cytotoxic studies of 1,7-bis(3-ethoxy-4-hydroxy phenyl)hepta-1,6-diene-3,5-dione(L_1) and their metal complexes towards EAC**

Table 4.3.2 ***In vitro*** **Cytotoxic studies of 1,7-bis(3-ethoxy-4-hydroxy phenyl)hepta-1,6-diene-3,5-dione(L_1) and their metal complexes towards DLA**

Drug Con. µg/ml	% Cell death				
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$	$VO(L_1)_2$
200	28	87	73	41	82
100	19	75	61	31	70
50	11	56	42	22	51
20	7	44	29	11	38
10	2	21	7	2	17

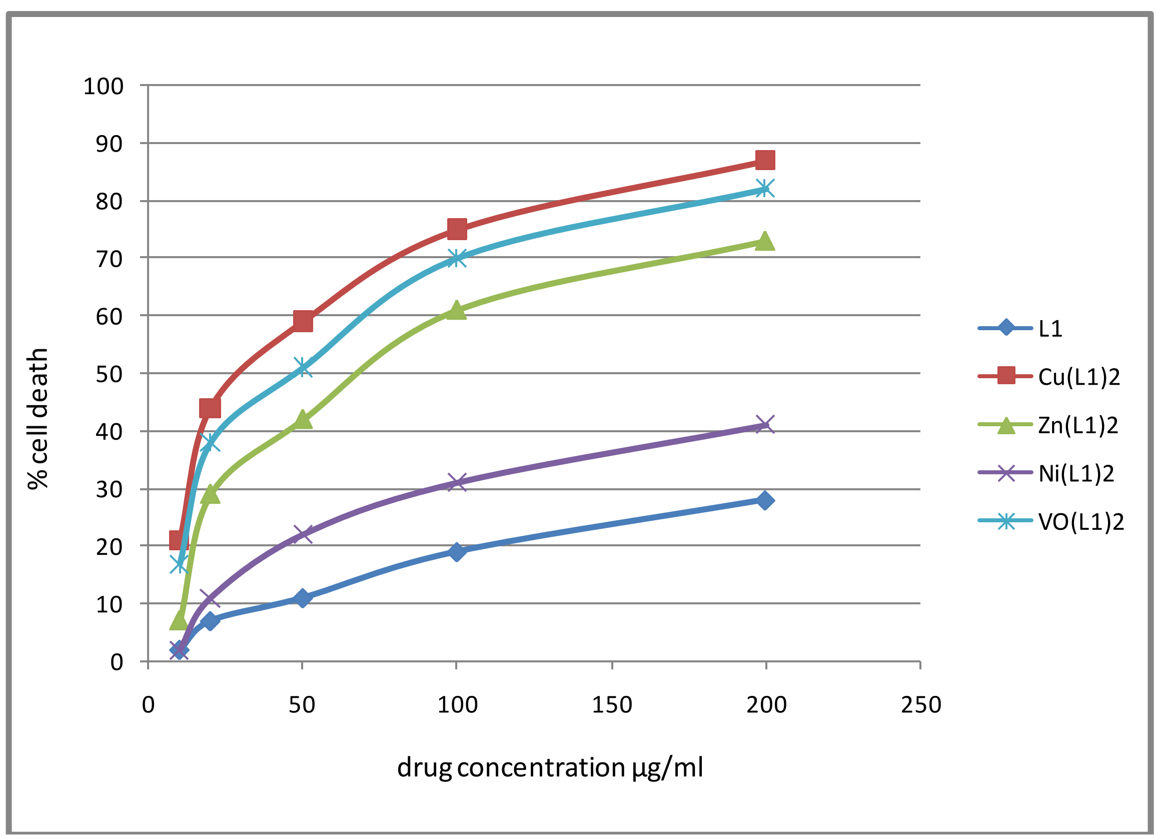


Fig.4.3.2. ***In vitro*** **Cytotoxic studies of 1,7-bis(3-ethoxy-4-hydroxy phenyl)hepta-1,6-diene-3,5-dione(L_1) and their metal complexes towards DLA**

The ligand 1,7-bis(3-ethoxy-4-hydroxy phenyl)hepta-1,6-diene-3,5-dione have shown comparable % cell death towards EAC and DLA with 26 and 28 % respectively.The Cu(II) and VO(IV) complexes of the ligand were found to be significant in their cytotoxic activity with a % cell death of 87 and 82 % respectively.The cell death produced was slightly greater with DLA cells.The Cu(II) and VO(IV) complexes were almost thrice more active than the ligand.The Zn(II) complex has also produced a % cell death in the range 73%.The Ni(II) complex has presented greater activity than the ligand (41%) but has least activity among the complexes.The cytotoxic nature of copper and Vanadyl complexes were high.Metal complexation results in an increase of the cytotoxic activity of the ligand.

4.3.2. *In vitro* Cytotoxic studies of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione(4b) and their metal complexes

In vitro Cytotoxic studies of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione(**4b**) and their metal complexes Cu(II),Zn(II),Ni(II) & VO(IV) were extensively carried out using EAC and DLA cells. All the results are projected in **Table 4.3.3** & **Table 4.3.4.**

Table 4.3.3. *In vitro* Cytotoxic studies of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione(L_2) and their metal complexes towards EAC

Drug Con. µg/ml	% Cell death				
	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$	$VO(L_2)_2$
200	30	52	40	30	45
100	15	35	23	18	28
50	10	17	8	10	10
20	5	10	5	5	7
10	0	0	0	0	0

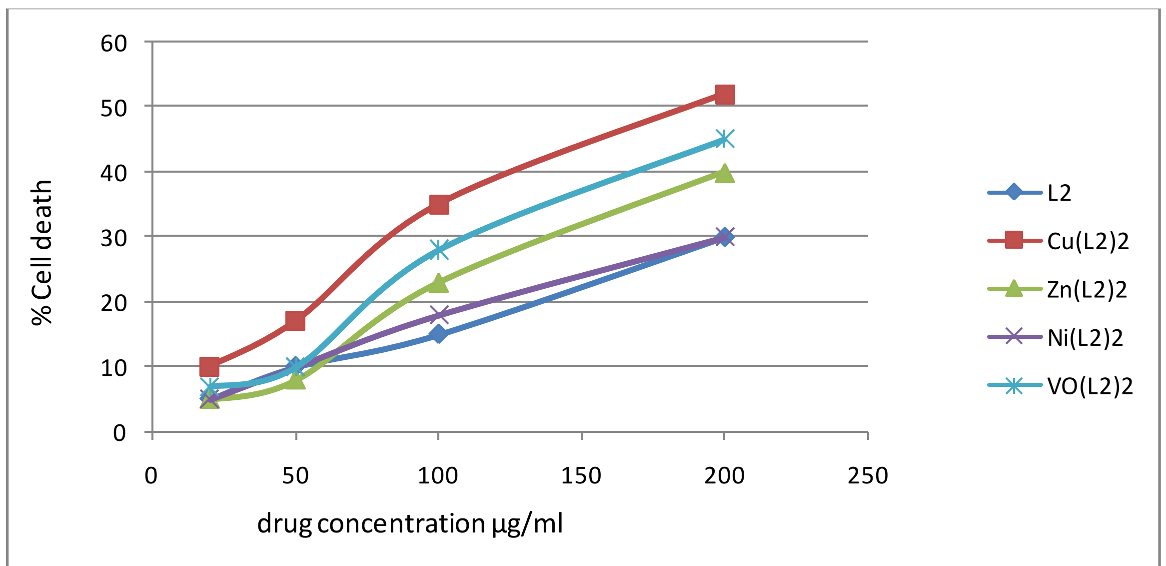


Fig.4.3.3. In vitro Cytotoxic studies of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione(L_2) and their metal complexes towards EAC

Table 4.3.4 *In vitro* Cytotoxic studies of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6 diene3,5-dione(L_2) and their metal complexes towards DLA

Drug Con. µg/ml	% Cell death				
	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$	$VO(L_2)_2$
200	34	55	44	35	49
100	18	38	27	22	32
50	10	20	12	10	14
20	4	13	9	7	11
10	0	0	0	0	0

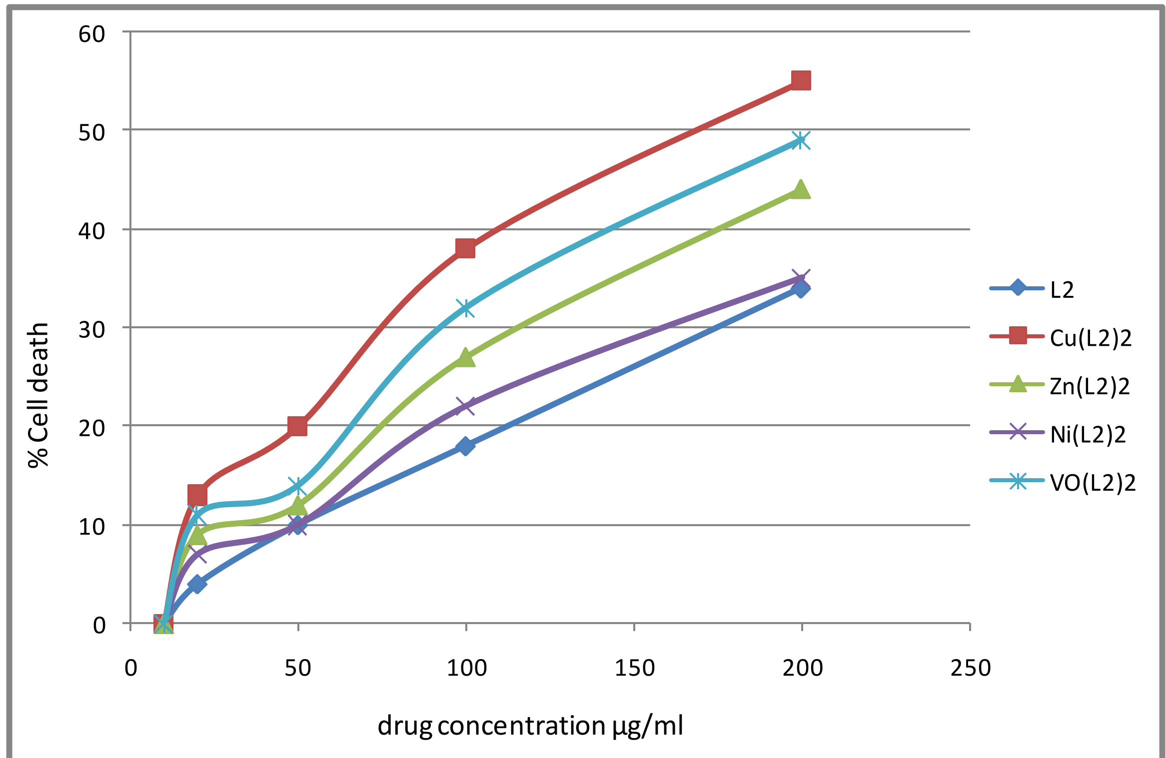


Fig.4.3.4. In vitro Cytotoxic studies of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione(L_2) and their metal complexes towards DLA

The ligand 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione has given a % cell death of 30% at concentration 200 μg/ml.The metal complexation has not increased the activity much.The Cu(II) complex has shown % cell death of 50%.All other metal complexes have shown very less activity which is less than 50%.The conc.of the metal complexes needed to bring about 50% cell death was 200 μg/ml.The Cu(II) and VO(IV) complexes possess almost comparable activities against cancer cells.The Ni(II) complex had shown almost same activity as that of the ligand.The ligand as well as the metal complexes were not at all active at lower concentrations.Even at a conc.of 50 μg/ml their activity is negligible.The cytotoxic nature has increased only slightly even after metal complexation.

4.3.3. *In vitro* Cytotoxic studies of 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione(4c) and their metal complexes

In vitro Cytotoxic studies of 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione(**4c**) and their metal complexes towards EAC are depicted in **Table 4.3.5** & **Fig.4.3.5**. The activity of the compounds with DLA cells are given in **Table 4.3.6** & **Fig.4.3.6**.

Table 4.3.5. *In vitro* Cytotoxic studies of 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione(L_3) and their metal complexes towards EAC

Drug Con. μg/ml	% Cell death				
	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$Ni(L_3)_2$	$VO(L_3)_2$
200	40	90	75	45	85
100	24	78	63	35	73
50	16	59	42	26	54
20	11	46	31	15	41
10	5	25	10	6	20

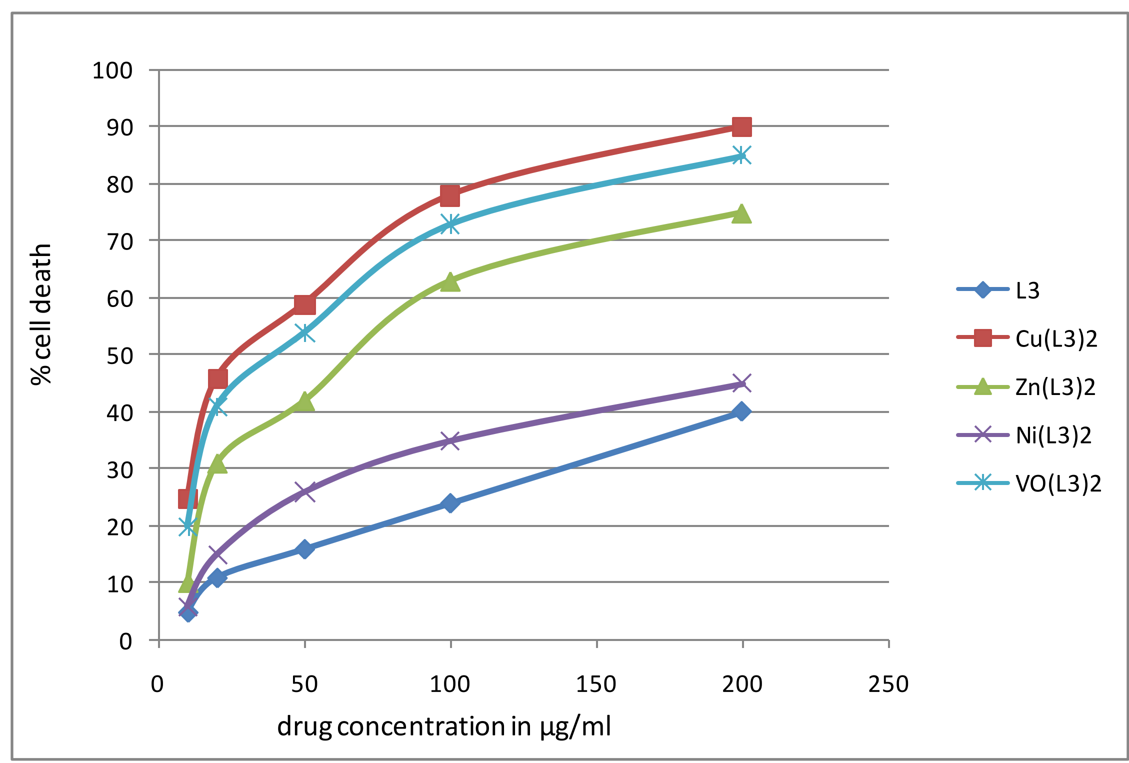


Fig.4.3.5. In vitro Cytotoxic studies of 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione(L_3) and their metal complexes towards EAC

Table 4.3.6. In vitro Cytotoxic studies of 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione(L_3) and their metal complexes towards DLA

Drug Con. µg/ml	% Cell death				
	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$Ni(L_3)_2$	$VO(L_3)_2$
200	42	92	76	47	88
100	26	80	64	37	75
50	18	61	44	28	57
20	13	49	33	17	44
10	7	28	12	8	21

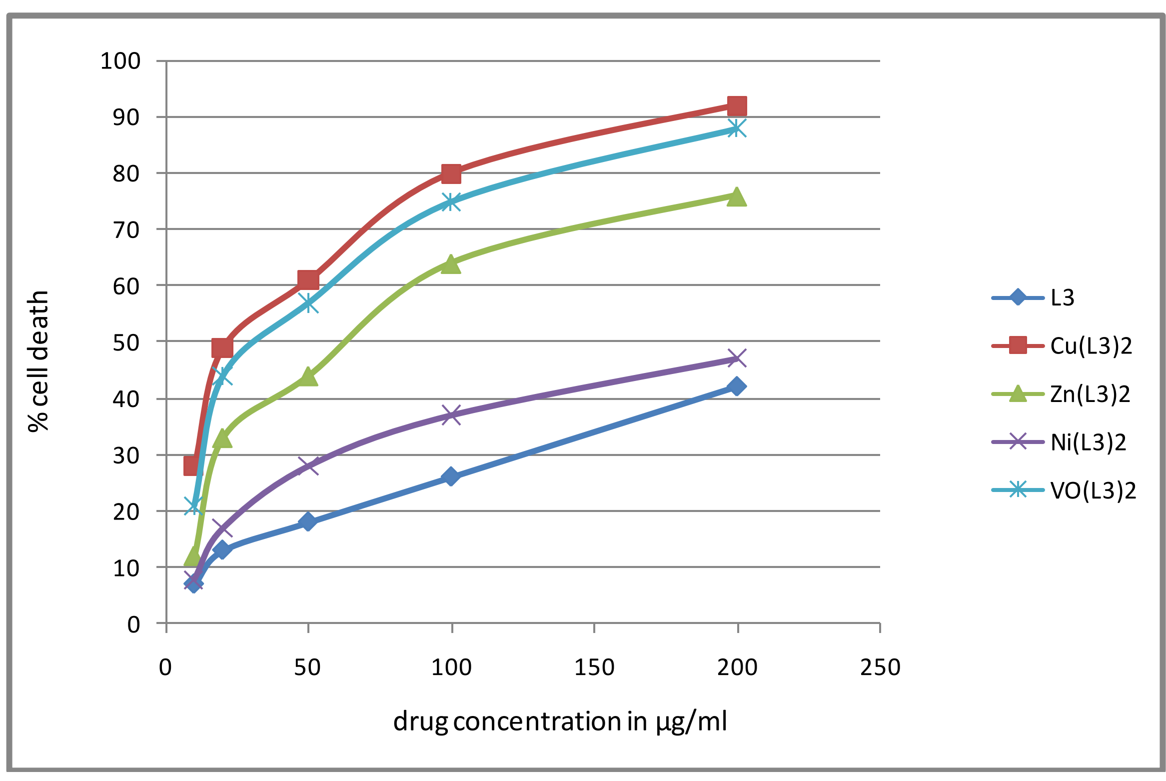


Fig.4.3.6 In vitro Cytotoxic studies of 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione(L_3) and their metal complexes towards DLA

The data given in Table revealed that the ligand 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione(L_3) possess good cytotoxic activity against both EAC and DLA cells at high concentration.All the metal complexes except Ni was found to be highly active against cancerous cells. Metal complexation has enhanced the cytotoxic nature significantly.The metal complexes especially that of Cu and Vanadyl were quite cytotoxic to both DLA and EAC cells.The effect of ligands and metal complexes against both DLA and EAC cells gave similar results.The complexes showed cytotoxic activity even at lower concentrations.The activity of the metal complexes were doubled when compared with the ligand.The Cu(II) and VO(IV) complex gave almost 92 and 88% cell death at a concentration of 200 µg/ml.

Conclusion

In this chapter the *in vitro* studies of three ligands **4a**, **4b** & **4c** and their Cu,Zn,Ni and Vanadyl complexes have been discussed. A comparison among the ligands shows that the cytotoxic activity followed the order 4c >4b >4a.The ligand with trimethoxy substituent on phenyl ring was most active and the one with hydroxyl substituent on phenyl ring was moderately active.The ligand with ethoxy and hydroxyl substituted phenyl ring exhibited least activity.The comparison of the activities of the metal complexes shows that maximum activity was observed with the metal Cu.The most cytotoxic compound was found to be the Cu(II) complex of 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione producing 90% cell death. A comparative study of ligand and its Cu(II) complex is given in **Fig.4.3.7**

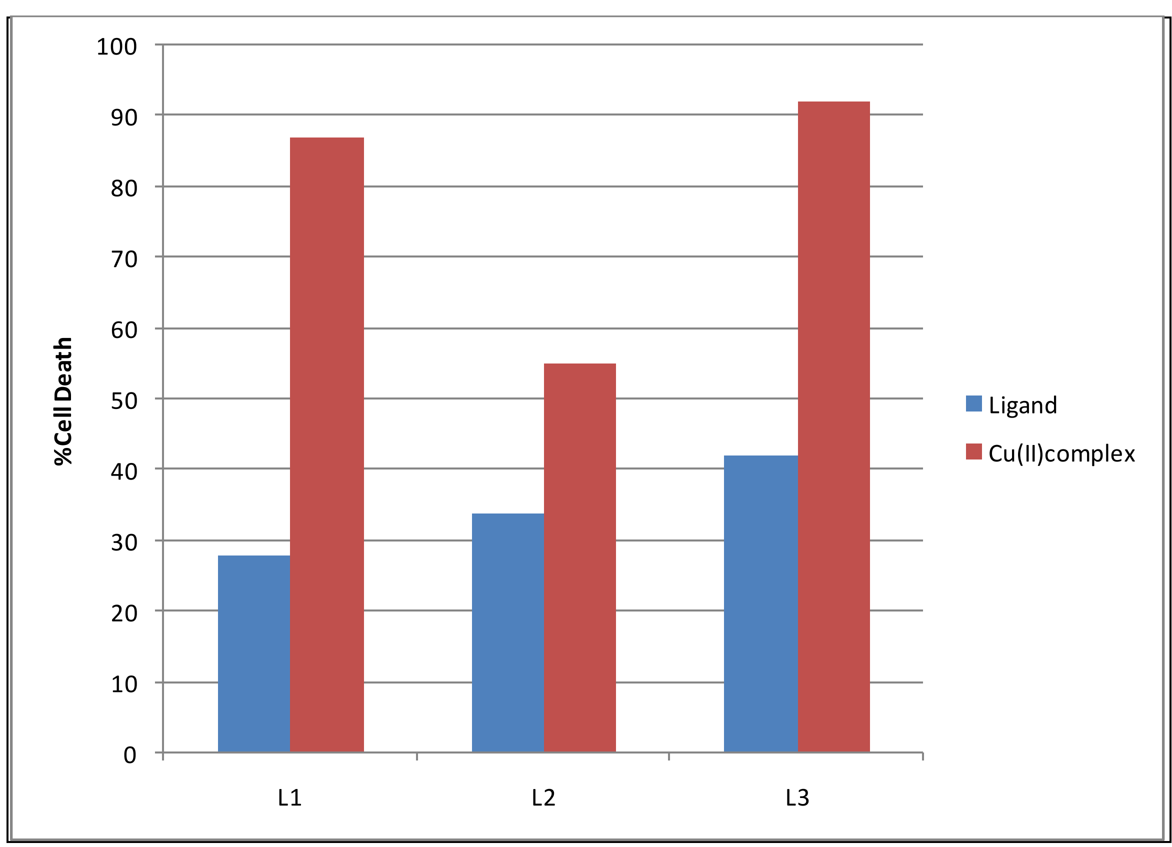


Fig.4.3.7 Comparison of *in vitro* cytotoxicity studies of ligands and Cu(II) complex towards DLA

THE *IN VIVO* ANTITUMOUR STUDIES OF 1,7-BIS(2,4-DIHYDROXY PHENYL)-1,6-HEPTADIENE-3,5-DIONE AND THE Cu(II) COMPLEX

Animals were divided into 8 groups and ascites tumour was induced in them.The ligand **1,7-bis(2,4-dihydroxy phenyl)-1,6-heptadiene-3,5-dione and its Cu(II) complex** were given as drug to tumour bearing mice in different concentrations namely 20,10,5 µg/ml.Antitumour study was done and the number of days survived by the animals treated with drug was noted and the increase in life span of animals were found. The values of No. of days survived are means of five determinations $\pm SD$ (standard deviation).The results of the study are given in Table **4.3.7**

Table 4.3.7 Effect of 1 ,7-bis(2,4-dihydroxy phenyl)-1,6-heptadiene-3,5-dione(L) and its Cu(II)complex 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	30.6±3.1	76.88
3. L	20	5/5	21.0±2.4	21.38
4. L	10	5/5	19.2±1.23	10.98
5. L	5	5/5	18±1.16	4.046
6. Cu(L)$_2$	20	5/5	24.4±1.15	41
7. Cu(L)$_2$	10	5/5	22.1±0.99	27.74
8. Cu(L)$_2$	5	5/5	18.7±0.87	8.09

The percentage increase in life span of ascites tumour burden animals was 76% when treated with standard drug cyclophosphamide.Compared with the std.drug the %ILS produced by the ligand L was one third its value ,21% at concentration 20µg/ml and that produced by its Cu(II) complex was nearly half of its value,41% at concentration 20µg/ml.For the complex

the no:of days survived by the animals was 24.4±1.15 days.Both the ligand and metal complex were more active at higher concentrations.The Cu(II) complex was twice more active than the ligand in increasing the life span of tumour burden animals.

THE *IN VIVO* ANTITUMOUR STUDIES OF 1,7-BIS(3,4,5-TRIMETHOXY PHENYL)-1,6-HEPTADIENE-3,5-DIONE AND Cu(II) COMPLEX

The compound 1 ,7-bis(3,4,5-trimethoxy phenyl)-1,6-heptadiene-3,5-dione(**L1**) and its Cu(II) complex was given by intraperitoneal injection from the first day of tumour induction as drug to mice groups. The death pattern of animals due to tumor burden was noted and the percentage increase in life span was found.The results are discussed in **Table 4.3.8**

Table 4.3.8 Effect of 1 ,7-bis(3,4,5-trimethoxy phenyl)-1,6-heptadiene-3,5-dione(L1) 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			17.3±1.1	
2.Std.drug			30.6±3.1	76.88
3. L1	20	5/5	26.2±2.1	51.45
4. L1	10	5/5	24.1±2.6	39.31
5. L1	5	5/5	18.0±2.60	8
6. Cu(L1)2	20	5/5	29.3±3.1	70.01
7. Cu(L1)2	10	5/5	26.7±2.4	54.34
8. Cu(L1)2	5	5/5	19.6±2.65	20.1

The compound and its Cu(II) complex when administered intraperitoneally could produce significant increase in the life span of mice bearing ascites tumour. The animals of the

control group survived for a period of 17.3±1.1 days and those treated with std.drug cyclophosphamide for a period of 30.6±3.1days. Cu(II) complex of the compound produced an increase in life span of tumour bearing mice compared with that of the ligand . The percentage increase in life span(%ILS) of tumour bearing mice were 51.45, 39.31 and 8% for Ligand at diffferent concentrations namely 20,10,5 µg/ml respectively. For the Cu(II) complex the % increase was 70.01 % at a concentration of 20µg/ml.There was an increase in the average life span of animals for both the ligand and the metal complex.But the studies reveal that Cu(II) complex is very effective in reducing tumour development in mice and increasing the life span of the animal.The Cu(II) complex exhibits significant antitumour activity in invivo studies.

SECTION-IV

ANTIBACTERIAL STUDY OF 1,7-DIARYL HEPTANOIDS WITH DI&TRISUBSTITUTED PHENYL RING AND THEIR Cu(II), Zn(II)&VO(IV)METAL COMPLEXES

4.4.1 Antibacterial studies of 1,7-bis(3-ethoxy-4-hydroxy phenyl)hepta-1,6-diene-3,5-dione(4a), 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione (4b) and 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione (4c) and their Cu(II),Zn(II)&VO(IV) complexes

The compounds and their complexes were evaluated for their antibacterial activities.The invitro antibacterial studies were performed on three types of bacterial strains namely Escherichia Coli, Klebsiella Pneumoniae & Bacillus Subtilis using Agar well diffusion method.The test compounds showed varying degree of inhibition against different bacterial strains. The results demonstrated promising antibacterial activity for hydroxyl and methoxy substituted 1,7-diaryl heptanoids and their complexes.The study revealed that the ligands and their complexes possess comparable antibacterial activity to that of standard drug streptomycin .The activity is expressed in terms of diameter of zone of inhibition in mm. In all the cases metal complexes possess better antibacterial activity than that of ligands, which means that metal complexation enhance the activity . The results of antibacterial activity are given in the Tables below.

Table 4.4.1 Antibacterial studies of 1,7-bis(3-ethoxy-4-hydroxy phenyl)hepta-1,6-diene-3,5-dione(L1) and their Cu(II),Zn(II)&VO(IV) complexes

Bacteria	Diameter of zone of inhibition in mm			
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$VO(L_1)_2$
E Coli	13	17	14.5	16
Klebsiella	13.5	17.5	15	17
Bacillus	12	16	13.5	15.5
Standard	20	20	20	20

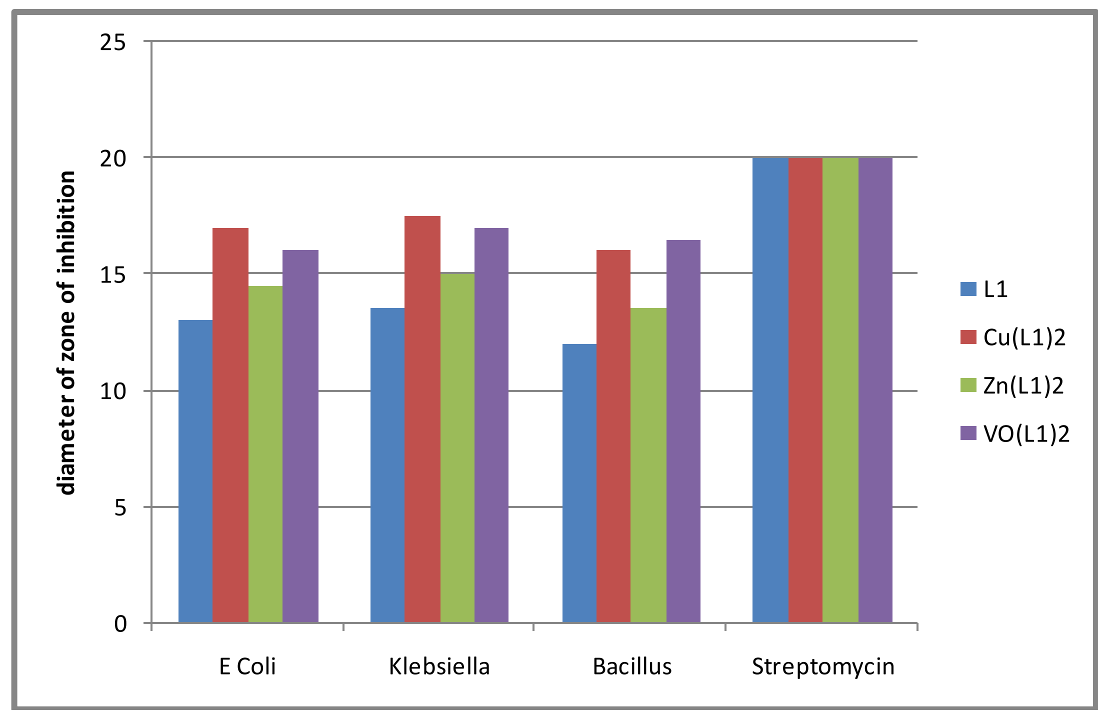


Fig. 4.4.1 Antibacterial studies of 1,7-bis(3-ethoxy-4-hydroxy phenyl)hepta-1,6-diene-3,5-dione(L1) and their Cu(II),Zn(II)&VO(IV) complexes

Table 4.4.2 Antibacterial studies of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione(L2) and their Cu(II),Zn(II)&VO(IV) complexes

Bacteria	Diameter of zone of inhibition in mm			
	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$VO(L_2)_2$
E Coli	17	19.5	16.5	18.5
Klebsiella	15	18	15	17
Bacillus	14	16.5	13	16.5
Standard	20	20	20	20

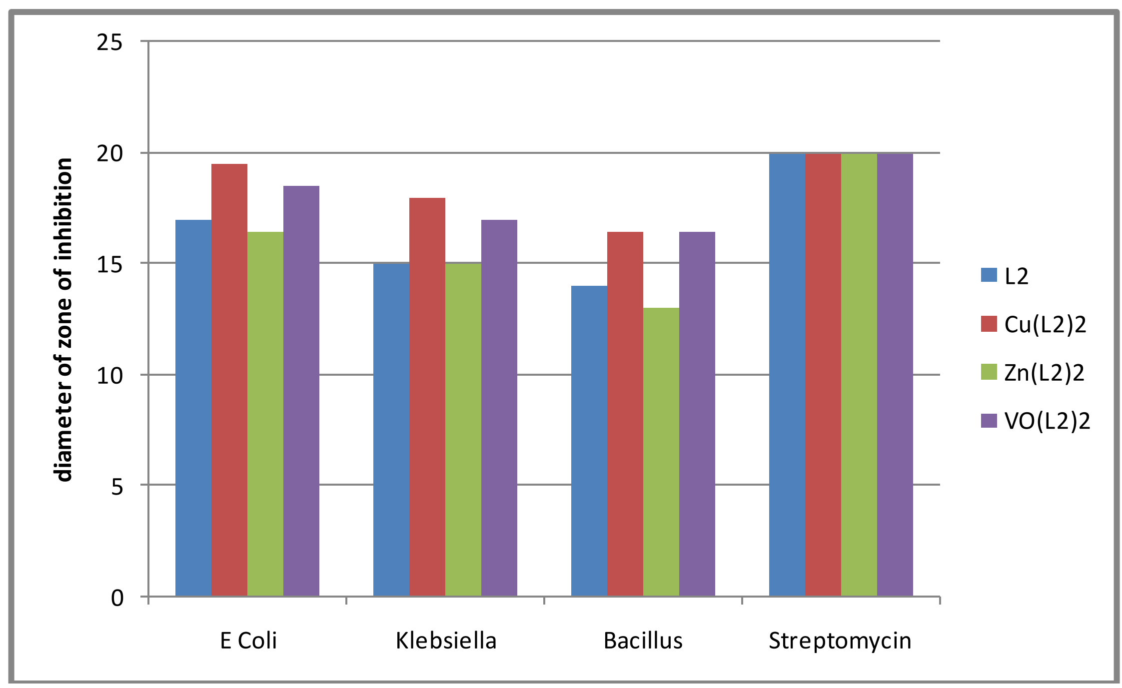


Fig. 4.4.2 Antibacterial studies of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione(L_2)and their Cu(II), Zn(II) & VO(IV) complexes

Table 4.4.3 Antibacterial studies of 1,7-bis(3,4,5-trimethoxy phenyl)hepta-1,6-diene-3,5-dione(L3) and their Cu(II),Zn(II)&VO(IV) complexes

Bacteria	Diameter of zone of inhibition in mm			
	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$VO(L_3)_2$
E Coli	15.5	18.5	15.5	17
Klebsiella	13.5	16.5	13.5	16
Bacillus	12	15	12.5	14.5
Standard	20	20	20	20

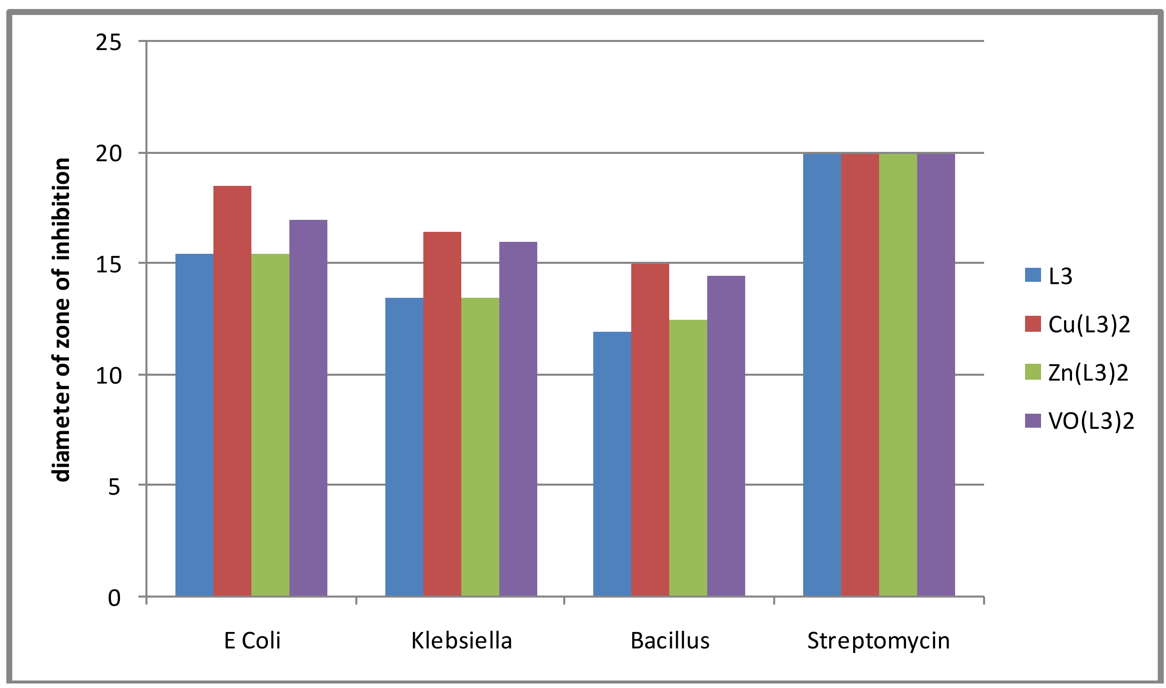


Fig.4.4.3 Antibacterial studies of 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione (L_3)and their Cu(II),Zn(II)&VO(IV) complexes

Among the three ligands discussed in this chapter,the one with dihydroxy substituted benzene ring (L2) was found to be very active against all bacterial strains.It gave a zone of inhibition of 17mm,15mm and 14mm against E.coli,Klebsiella and Bacillus species.The ligand with trimethoxy substituted benzene ring (L3)had also shown significant anti bacterial activity.The L3 compound with methoxy groups was less active than L2 compound with hydroxyl groups.It has been proved in earlier studies that hydroxyl groups and methoxy groups play an important role in antibacterial activity.The least activity was exhibited by 1,7-bis(3-ethoxy-4-hydroxy phenyl)hepta-1,6-diene-3,5-dione(L1).

All the metal complexes displayed better antibacterial activity than the corresponding ligands.The Cu(II) complex of all the ligands had shown maximum activity among metal complexes.The Cu(II) complex is a potent antibacterial compound against E.coli species.The Cu(II) complex of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione(L2)has produced

a zone of inhibition of 19.5mm which is almost same as the activity of the standard drug.Antibacterial nature of the metal complexes follow the order Cu(II)>VO(IV)>Zn(II).The same order is observed in the case of metal complexes of all ligands.The VO(IV) complexes also possess significant activity with a zone of inhibition in the range 17mm.The std.drug streptomycin has given a zone of inhibition of 20mm against all bacterial strains.The Zn(II) complexes had shown a slight marginal increase in activity when compared with the corresponding ligands.

SECTION-V

ANTIFUNGAL STUDY OF 1,7-DIARYL HEPTANOIDS WITH DI&TRISUBSTITUTED PHENYL RING AND THEIR VO(IV)METAL COMPLEXES

4.5.1 Antifungal studies of di & tri substituted 1,7-diaryl heptanoids (4a, 4b & 4c) and their VO(IV) complexes

The curcuminoid analogues with dihydroxy substituted phenyl ring,trimethoxy substituted phenyl ring and ethoxy hydroxy substituted phenyl ring and their VO(IV) metal complexes were studied for their antifungal activity against three fungal cultures namely Aspergillus Niger, Penicillium Chrysogenum and Alternaria Alternate.Kirby Baurer disc plate method was used to test the susceptibility of the fungi species to the test compounds. Different concentrations [100, 250, 500µg/ml] by dissolving in 2% DMSO solvent were used for all the test compounds and results were compared with the std.drug flucanozole.The antifungal activities are measured in terms of zone of inhibition in mm.The data of the study revealed that the synthesized 1,7-diaryl heptanoids with di and trisubstituted phenyl rings and VO(IV) complexes possess comparable antifungal activities to that of std.drug.

The inhibition zone of the test compounds with the three fungi species in comparison to flucanazole(std.drug) is shown in Table **4.5.1,**Table **4.5.2** &Table **4.5.3**.

Table 4.5.1 Antifungal studies of 1,7-bis(3-ethoxy-4-hydroxy phenyl)hepta-1,6-diene-3,5-dione(L_1), and its VO(IV) complexes

Fungi	Diameter of zone of inhibition in mm					
	L_1			$VO(L_1)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	11.5	13	14.5	12.5	15	17
Penicillium	10.5	12	14	11	13	15
Alternaria	11	13.5	15	11.5	14	17.5

Table 4.5.2 Antifungal studies of 1,7-bis(2,4-dihydroxy phenyl)hepta-1,6-diene-3,5-dione (L_2) and its VO (IV) complexes

Fungi	Diameter of zone of inhibition in mm					
	L_2			$VO(L_2)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	13	16.5	18	14	17	19
Penicillium	12.5	16	17	14	17.5	19
Alternaria	12	15	16	13	18	19.5

Both ligands and metal complexes have shown maximum antifungal activity at a higher concentration of 500µg.The ligand L_1 has shown almost comparable and moderate activities against all fungi species producing a zone of inhibition in the range 15mm.Among the fungi species,Alternaria was most sensitive to L_1 and its VO(IV) complex.The Vanadyl complex exhibited significant activity with a zone of inhibition of 17.5mm.

The ligand L_2 had shown appreciable antifungal nature against all fungi species.It was most effective against Aspergillus species .The VO(IV) complex of the ligand L_2 at 500 µg conc. exhibits promising antifungal activity.The complex showed highest activity against Alternaria species with zone of inhibition 19.5mm,where as zone of inhibition for std.drug is 21 mm.

Table 4.5.3 Antifungal studies of 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione (L_3) and its VO(IV) complexes

Fungi	Diameter of zone of inhibition in mm					
	L_3			$VO(L_3)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	14	17	19	15	18.5	20
Penicillium	13	16	18	15	17	19
Alternaria	13.5	17	19	14	18	20

The ligand 1,7-bis(3,4,5-trimethoxy phenyl) hepta-1,6-diene-3,5-dione (L_3) and its VO(IV) complex exhibited remarkable potency against all fungi species.They were most effective in their ability to inhibit the fungal cultures.The ligand produced a zone of inhibition of 19mm against both Aspergillus and Alternaria and has also given 18mm zone of inhibition against Penicillium species.Both the ligand as well as the complex were very effective even at lower concentrations.The VO(IV) complex of the ligand has proved to be the most active compound against all fungi species producing a zone of inhibition of 20 mm.The activity of the compound is almost the same as that of the std.drug.

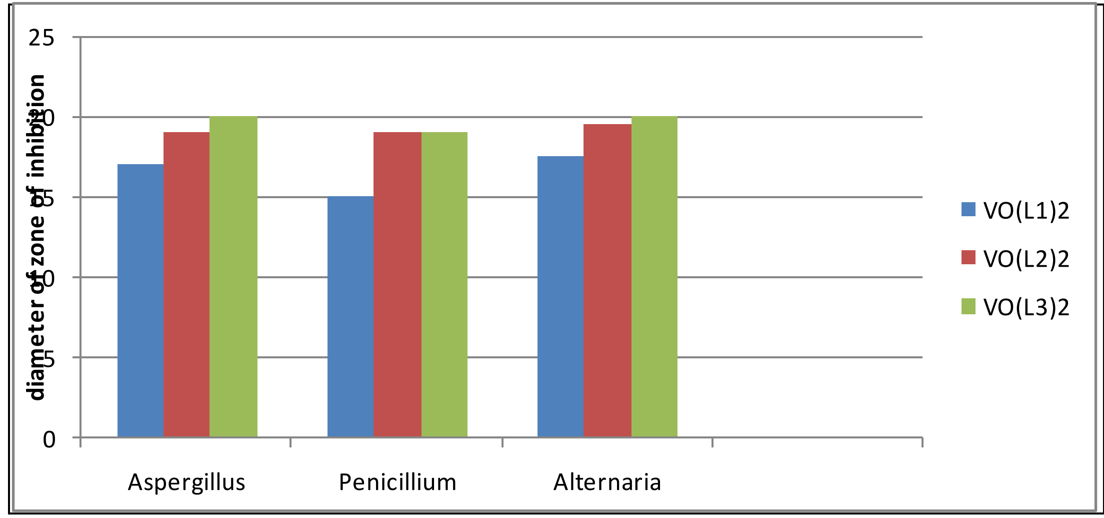


Fig.4.5.1 Comparative study of Antifungal activity of VO(IV)complexes

CHAPTER-V

SYNTHESIS, CHARACTERISATION AND BIOCHEMICAL ACTIVITIES OF 1,7-DIANTHRACENYLHEPTA-1,6-DIENE-3,5-DIONES AND THEIR TRANSITION METAL CHELATES WITH CU(II), Zn(II),Ni(II) & OXOVANADIUM(IV)

SECTION I

SYNTHESIS AND CHARACTERIZATION OF CURCUMINOID ALLIED WITH ANTHRACENYL RING

5.1.1. Synthesis of 1,7–dianthracenyl heptanoids

The compound 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione (5a)was synthesized by the condensation of anthracene 9-carboxaldehyde with acetyl acetone-boric oxide complex in ethyl acetate medium in presence of tributyl borate and n-butyl amine. The synthesized curcuminoid analogue with anthracenyl ring was purified by column chromatography over silica gel (60 – 120mesh) using 4:1 (v/v) chloroform: acetone mixture as the eluent and recrystallised twice from hot benzene to get pure crystalline material. The product formation can be represented in a schematic way in **Scheme 5.1.1.**

Ar-CHO + H_3C–CO–CH_2–CO–CH_3 + OHC-Ar → Ar–CH=CH–CO–CH_2–CO–CH=CH–Ar

Ar = 9-Anthracenyl

Scheme 5.1.1

The aldehyde used for synthesis, structure of the ligand, systematic name, colour and yield has been included in the table given below.

Table 5.1.1 Synthetic details of 1,7-dianthracenyl heptanoid (5a)

Compound	Aldehyde used for Synthesis	Structure of Ligand	Systematic name	Yield %	Colour
5a	Anthracene 9-carbox aldehyde		1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione	85	Orangish red

The compound is crystalline in nature and has sharp melting point.It is soluble in organic solvents like acetone, methanol, ethylacetate etc.The yield was maximum for the compound.The elemental analysis results and molecular weight determination of the compound is given in the Table below.The data suggests that two equivalents of aldehyde have condensed with one equivalent of acetyl acetone to form 1,7-dianthracenyl heptanoid.

Table 5.1.2 Analytical & UV spectral data of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione

Compound	M.P. (°C)	Elemental analysis (%) Found/(Calculated) C	H	Molecular weight	UV λmax (nm)
5a	190	87.35(88.23)	4.71(5.04)	473(476)	286, 420

5.1.2. Characterisation of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione

The compound 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione(5a) synthesized as per the above method was characterized by various spectral techniques like UV, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral techniques.The data collected from the various spectra help in establishing the correct structure of the compound.The different spectral techniques employed are given below.

UV spectra

The UV spectra of the compound 5a showed two absorption maxima which are assigned to the n→$\pi*$ and $\pi \rightarrow \pi*$ transitions. The high energy band at 286 nm is due to $\pi \rightarrow \pi*$ transition and at 420 nm is due to n→$\pi*$ transitions.The n→$\pi*$ absorption of carbonyl chromophore occurs at higher wavelength and the presence of α,β –unsaturation also increases the wavelength of carbonyl absorption maxima.The UV spectra of the compound is given below in Fig.5.1.1

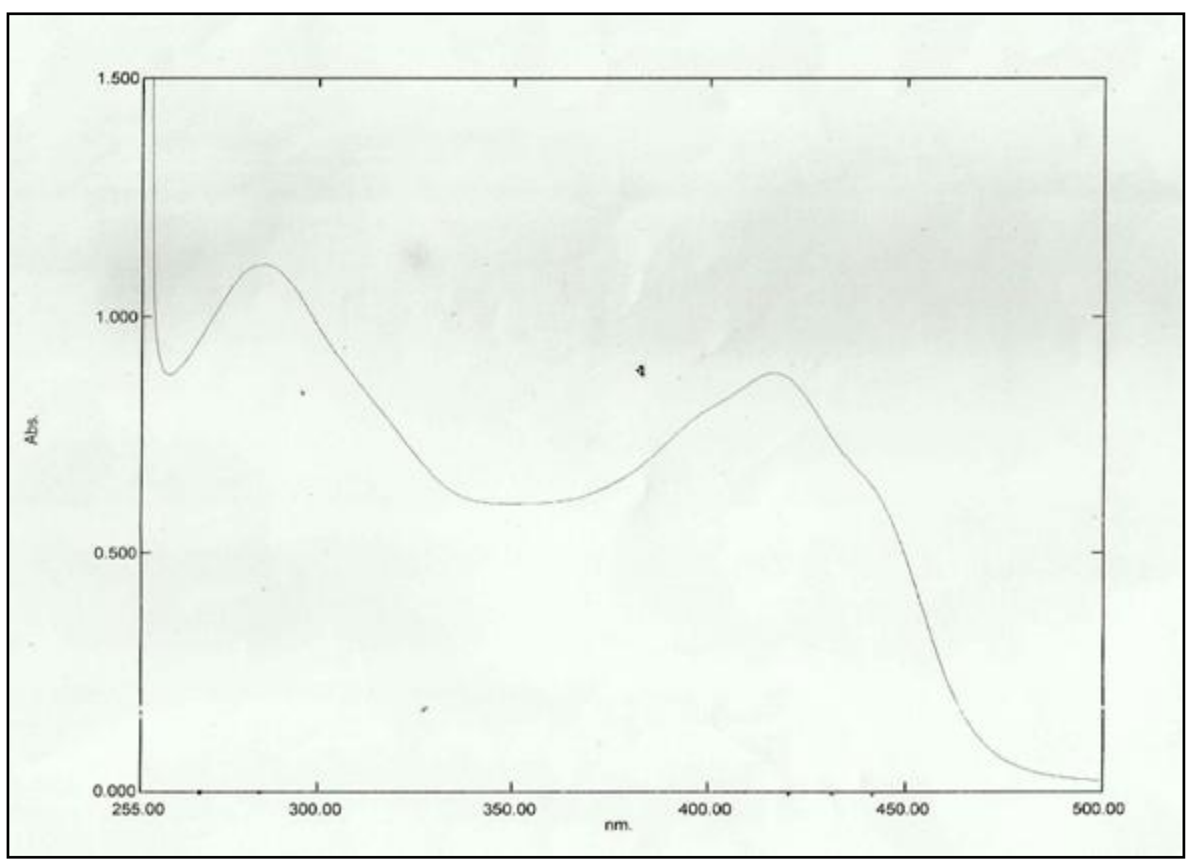


Fig.5.1.1 The UV spectra of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione

IR spectra

The IR spectra of 1,7-dianthracenyl heptanoid is characterized by the presence of a strong band in the region 1624 cm^{-1} which is due to the ν(C=O) chelated group.There is no other band in the region 1600-1800 cm^{-1}.This indicates that the carbonyl group in the compound is existing not as a free group but as intramolecularly hydrogen bonded enolic group.The IR spectra give peaks corresponding to ν(C=C) phenyl, ν(C-C) alkenyl, ν(C-C-C) chelate ring etc.A broad band in the region 3000-3500 cm^{-1} shows the presence of hydroxyl group which is present in the intramolecular hydrogen bonded enolic group.The peak at 957 cm^{-1} is due to ν(CH=CH) trans vibrations.Thus IR spectra is in favour of the intramolecularly hydrogen bonded enolic structure of the curcuminoid analogue.Characteristic IR data and the corresponding probable assignments are given in Table.5.1.3.The IR spectra of the compound is depicted below in Fig.5.1.2.

Table 5.1.3 IR spectral data of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione

5a (compound)	Probable IR assignments
1624	ν(C=O) chelated
1552	ν(C=C) phenyl
1542	ν(C-C) alkenyl
1506	ν_{as}(C-C-C) chelate ring
1440	ν_{s}(C-C-C) chelate ring
1163,1047	β(C-H) chelate ring
957	ν(CH=CH) trans

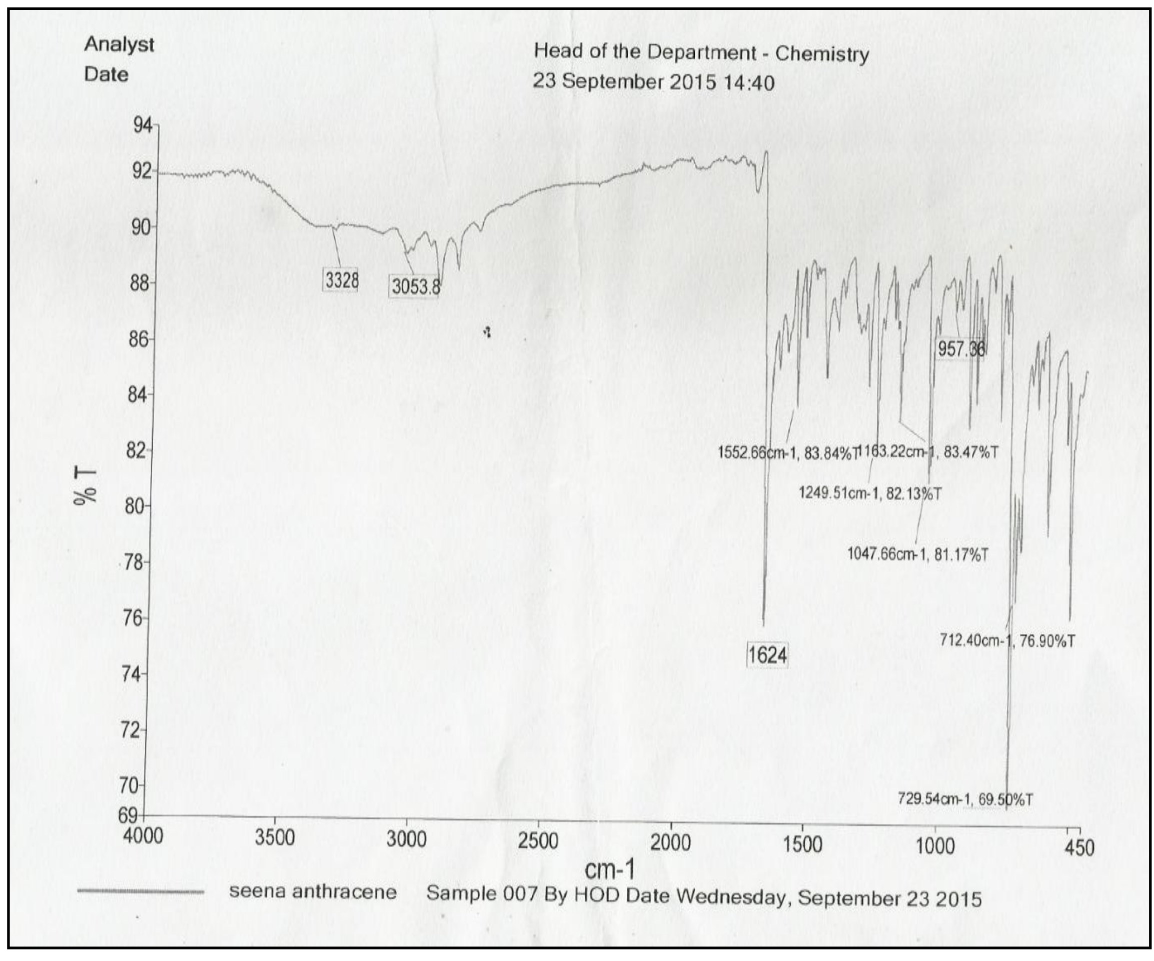


Fig.5.1.2 IR spectrum of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione

^{1}HNMR spectra

The enolic existence of the compound is supported by the fact that ^{1}H NMR spectra of 1,7-dianthracenyl heptanoid showed a one proton singlet at ~ 16ppm assignable to strong intra molecularly hydrogen bonded enolic proton and another one proton singlet at ~ 6ppm assignable to methine proton.The peaks due to alkenyl protons are seen distributed in the range 6.61-8.99 ppm and that due to aryl protons are seen distributed in the range 7.01-7.80 ppm.The NMR spectra of the compound is reproduced in Fig.5.1.4 and assignments of various signals are brought out in Table 5.1.4.The enolic structure of the compound as per spectral datas is given below.

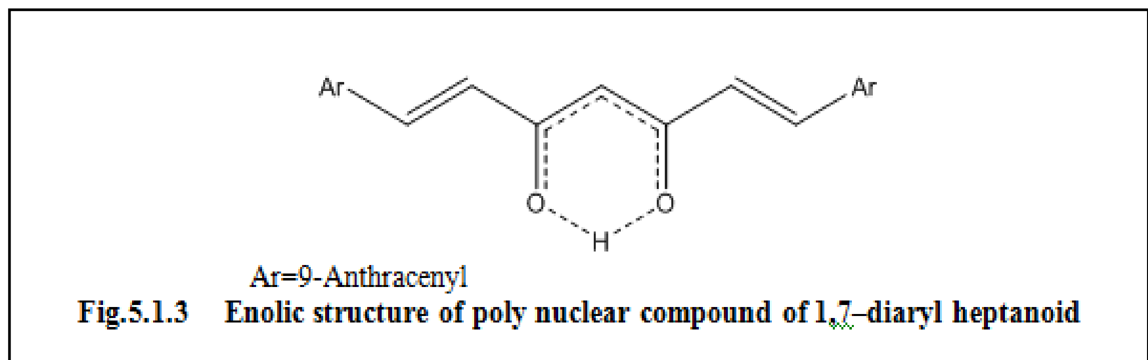


Fig.5.1.3 Enolic structure of poly nuclear compound of 1,7–diaryl heptanoid

Table 5.1.4 ^{1}H NMR spectral data of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione

Compound	Chemical shifts (δ ppm)			
5a	Enolic	Methine	Alkenyl	Aryl
	16.14	5.62	6.613-8.99	7.014-7.808

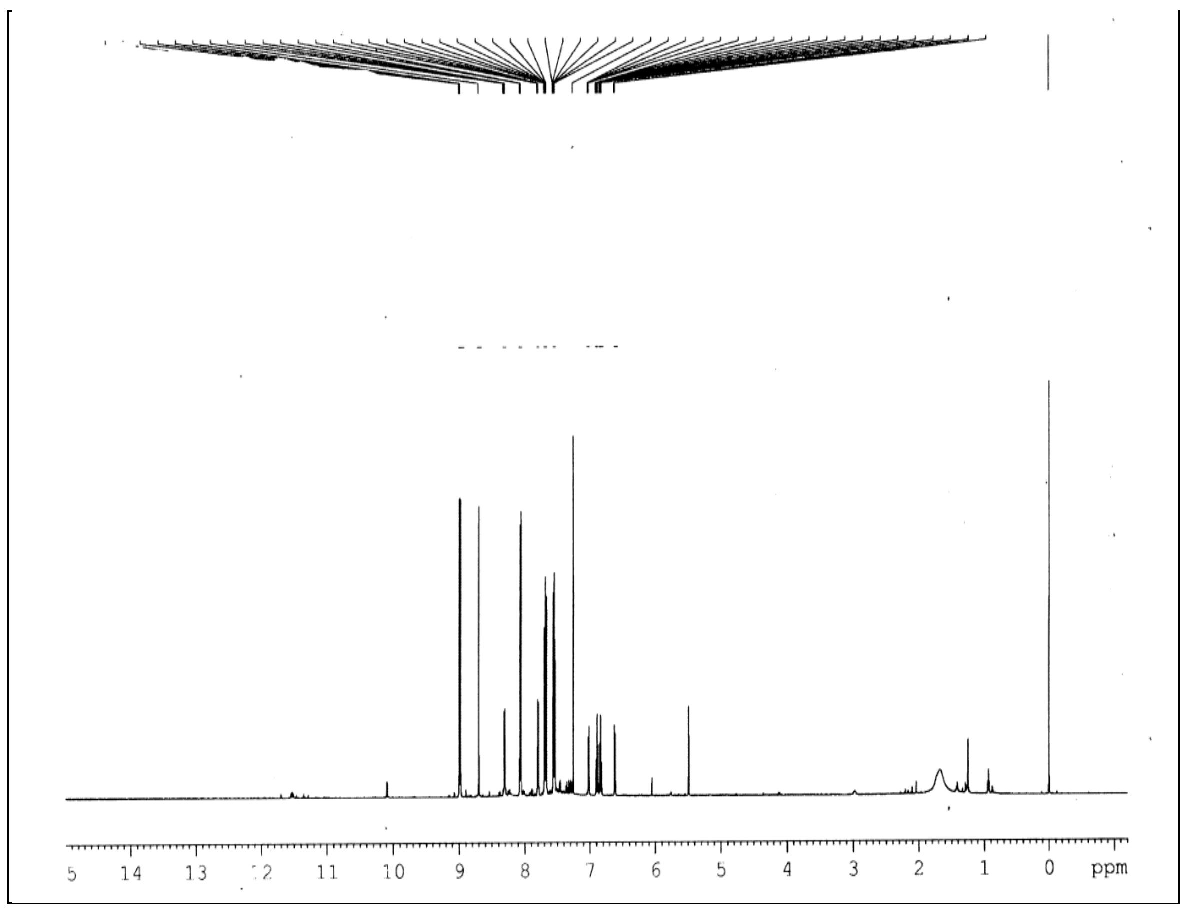


Fig.5.1.4 ^{1}H NMR spectrum of 5a

^{13}C NMR spectra

The ^{13}C NMR spectral data of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione is given in Table.5.1.5 below.The Structure representing different non equivalent C atoms in the compound is given in Fig.5.1.5.The **^{13}C NMR spectra** of the compound is given in Fig.5.1.6.

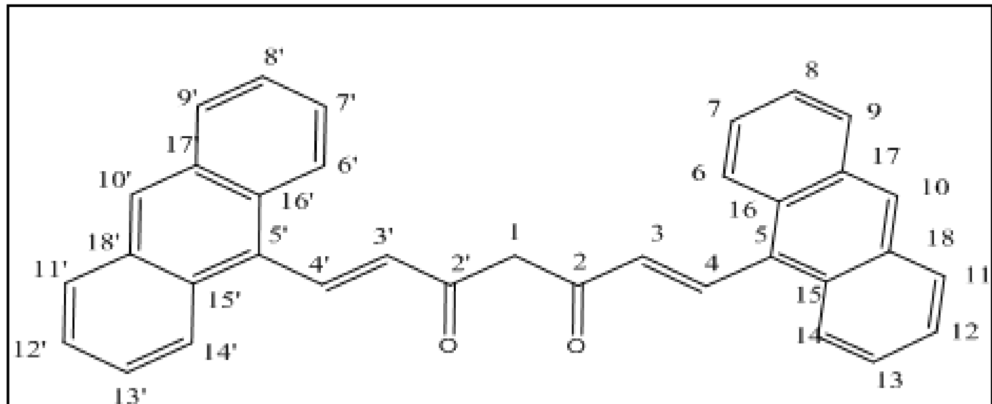


Fig.5.1.5 Different non equivalent C atoms in the compound.

Table 5.1.5 ^{13}CNMR spectral data of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione

C1	C2,C2’	C3,C3’	C4,C4’	C5,C5’	C6,C6’
104.68	193.037	135.25	123.83	134.12	129.30
C7,C7’	**C8,C8’**	**C9,C9’**	**C10,C10’**	**C11,C11’**	**C12,C12’**
124.76	127.23	131.11	132.16	131.11	125.716
C13,C13’	**C14,C14’**	**C15,C15’**	**C16,C16’**	**C17,C17’**	**C18,C18’**
124.76	129.15	123.65	122.58	121.55	121.35

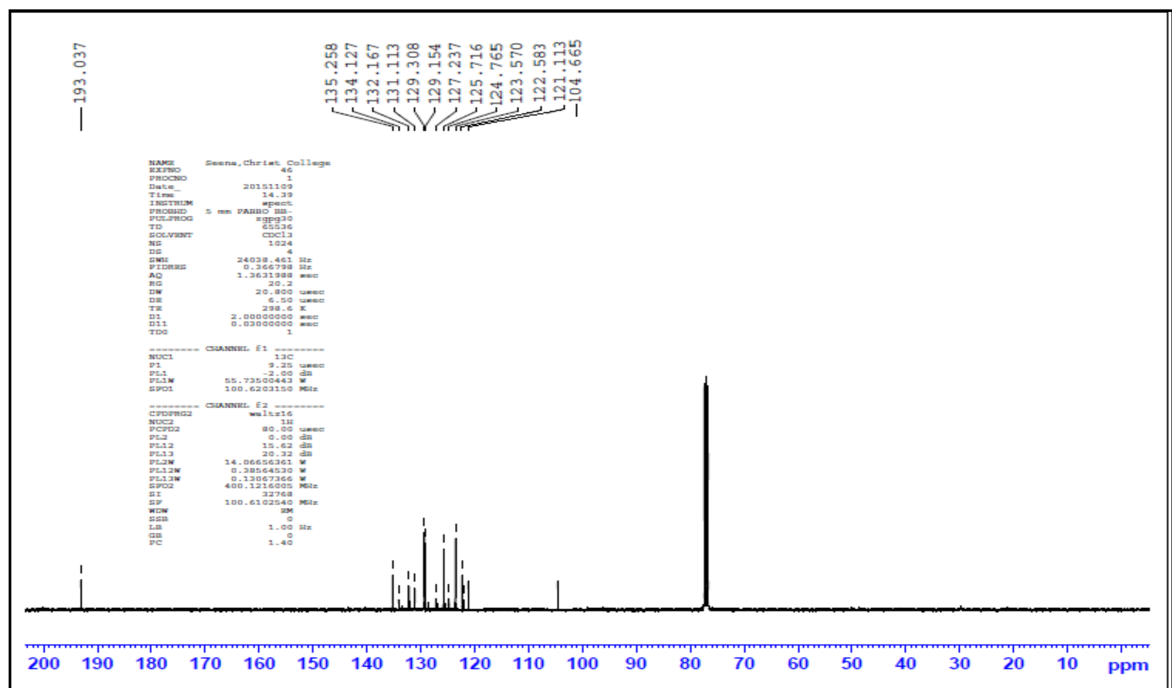


Fig.5.1.6 ^{13}C NMR spectral data of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione

In 5a all the aryl carbon atoms are present in the range between 120ppm and 132ppm. Only the carbon which is attached to the alkenyl group is downshielded and present at 134.12ppm. The C1 carbon atom which is flanked between two carbonyl group is present at position 104.68 ppm & C2 carbon atom which is present in the carbonyl group is seen at 193.037 ppm .

Mass spectra

The mass spectrum of the compound shows molecular ion peak at 476.The other peaks in the spectrum are due to fragment ions shown according to the scheme below.The mass spectrum is given in Fig.5.1.7 and mass spectral data is represented in Table.5.1.6.

Table 5.1.6 Mass spectral data of 5a

Fragments*	Ligand	M+/ (M+1)ion	A	B	C	D	E	F	G	H
Mass pattern	5a	476	245	232	189	205	178	204	298	278

*The alphabets corresponds to the fragments given in **Scheme 5.1.2**

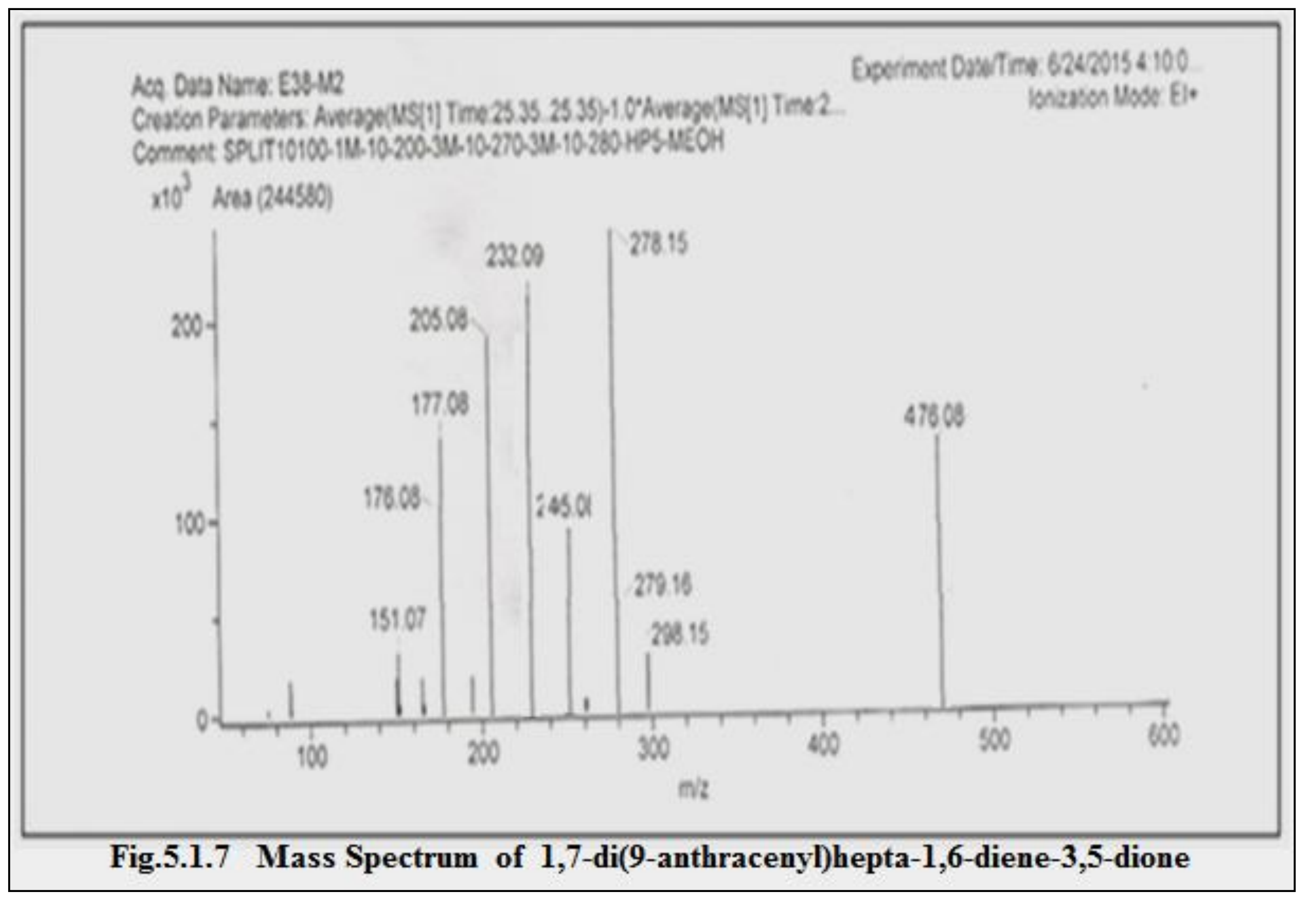
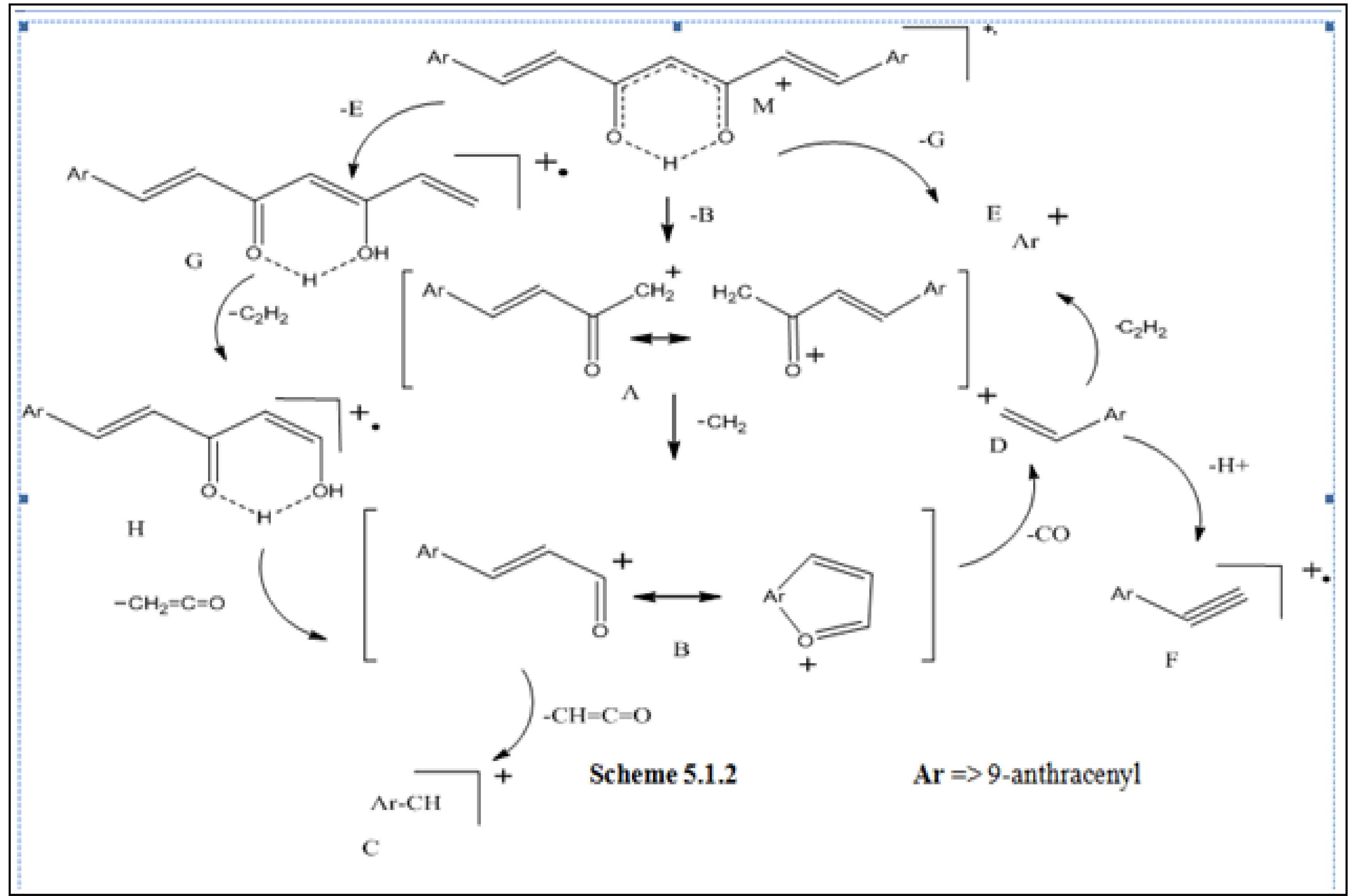


Fig.5.1.7 Mass Spectrum of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione


Ar
Ar
M
-E
-G
G
O
H
OH
-B
E
Ar
$-C_2H_2$
CH_2
H_2C
A
$-C_2H_2$
-CH_2
D
H
-H+
-CO
$-CH_2=C=O$
B
F
-CH=C=O
Ar-CH
C
Scheme 5.1.2
Ar => 9-anthracenyl

SECTION-II

SYNTHESIS AND CHARACTERISATION OF TRANSITION METAL CHELATES OF 1,7-DIANTHRACENYL HEPTANOIDS

5.2.1 Synthesis of transition metal complexes of 1,7-dianthracenyl heptanoids

Copper(II), Zinc(II), Nickel(II) and Oxovanadium(IV)complexes of curcuminoid allied with polynuclear anthracenyl ring were synthesized by the following general method.

To a refluxing solution of the ligand(0.002 mol) in methanol(25ml),a methanolic solution of metal salt (0.001mol)was added and the reaction mixture was refluxed for nearly 2hrs and cooled to room temperature.The metal salts used were Copper acetate,Zinc acetate,Nickel acetate and Vanadium (IV)oxide sulphate for the preparation of Cu(II),Zn(II),Ni(II) &VO(IV) complexes respectively.The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol.

5.2.2 Characterisation of metal complexes of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione

Metal chelates (Cu,Zn,Ni,Vanadyl) of ligand 5a were characterized using analytical and various spectral techniques like UV, IR, NMR and mass data. Elemental analysis (C, H and metal percentages), physical data, UV and IR spectral data are given in Table 5.2.1. The analytical data together with mass spectral data suggest a ML_2 stoichiometry for all the synthesized complexes.

Table 5.2.1 Analytical and spectral data of metal complexes of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione

Metal chelates	M.P. (°C)	Elemental analysis (%) Found/(calculated)			UV λmax nm	Characteristic IR stretching bands (cm^{-1})		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu(II)	185	81.98 (82.88)	4.37 (4.53)	6.15 (6.265)	288, 421	1594	1520	466, 427
Zn(II)	195	82.11 (82.72)	4.31 (4.53)	6.301 (6.44)	287, 422	1593	1521	462, 426
Ni(II)	198	82.73 (83.27)	4.33 (4.56)	5.75 (5.818)	287, 421	1590	1512	458, 424
VO(IV)	202	82.31 (82.600)	4.07 (4.52)	4.97 (5.009)	289, 424	1596	1517	475, 425

UV spectra

The UV spectra of metal complexes closely resembles the spectra of respective ligands. This indicates that no structural change has taken place during complex formation. In certain cases there is a slight shift of absorption maxima to longer wavelength which indicate the involvement of the carbonyl oxygens in metal complexation.

IR spectra

In the IR spectra of metal complexes, the peak of carbonyl moiety which is present at ~ 1630 cm^{-1} disappeared and a new band appeared at ~ 1590 cm^{-1}. The broad band in the region of 2600 -3500 cm^{-1} present in the ligand also reduced in the spectra of metal complexes. This is an indication of the replacement of the chelated proton by the metal ion during complexation. The new bands occurred in the range 460 and 420 cm^{-1} further supports the formation of M-O bond(metal-oxygen). The IR spectrum of Ni(II) complex of 5a is depicted below in Fig.5.2.1

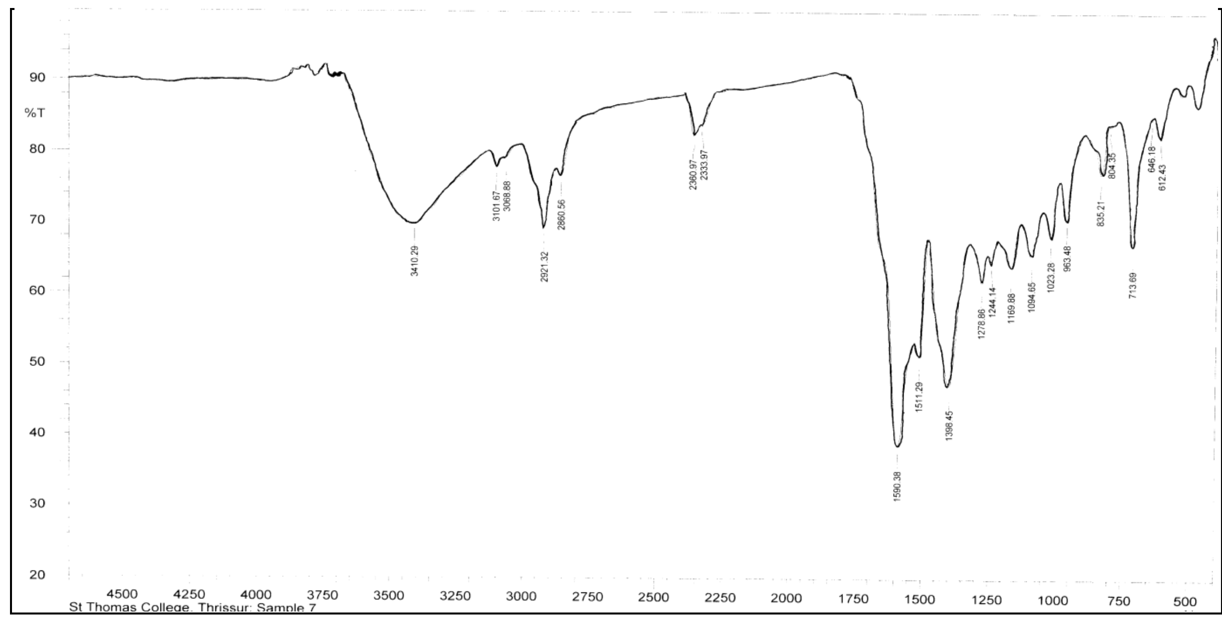


Fig.5.2.1 IR spectrum of Ni(II) complex of 5a

^{1}H NMR spectra

The enolic proton present in the ligands is replaced by metal atom in metal complexes. This is evident by the disappearance of the signal at $\delta \sim 16$ppm in metal complexes. The phenyl and alkenyl protons are not altered much since they are not involved in metal complexation. There is a slight shift of methine signals to the downfield of the spectra. Thus the spectra of ligand and complexes are much similar except those of enolic proton.

Mass spectra

The use of mass spectroscopy for the establishment of the stochiometry of metal 1,3-diketonates has been well established.Mass spectral analysis shows that stepwise removal of aryl group is a characteristic feature of the complexes.Electronic and steric effect of the group attached to the diketo function strongly influences the stability of various fragments formed under mass spectral condition.The suggested structure of the complexes are in agreement with the observed spectra of complexes.From the observed peaks in the spectrum of complexes ,it is suggested that certain fragments rearrange to form stable cyclic species.Peaks

due to [ML]+,L+(fragment F in Table) and fragments of L+ are also detected in the spectrum.The spectrum gives strong evidence for ML_2 stochiometry for the complex.The Mass spectrum of Cu(II) complex of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione is given in Fig.5.2.2.

Table 5.2.2 Mass spectral fragmental pattern of metal chelates of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione

Fragments	Metal chelates	M+/ (M+1)/ (M+2) ion	A	B	C	D	E	F	G
Mass Pattern	Cu(II)	1014	660	539	120	306	185	476	354
	Zn(II)	1016	662	541	120	308	187	476	354
	Ni(II)	1009	655	534	120	301	180	476	354
	VO(IV)	1017	663	542	120	309	188	476	354

*The alphabets corresponds to the fragments given in **Scheme 5.2.1**

Scheme 5.2.1

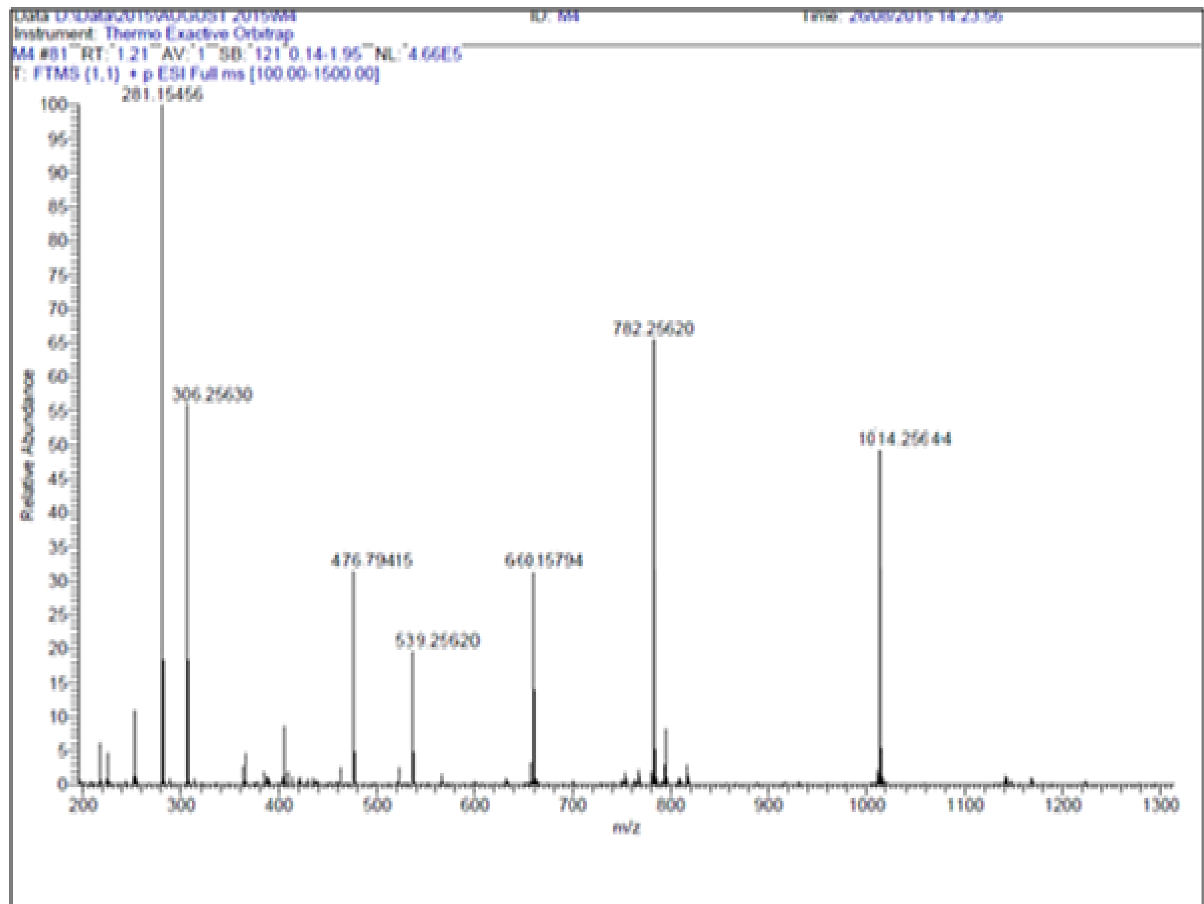


Fig.5.2.2 Mass spectrum of Cu(II) complex of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione

The mass spectrum of Cu(II)complex shows an intense molecular ion peak at 1014.The peak at 660 is due to the removal of two anthracenyl groups from the complex and the peak at 306 is due to the removal of four anthracenyl groups from the complex.The peak at 476 is that of the ligand.The base peak at 281 is due to fragment ion of the ligand and is observed in the spectrum of the ligand.

SECTION III

INVITRO CYTOTOXIC STUDIES OF CURCUMINOID ANALOGUES WITH ANTHRACENYL RING AND THEIR TRANSITION METAL COMPLEXES

5.3.1. *In vitro* Cytotoxic studies of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione and their metal complexes Cu(II), Zn(II), Ni(II) and VO(IV)

In vitro cytotoxic activity of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione and their transition metal complexes against DLA and EAC cancer cells were evaluated.The cytotoxic nature was determined in terms of the % cell death produced by them.Both the ligand and all the metal complexes showed considerable cytotoxic activity in both DLA and EAC cell lines in a dose dependant manner.The results of the study with EAC and DLA cancer cell lines are given in **Table 5.3.1** & **Table 5.3.2** respectively**.**

Table 5.3.1. In vitro Cytotoxic studies of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione (L) and their metal complexes towards EAC

Drug Con. µg/ml	% Cell death				
	L	$Cu(L)_2$	$Zn(L)_2$	$Ni(L)_2$	$VO(L)_2$
200	70	98	86	80	90
100	45	75	68	65	70
50	25	60	55	50	58
20	15	46	42	40	44
10	5	25	20	18	23

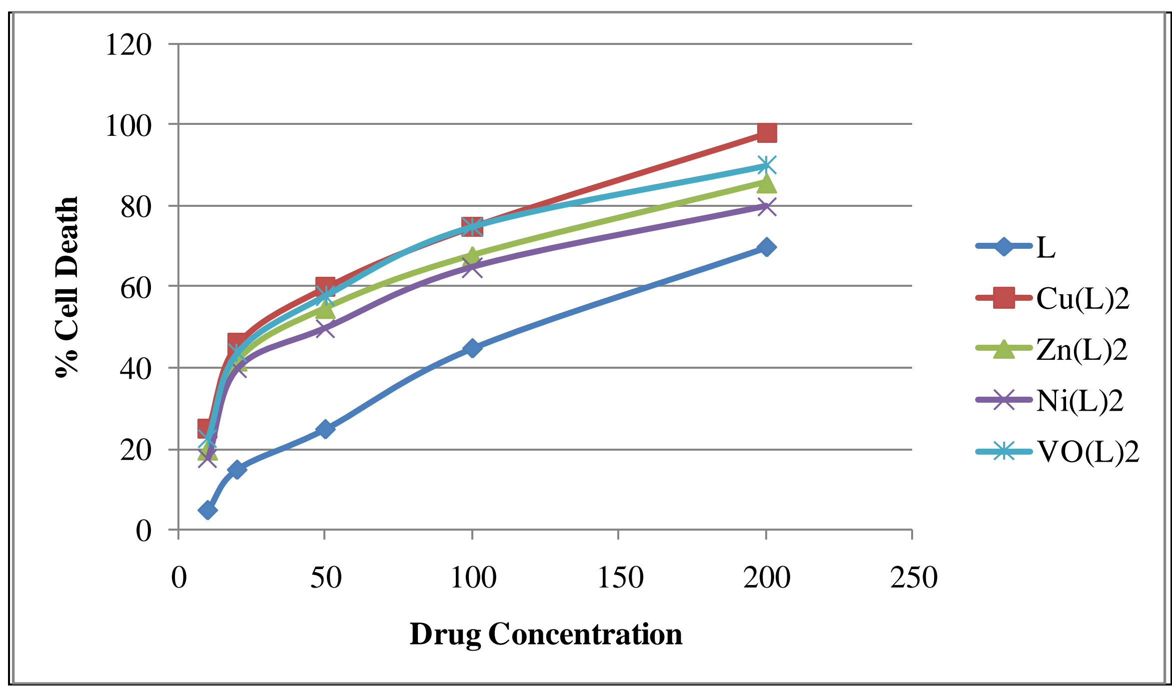


Fig.5.3.1. In vitro Cytotoxic studies of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione(L) and their metal complexes towards EAC

Table5.3.2. *In vitro* Cytotoxic studies of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione(L) and their metal complexes towards DLA

Drug Con. µg/ml	% Cell death				
	L	$Cu(L)_2$	$Zn(L)_2$	$Ni(L)_2$	$VO(L)_2$
200	68	96	84	80	89
100	42	74	66	63	69
50	20	58	53	50	56
20	10	44	40	39	43
10	5	24	19	17	21

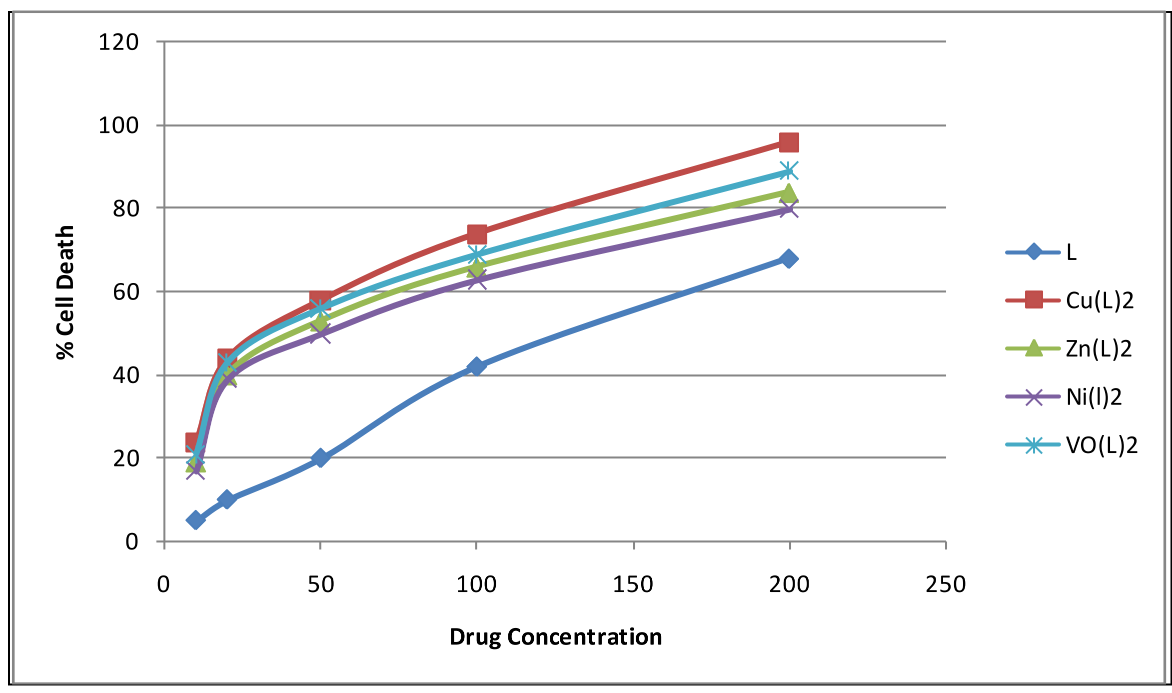


Fig.5.3.2. ***In vitro*** **Cytotoxic studies of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione(L) and their metal complexes towards DLA**

The results reveal that the compound and its metal complexes display significant cytotoxic property.70 % cytotoxicity to DLA and EAC was observed at a concentration of 200 µg/ml for the ligand and 98 % cytotoxicity was given by its Cu(II) complex at the same concentration.All the metal complexes gave good results with more than 80 % cell death at higher concentrations.The metal complexes were quite effective even at lower concentrations.The concentration of metal complexes required for 50 % cell death (IC 50) was found to be 50 µg/ml.The metal chelates presented activity in the order Cu ,Vanadyl,Zn and Ni.But the activity of Cu and Vanadyl complexes are comparable producing 90 % cell death.The activity of the compounds towards DLA cells is slightly less than their activity towards EAC cells.Thus it is concluded that the ligand and metal complexes with the polynuclear anthracenyl ring has profound effect in bringing about cell death.

IN VIVO ANTITUMOUR STUDIES OF 1,7-DI(9-ANTHRACENYL)-1,6-HEPTADIENE-3,5-DIONE AND THEIR METAL COMPLEXES

In this study the anticarcinogenic activities of the polynuclear ligand 1,7-di(9-anthracenyl)-1,6-heptadiene-3,5-dione and their Cu & Vanadyl complexes were evaluated in vivo.The effect of these compounds to increase the life span of ascites tumour bearing animals were studied.Viable EAC cells were injected into the peritoneal cavity of mice so as to develop tumour in them.Drugs (ligand and metal complexes) were administered as ip injection at different concentrations for 10 days after tumour injection.The death pattern of animals due to tumour burden was noted and the % increase in life span calculated. The results of the study are given in **Table 5.3.3.**

Table 5.3.3 Effect of 1,7-di(9-anthracenyl)-1,6-heptadiene-3,5 dione(L) and their metal complexes (Cu(II) & VO(IV)) 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	**30.6±3.1**	**76.88**
3.L	20	5/5	**28.8±2.7**	**66.47**
4. L	10	5/5	**27.8±3.1**	**60.71**
5. L	5	5/5	**24.9±2.10**	**43.93**
6.Cu(L)$_2$	20	5/5	**30.9±2.2**	**78.62**
7. Cu(L)$_2$	10	5/5	**29.6±3.0**	**71.70**
8. Cu(L)$_2$	5	5/5	**27.6±2.8**	**56.59**
9.VO(L)$_2$	20	5/5	**29.0±2.2**	**67.63**
10.VO(L)$_2$	10	5/5	**28.1±1.75**	**62.42**
11.VO(L)$_2$	5	5/5	**25.0±2.01**	**44.50**

The study revealed that intraperitoneal administration of ligand, its Cu(II) and Vanadyl complexes significantly increased the life span of EAC induced ascites tumour bearing mice.The ligand as well as the metal complexes exhibited very prominent antitumour activity.The life span of animals was found to be significantly increased to 28,31 and 29 days for ligand,Cu(II) and VO(IV) complexes when the control animals survived for an average of 17 days after tumour induction. The % ILS for ligand,Cu(II) and Vanadyl complexes with concentration 20 µg/ml was found to be 66.47 ,78.62 and 67.63 % respectively which is comparable with the value 76.8 % for the standard drug cyclophosphamide. The maximum value for the no.of days of survival was observed with Cu(II) complex of 1,7-di(9-anthracenyl)-1,6-heptadiene–3,5–dione ie 30.9 days which is greater than 30.6 days for the standard drug.The Cu(II) complex exhibited almost the same activity as the std.drug.So the studies reveal that Cu(II) complex is very effective in reducing tumour development in mice and increasing the life span of the animal.

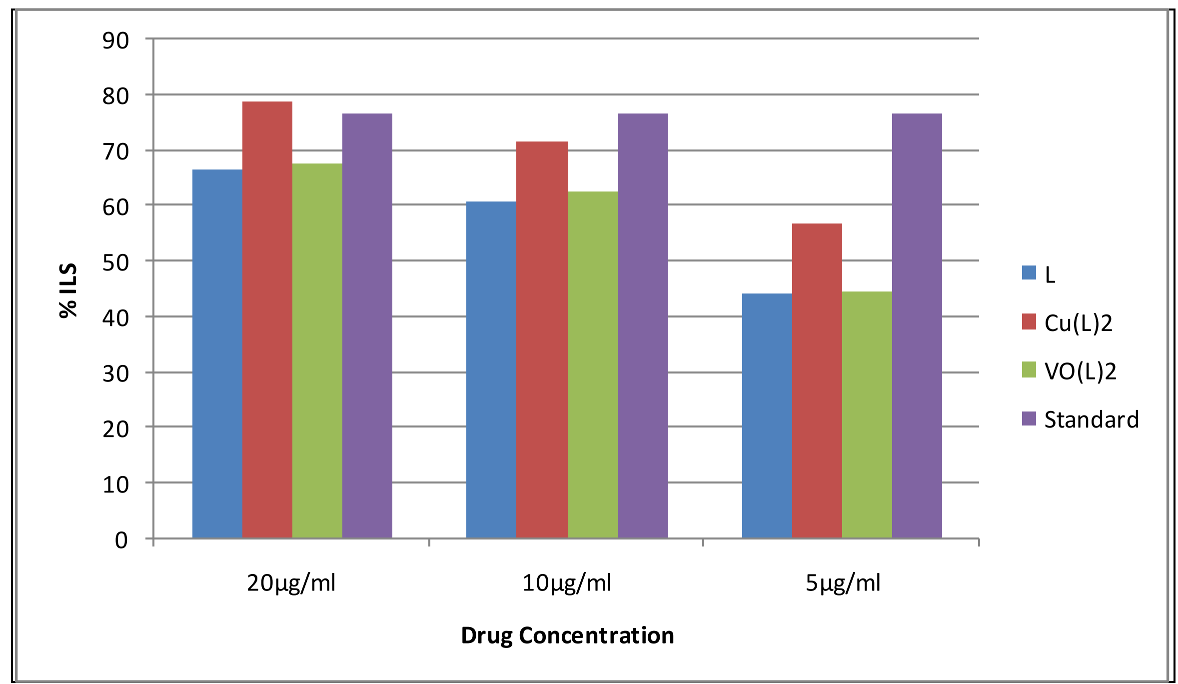


Fig.5.3.3 The % ILS with different conc. of 1,7-di(9-anthracenyl)-1,6-heptadiene-3,5 dione(L) and their metal complexes (Cu& VO(IV))

INVIVO CYTOTOXIC STUDY ON SOLID TUMOUR DEVELOPMENT

Effect of 1,7-di(9-anthracenyl)-1,6-heptadiene-3,5 dione(L) and its Cu(II) complex on solid tumour

Swiss albino mice (5-6 weeks old) weighing 20-25 g were divided into four groups comprising of 5 animals for this study.Tumour was induced by injecting DLA cells on to the right hind limb of all animals.Group I was kept as control without drug treatment and group II was treated with the std.drug cyclophosphamide.Group III and IV were treated with1,7-di(9-anthracenyl)-1,6-heptadiene-3,5dione and its Cu(II) complex at a concentration of 200 μmol /kg body weight.The drugs were administered by intraperitoneal injection from the first day of tumour induction and continued for next 10 days.The tumour development on animals in each group was determined by measuring the diameter of tumour growth from seventh day of tumour induction upto 31 rst day.The tumour volume was calculated using 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 significant reduction of solid tumour volume in mice treated with 1,7-di(9-anthracenyl)-1,6-heptadiene-3,5 dione and its Cu(II) complex .Compared to ligand ,the respective Cu(II) chelate was more effective in bringing about reduction in solid tumour volume.The measured tumour volume was 5.042 cm^3 for the control group on the 31 rst day.The std.drug treated mice showed the reduced tumour volume 1.982 cm^3.The ligand treated group significantly decreased the tumour volume to2.25 cm^3 . Comparing with that of the control group,the ligand produced a decrease in volume of 2.792 cm^3 . The tumour volume on day 31 for copper complex of ligand was 1.985 cm^3 .The decrease in tumour volume was 3.057 cm^3 with respect to control group.The tumour burden was comparatively less in case of treated animals than control group. The decrease in tumour volume for std.drug was 3.060 cm^3 .The Cu(II) complex of 1,7-di(9-anthracenyl)-1,6-heptadiene-3,5

dione had shown a prominent effect in reducing tumour volume comparable with the std.drug.The results of the study are given below in **Table.5.3.4**

Table 5.3.4 Effect of Compound on solid tumour development

Compounds	Tumour volume on 31st day
Control group	5.042 cm^3
5a (L)	2.25 cm^3
Cu $(L)_2$	1.985 cm^3
Std.group	1.982 cm^3

SECTION-IV

ANTIBACTERIAL STUDY OF 1,7-ANTHRACENYL HEPTANOIDS AND THEIR Cu(II),Zn(II)&VO(IV)METAL COMPLEXES

Antibacterial studies of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione and their metal complexes Cu(II), Zn(II) and VO(IV)

Antibacterial screening of ligand and metal complexes were carried out by using Agar well diffusion method.Bacterial cultures included in the study are Escherichia Coli, Klebsiella Pneumoniae & Bacillus Subtilis.The test compounds showed varying degree of inhibition against different bacterial strains.All synthesized compounds have shown to be susceptible to excellent potency against the different bacterial strains. The results of the antibacterial activity of synthesized compounds revealed that the ligand and the complexes possess comparable antibacterial activity to that of standard drug streptomycin.The activity is expressed in terms of diameter of zone of inhibition in mm. In all the cases metal complexes possess better antibacterial activity than that of ligands, which means that metal complexation enhance the activity .

5.4.1 Antibacterial studies of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione and their metal complexes Cu(II), Zn(II) and VO(IV)

The ligand exhibited considerable antibacterial activity against all the three bacterial strains.It was most effectual against E.coli species producing a zone of inhibition of 15mm.All the complexes elicited inhibitory activities against all three bacterial strains and were more effective than ligand.The Cu(II) complex exhibited maximum inhibitory activity against E.coli species with a zone of inhibition of 19mm which is comparable with the activity of Streptomycin.It was found that Cu(II) complex was also very effective against Bacillus and Klebsiella with a zone of inhibition of 18mm and 16.5mm respectively.The std.drug produced a zone of inhibition of 20 mm against all bacterial strains.The Zn(II) complex had

shown a slight marginal increase in activity compared with the ligand.The Vanadyl complex was quite effective against all the three bacterial strains.The results of antibacterial study of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione (L)and its metal complexes are given in **Table.5.4.1**

Table 5.4.1 Antibacterial studies of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione (L) and their metal complexes Cu(II), Zn(II) and VO(IV)

Bacteria	Diameter of zone of inhibition in mm			
	L	$VO(L)_2$	$Zn(L)_2$	$Cu(L)_2$
E Coli	15	17.5	16	19
Klebsiella	14.5	15.5	14.5	16.5
Bacillus	13	15	13.5	18
Standard	20	20	20	20

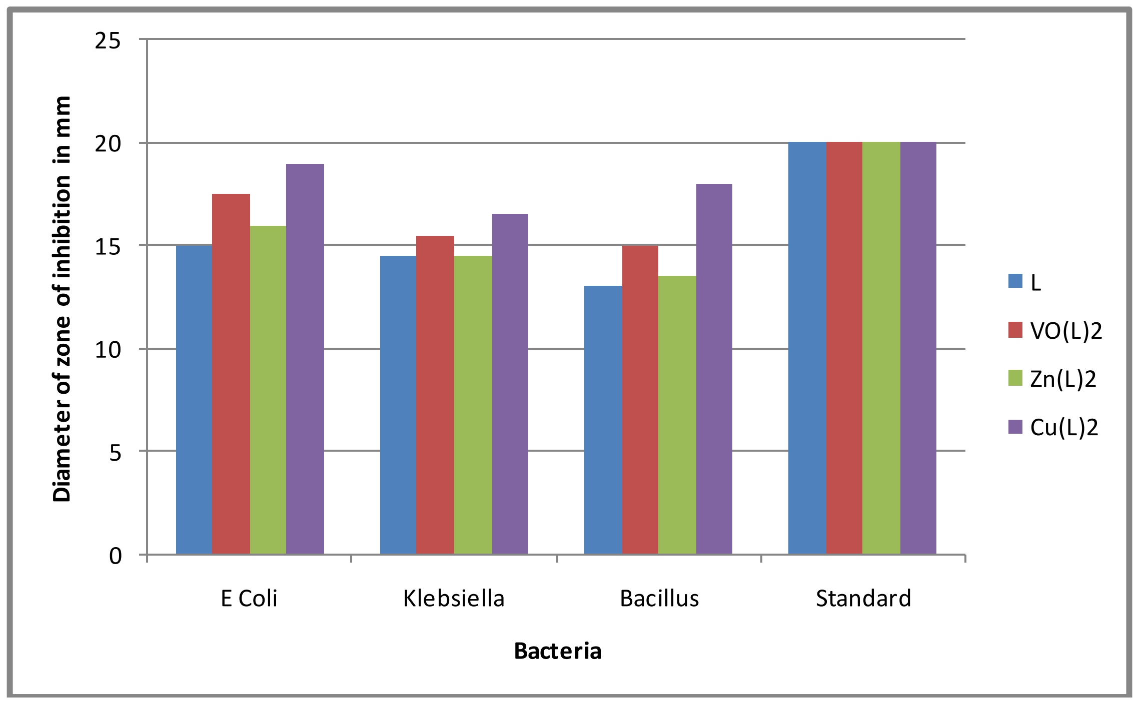


Fig. 5.4.1 Antibacterial studies of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione (L) and their metal complexes Cu(II), Zn(II) and VO(IV)

SECTION-V

ANTIFUNGAL STUDY OF 1,7-ANTHRACENYL HEPTANOIDS AND VO(IV)METAL COMPLEXES

Antifungal Activity of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione (L)and their VO(IV) complexes

The curcuminoid analogue with anthracenyl ring and the Vanadyl complex were studied for their antifungal activity against three fungal cultures namely Aspergillus Niger, Penicillium Chrysogenum and Alternaria Alternate.Kirby Baurer disc plate method was used to test the susceptibility of the fungi species to the test compounds. Different concentrations [100, 250, 500µg/ml] by dissolving in 2% DMSO solvent were used for all the test compounds and results were compared with the std.drug flucanozole.The antifungal activities are measured in terms of zone of inhibition in mm.The data of the study revealed that the synthesized 1,7-dianthracenyl heptanoids and the VO(IV) complex possess comparable antifungal activities to that of std.drug.

5.5.1 Antifungal Activity of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione (L) and their VO(IV) complexes

The diameter of the inhibition zone of the test compounds with the three fungi species in comparison to flucanazole(std.drug) is shown in Table **5.5.1**

Table 5.5.1. Antifungal studies of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione (L) and their VO(IV) complexes

Fungi	Diameter of zone of inhibition in mm					
	L			$VO(L)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	16	18.5	21	17	19	24
Penicillium	15	17	20	16	18	21
Alternaria	14.5	16	19	15.5	18	20

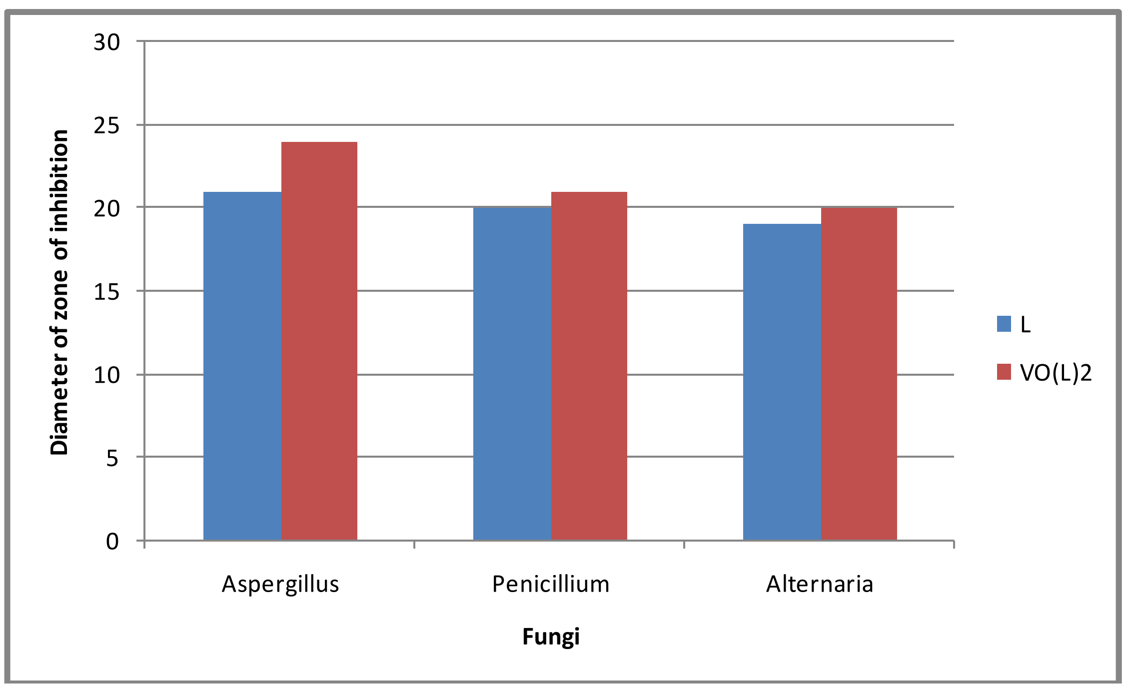


Fig 5.5.1. Antifungal studies of 1,7-di(9-anthracenyl)hepta-1,6-diene-3,5-dione (L) and their VO(IV) complexes at 500µg/ml.

The inhibitory effect of ligand and its metal complexes against the fungal cultures is given in **Table 5.5.1.**For all the tested compounds they show maximum antifungal activity at a higher concentration of 500µg/ml.It is observed that antifungal nature increases with the concentration of the compounds.The ligand exhibited a zone of inhibition of 21mm against Aspergillus where as zone of inhibition produced is 20 and 19 mm against Penicillium and Alternaria respectively at higher conc.The ligand exhibited more antifungal activity against Aspergillus..The VO(IV) complexes were quite effective against all fungi at all concentrations.The vanadyl complex of ligand confirmed promising antifungal activity producing a zone of inhibition of 24mm with Aspergillus species. It was also very effective against Penicillium and Alternaria with the zone of inhibition in the range 20mm.The Vanadyl complex has shown greater activity than the std.drug which produced a zone of inhibition of 21mm.The ligand as well as the metal complexes has proved to be potent antifungal compounds.

CHAPTER-VI

SYNTHESIS, CHARACTERISATION AND BIOCHEMICAL ACTIVITIES OF METHOXY AND HYDROXY SUBSTITUTED 1,7-DINAPHTHYLHEPTA-1,6-DIENE-3,5-DIONES AND THEIR TRANSITION METAL CHELATES WITH Cu(II), Zn(II), Ni(II) & OXOVANADIUM(IV)

SECTION I

SYNTHESIS AND CHARACTERIZATION OF CURCUMINOID ANALOGUES WITH NAPHTHYL AND SUBSTITUTED NAPHTHYL RINGS

This chapter deals with the synthesis and characterization of curcuminoid analogues with naphthyl and substituted naphthyl rings instead of phenyl rings in natural curcuminoids.The ligands synthesized here include 1,7-dinaphthyl-1,6-heptadiene-3,5-dione(6a),1,7-bis(2-methoxynaphthyl)1,6-heptadiene-3,5-dione (6b), 1,7-bis(2-hydroxynaphthyl)1,6-heptadiene-3,5-dione(6c).Section II of this chapter deals with the synthesis and characterization of metal complexes of the above mentioned ligands with transition metal ions Cu(II),Zn(II),Ni(II) and VO(IV).Section III deals with the invitro and invivo antitumour activity of both ligands and metal complexes.In Section IV the antibacterial activity of the synthesized compounds are discussed.Section V presents the antifungal activity of both ligands and metal complexes prepared.Introduction of naphthyl ring system in the α,β unsaturated diketone moiety modifies the chemical and biochemical properties of the compounds.

6.1.1 Synthesis of substituted derivatives of 1,7-dinaphthyl-1,6-heptadiene-3,5-diones

The curcuminoid analogues 1,7-dinaphthyl-1,6- heptadiene-3,5-dione(6a),1,7-bis(2-methoxynaphthyl)1,6-heptadiene-3,5-dione(6b),1,7-bis(2-hydroxynaphthyl)1,6-heptadiene-3,5-dione (6c) were prepared by the condensation of aldehydes (1-naphthaldehyde, 2-methoxynaphthaldehyde and 2-hydroxynaphthalehyde) with acetyl acetone-boric oxide complex in ethyl acetate medium in presence of tributyl borate and n-butyl amine.The product was purified by column chromatography over silica gel (60–120 mesh) using 4:1 (v/v) chloroform : acetone mixture as the eluent and recrystallised twice from hot benzene to

get pure crystalline material.The synthesis of naphthyl derivatives of curcuminoids described in this section is outlined in Scheme **6.1.1**.

Ar-CHO + H_3C-CO-CH_2-CO-CH_3 + OHC-Ar

6a – Ar = 1-Naphthyl

6b – Ar = 2-Methoxy naphthyl

6c – Ar = 2-Hydroxy naphthyl

↓

Ar-CH=CH-CO-CH_2-CO-CH=CH-Ar

Scheme 6.1.1

The aldehydes used for synthesis, structure of the ligand, systematic name, colour and yield has been included in the table given below.

Table 6.1.1 Synthetic details of 1,7-dinaphthyl heptanoids (6a-6c)

Compound	Aldehyde used for Synthesis	Structure of Ligands	Systematic name	Yield %	Colour
6a	1-Naphthaldehye		1,7-dinaphthyl hepta-1,6-diene-3,5-dione	62	Orange red
6b	2-methoxynaphth-aldehyde		1,7-bis(2-methoxy naphthyl) hepta-1,6-diene-3,5-dione	75	black
6c	2–hydroxynaphth-aldehyde		1,7-bis(2-hydroxy naphthyl) hepta-1,6-diene-3,5-dione	65	green

All the compounds prepared(6a, 6b, 6c) are crystalline solids and show sharp melting point. They are insoluble in water and are soluble in organic solvents like acetone,chloroform,ethyl acetate,methanol etc.The elemental analysis results,determined molecular weight and melting point of the compounds are given in **Table 6.1.2.** The UV spectral data of the compounds has also been included in the table below.

Table 6.1.2 Analytical & UV spectral data of 1,7–dinaphthyl heptanoids

Compounds	M.P(^{o}C)	Elemental analysis(%) C	Elemental analysis(%) H	Molecular weight	UV λmax (nm)
		Found/(calculated)			
6a	165	85.70(86.23)	4.98(5.21)	373(376)	260,386
6b	172	78.74(79.81)	5.12(5.50)	433(436)	266,392
6c	181	80.01(79.41)	4.32(4.90)	410(408)	270,395

6.1.2. Spectral Characterisation of 1,7–dinaphthyl heptanoids

1,7-dinaphthyl-1,6-heptadiene-3,5-dione(6a),1,7-bis(2-methoxynaphthyl)1,6-heptadiene-3,5-dione(6b),1,7-bis(2-hydroxynaphthyl)1,6-heptadiene-3,5-dione(6c)were synthesized and are characterized by various spectral techniques like UV, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral techniques.

UV spectra

The electronic spectra of the compounds were recorded in methanol and the spectra of 1,7-bis(2-methoxynaphthyl)1,6-heptadiene-3,5-dione(6b)is shown in **Fig 6.1.1.** and the data is given in the Table above.The curcuminoid analogues with naphthyl ring in methanolic solution showed two characteristic UV-Visible absorption maxima in the range 260-400nm. The bands observed in the spectra corresponds to the $n \rightarrow \pi *$ and $\pi \rightarrow \pi *$ transitions . The absorption bands at 260-270 nm are due to $\pi \rightarrow \pi *$ transition and at 380-400nm are due to $n \rightarrow \pi *$ transitions.

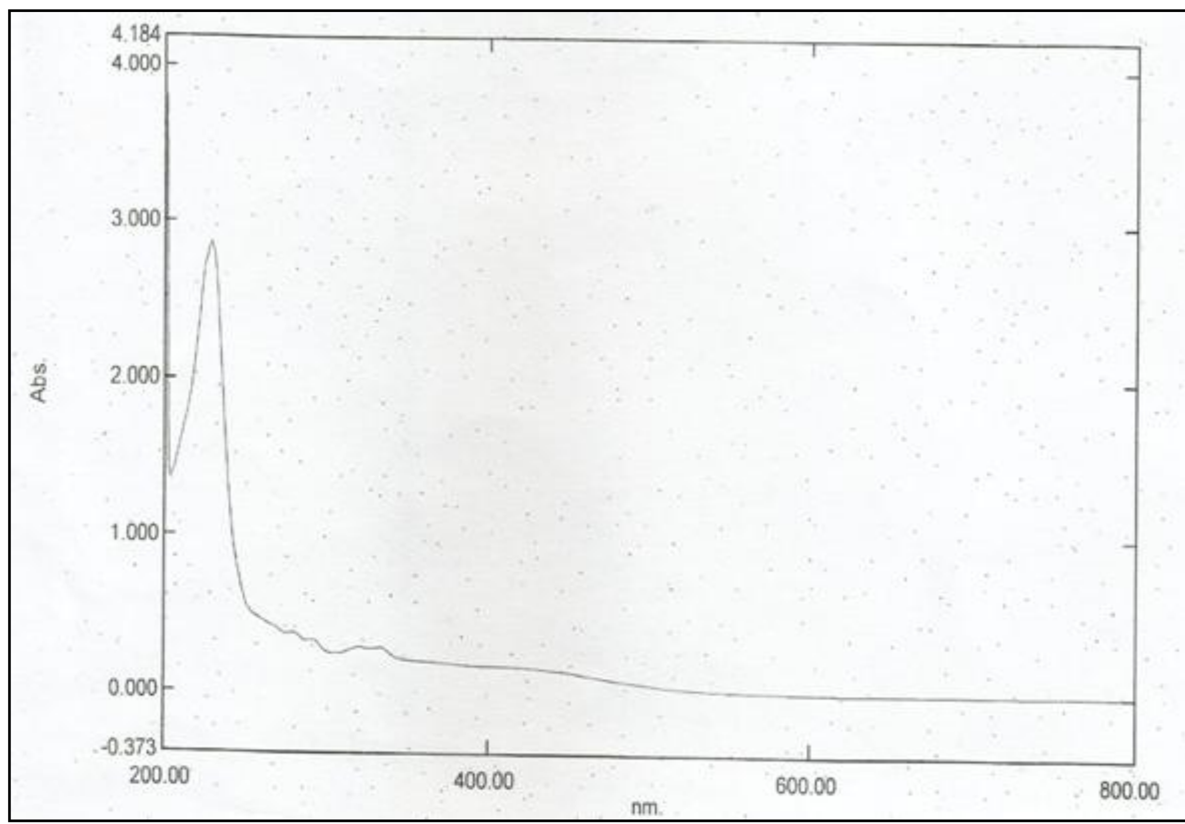


Fig.6.1.1 UV Spectrum of 1,7-bis(2-methoxynaphthyl)1,6-heptadiene-3,5-dione(6b)

IR spectra

In these compounds the position and intensity of the carbonyl stretching frequency depends on the molecular structure in its immediate vicinity and is useful in characterizing the type of carbonyl function.Presence of α,β– unsaturated 1,3 - diketo moiety present in these molecules can be very well established using IR spectroscopy.All the compounds are quite symmetrical with the carbonyl groups attached to aliphatic carbons,the olefinic groups are interposed between the naphthyl and carbonyl group.

IR spectra of 6a ,6b and 6c are characterized by the presence of strong bands at 1620 cm^{-1},1662 and 1637 cm^{-1} respectively due to the enolised conjugated C=O group. The decrease in C=O frequency from the normal value can be explained due to hydrogen bonding and increased conjugation. There is no other band in the region 1600-1800 cm^{-1} which is assignable due to free or bound C=O group.This shows that the compound exist in the intramolecularly hydrogen bonded enolic form.All the compounds show bands in the region

1590 cm^{-1} which are assigned to C=C bonds in naphthyl ring and in the range 1540cm^{-1} which are assigned to C=C bonds of olefinic groups.

In the spectra, the intramolecular hydrogen bonded enolic group shows a broad band in the region 2550-3600 cm^{-1}. There are a number of medium intensity vibrations observed in the region 1050-1520 cm^{-1} due to various stretching vibrations of the phenyl group, alkenyl & chelate ring.The bands in the region 964, 992 and 985 cm^{-1} are assigned to the trans CH=CH vibrations. The important IR absorptions and their probable assignments are given in Table.6.1.3. IR spectrum of compounds 6a, 6b and 6c are depicted in Fig.6.1.2,Fig.6.1.3 &Fig.6.1.4 respectively

Table 6.1.3 IR spectral data of 1,7–dinaphthyl heptanoids

Compounds			**Probable IR assignments**
6a	**6b**	**6c**	
1620	1662	1637	ν(C=O) chelated
1594	1583	1593	ν(C=C) phenyl
1542	1532	1541	ν(C-C) alkenyl
1506	1512	1514	ν_{as}(C-C-C) chelate ring
1458	1460	1463	ν_{s}(C-C-C) chelate ring
1134,1087	1123,1035	1167,1078	β (C-H) chelate ring
964	992	985	ν(CH=CH) trans

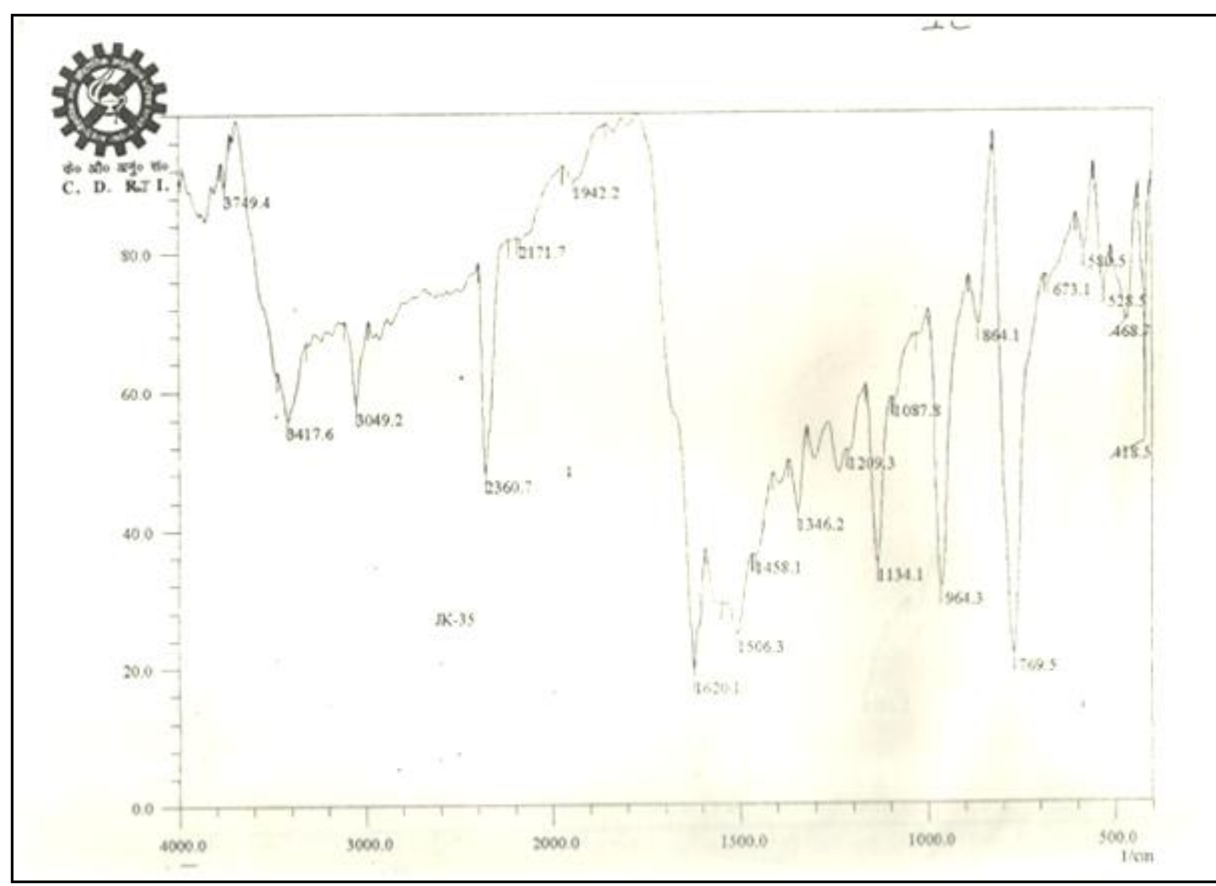


Fig.6.1.2 IR spectrum of 6a

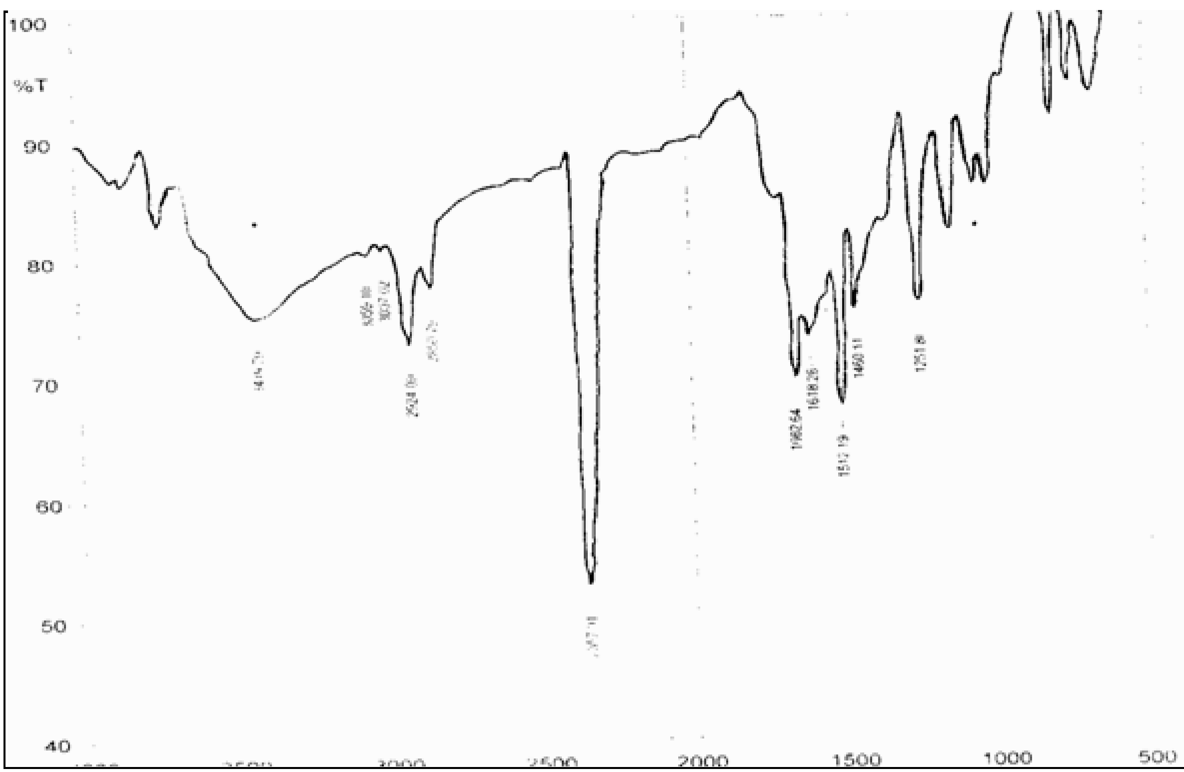


Fig.6.1.3 IR spectrum of 6b

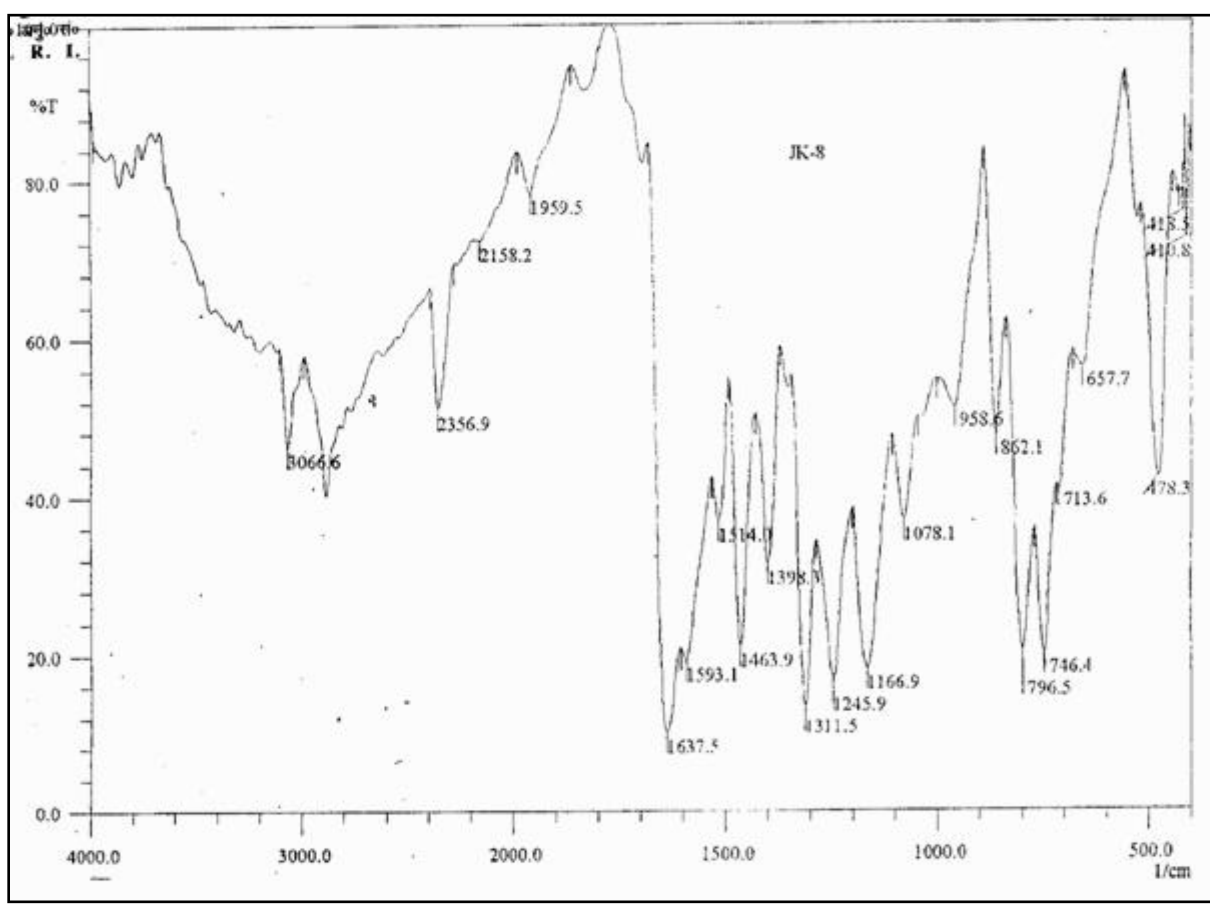


Fig.6.1.4 IR spectrum of 6c

^{1}H NMR spectra

The ^{1}H NMR spectra of all compounds 6a, 6b & 6c displayed a one proton singlet downfield at ~ 16ppm and another singlet peak at δ ~ 6.0ppm due to intra molecularly hydrogen bonded enolic proton and methine proton respectively.The position of these signals are varied slightly in different compounds because they are influenced by the electronic effects of groups attached to the carbonyl function.The ^{1}H NMR spectra of all compounds show specific peaks corresponding to enolic, methine, alkenyl, methoxy, phenyl and hydroxyl groups.The protons of the methoxy substituent on the naphthyl ring in compound 6b displayed a signal at 4.058 ppm . The protons of the hydroxy substituent on the naphthyl ring in compound 6c displayed a signal at 10.789 ppm. The assignments of various proton signals observed in the spectra of the compounds are given in **Table 6.1.4**. The enolic structure of the compound representing

enolic and methine proton is given below in Fig.**6.1.5**.The[1]**H NMR spectra** of compounds 6a,6b & 6c are brought out in figures **6.1.6 -6.1.8.**

Ar=1-Naphthyl,2-Methoxynaphthyl,2-hydroxynaphthyl
Fig.6.1.5 Enolic structure of 1,7–dinaphthyl heptanoids

Table 6.1.4 ^{1}H NMR spectral data of 1,7–dinaphthyl heptanoids

Compounds	Chemical shifts (δ ppm)					
	Enolic	Methine	Alkenyl	Aryl	Substituent	
					Methoxy	Hydroxyl
6a	15.98	5.952	6.7-8.56	7.58-8.28	-	-
6b	16.01	5.95	7.25-7.76	7.41-8.04	4.058	-
6c	16.041	5.909	7.142-7.976	7.413-8.330	-	10.789

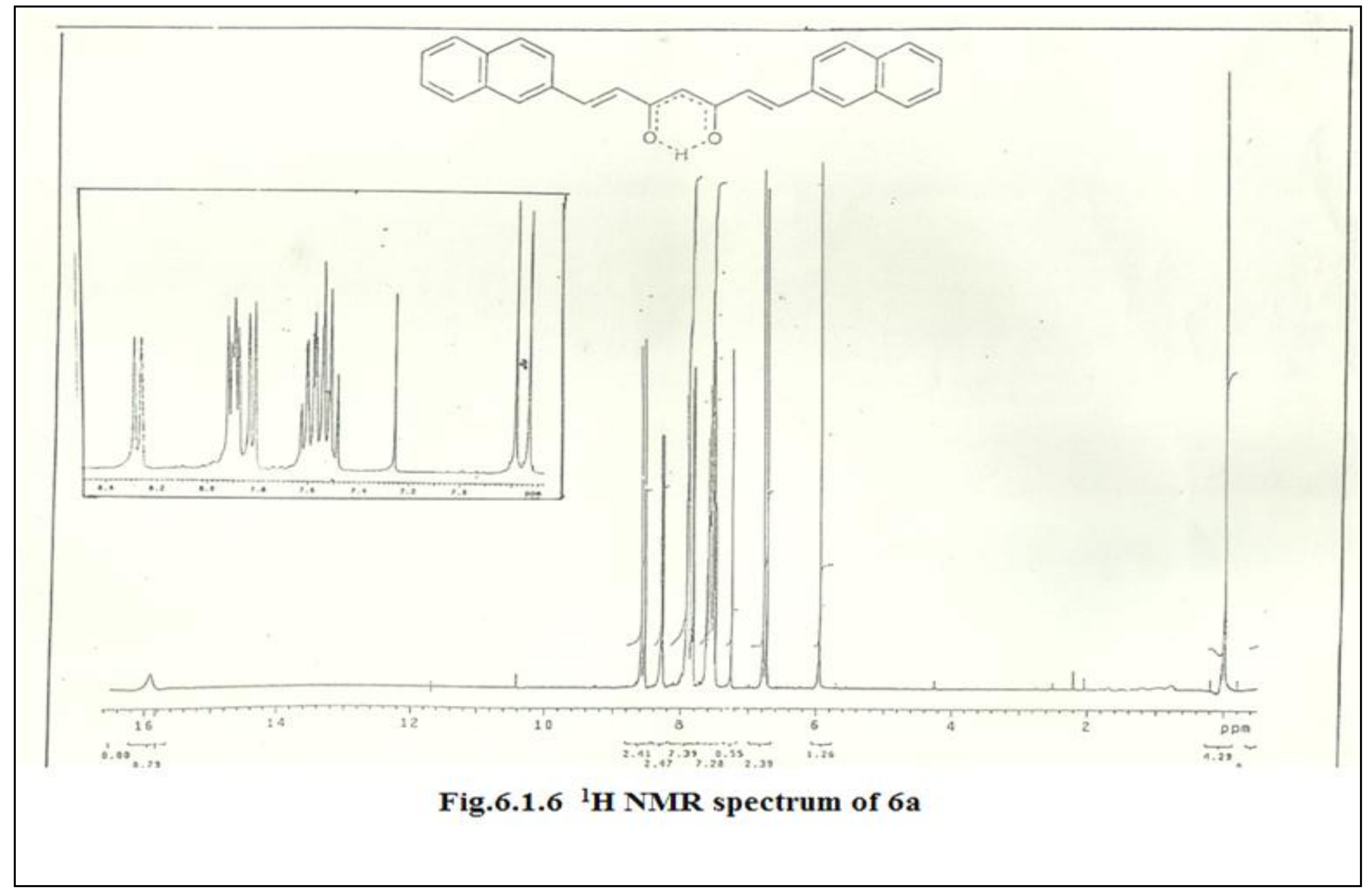


Fig.6.1.6 ^{1}H NMR spectrum of 6a

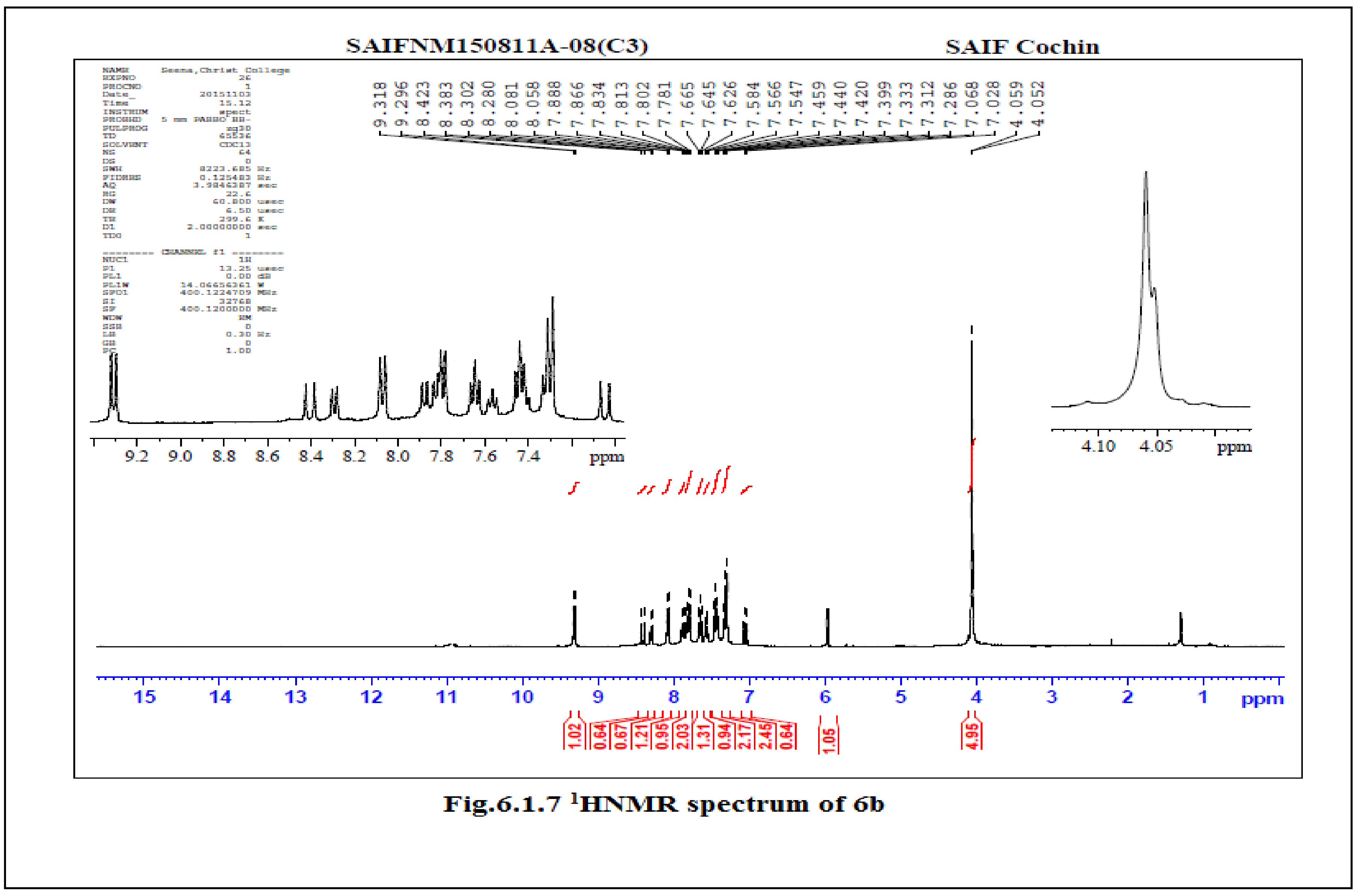


Fig.6.1.7 ^{1}HNMR spectrum of 6b

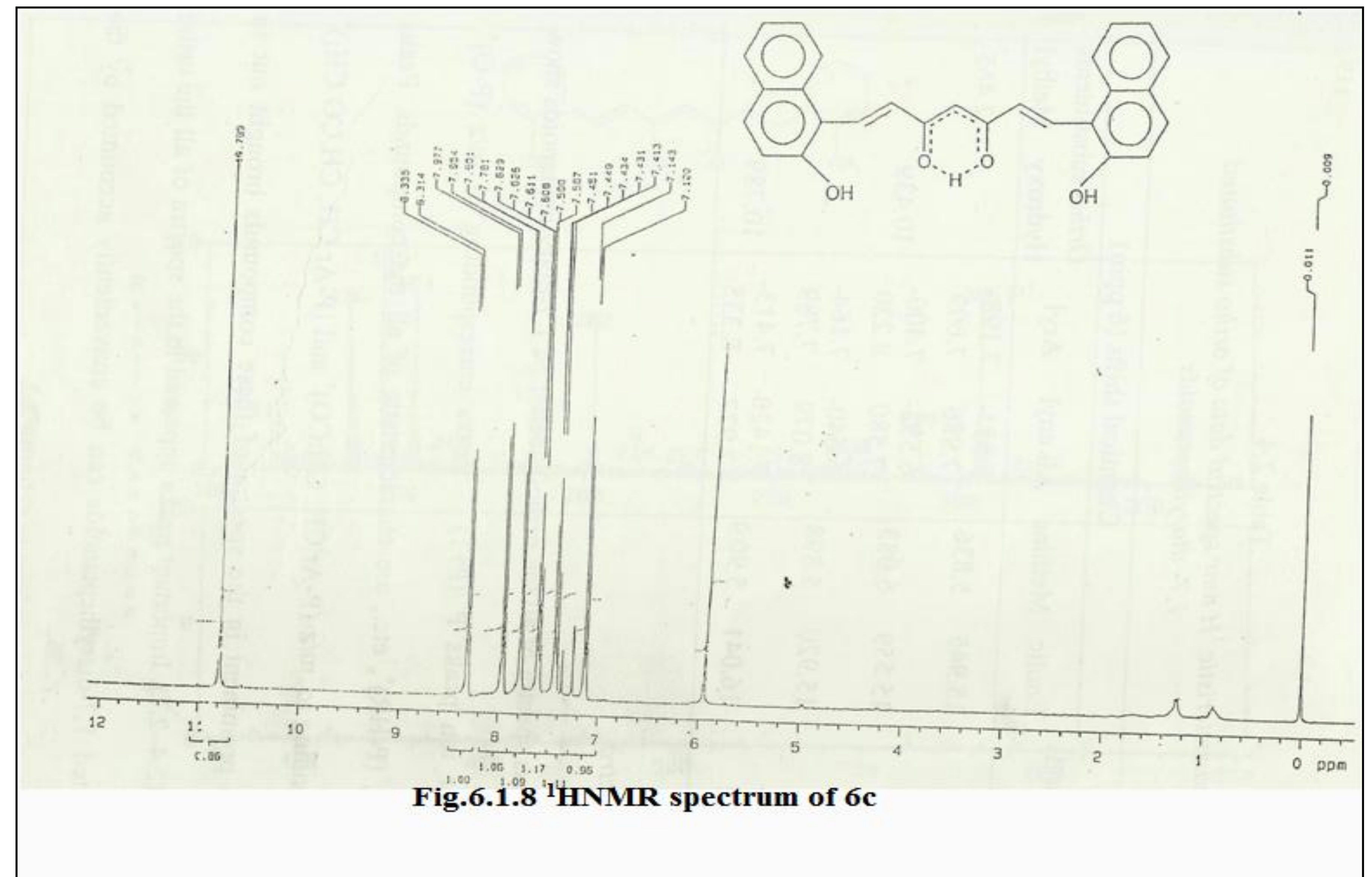


Fig.6.1.8 ^{1}HNMR spectrum of 6c

^{13}C NMR spectra

The ^{13}C NMR spectra of all the compounds 6a, 6b & 6c gives idea about the non equivalent carbon atoms in the molecule.Each non equivalent carbon atom gives rise to a peak with a different chemical shift. The ^{13}C NMR spectral data of all the compounds are given in Table 6.1.5,6.1.6 & 6.1.7.In the compound 6a, the peak corresponding to methine (C1) carbon is present ~ at 102ppm. The presence of keto-enol tautomerism makes the shift of C1 carbon to ~ at 102ppm. C2 carbon of carbonyl appears at the lowest field value,the position of the peak is at~ 193 ppm. The alkenyl carbon atoms(C3 &C4) are present at a position nearer to138 & 124ppm respectively. The C5 is part of the naphthyl ring and is also attached to olefinic carbon and gave a peak at 136 ppm.The aromatic carbon atoms are present between 122 – 136 ppm.

Fig. 6.1.9 Structure representing different non equivalent C atoms in 1,7-dinaphthyl-1,6- heptadiene-3,5-dione(6a)

Table 6.1.5 ^{13}CNMR spectral data of 1,7-dinaphthyl-1,6- heptadiene-3,5-dione(6a), (chemical shift in ppm)

C1	**C2,C2'**	**C3,C3'**	**C4,C4'**	**C5,C5'**	**C6,C6'**	**C7,C7'**
102.22	193.02	137.54	124.93	136.62	133.80	123.49
C8,C8'	**C9,C9'**	**C10,C10'**	**C11,C11'**	**C12,C12'**	**C13,C13'**	**C14,C14'**
131.63	126.91	128.99	130.54	132.39	135.30	125.51

The ^{13}**CNMR spectra** of 6a is given below in Fig.6.1.10 .

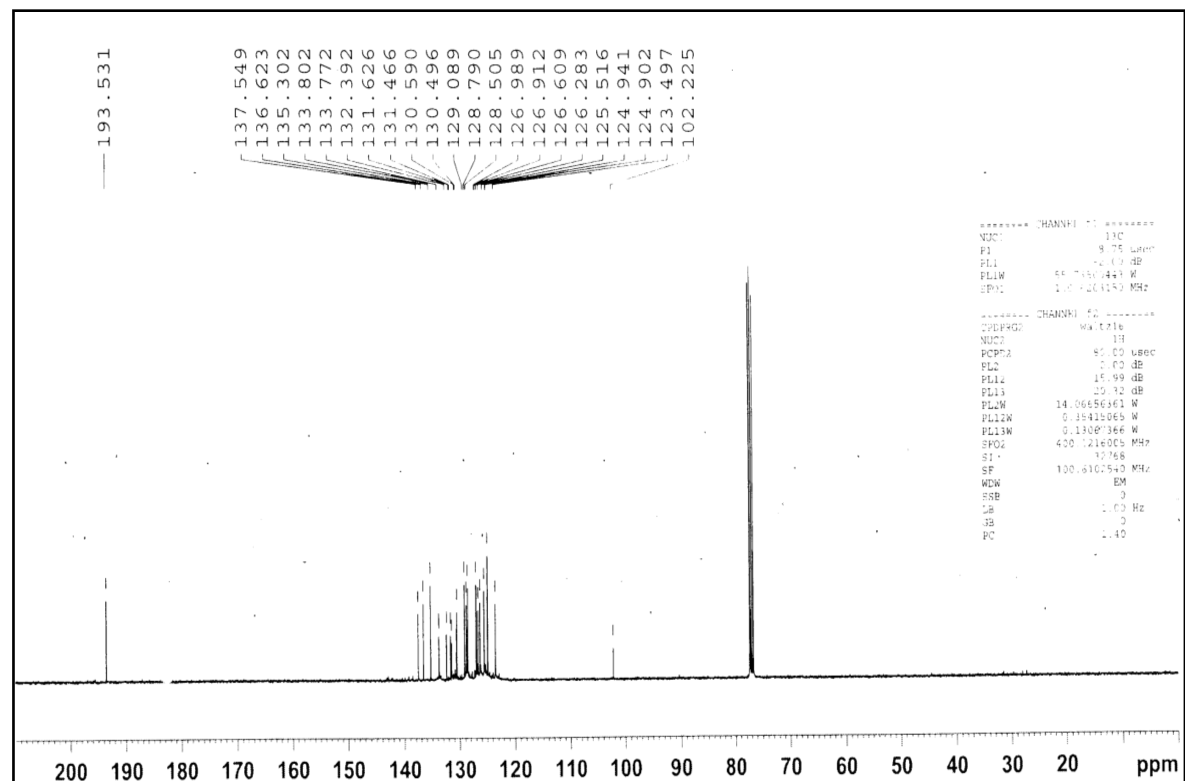


Fig 6.1.10 ^{13}C NMR spectra of 6a

In 1,7-bis(2-methoxynaphthyl)1,6-heptadiene-3,5-dione(6b),the methine carbon C1 gave a peak at 102 ppm.The carbonyl carbon C2,C2'produced a peak at downfield ,at 192 ppm.This carbon is attached to oxygen and the electronegativity of oxygen produces the deshielding effect.The alkenyl carbons C3 & C4 gave peaks at 137 & 112ppm respectively.The carbon atoms in the aromatic ring ie C5 to C14 are observed in the range 124 ppm -164 ppm.The C6 carbon which is attached to the methoxy group is seen downfield at 163.96ppm.The downfield position of this signal can be explained due to the electronegativity of oxygen attached to it. In 6b methoxy carbon atom is present at position 56.60ppm. The carbon which is attached to the alkenyl carbon atom(C5) is down shielded and present at a position ~ at 156 ppm. **^{13}C NMR spectra of 6b** is depicted in Fig.6.1.12 .The spectral data is given below in **Table 6.1.6.**

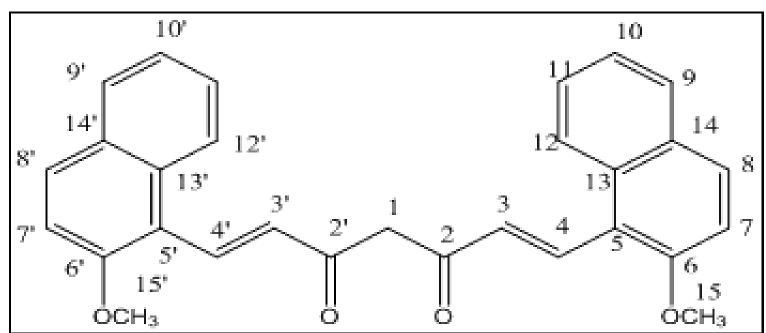


Fig. 6.1.11 Structure representing different non equivalent C atoms in 1,7-(2-methoxy)naphthyl-1,6- heptadiene-3,5-dione(6b)

Table 6.1.6 ^{13}C NMR spectral data of 1,7-Bis(2-methoxy)naphthyl-1,6- heptadiene-3,5-dione(6b),(chemical shift in ppm)

C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'	C6,C6'	C7,C7'	C8,C8'
102.38	192.98	137.56	112.74	156.25	163.96	133.59	131.5
C9,C9'	**C10,C10'**	**C11,C11'**	**C12,C12'**	**C13,C13'**	**C14,C14'**	**C15,C15'**	
123.94	129.89	128.23	124.79	132.88	129.09	56.60	

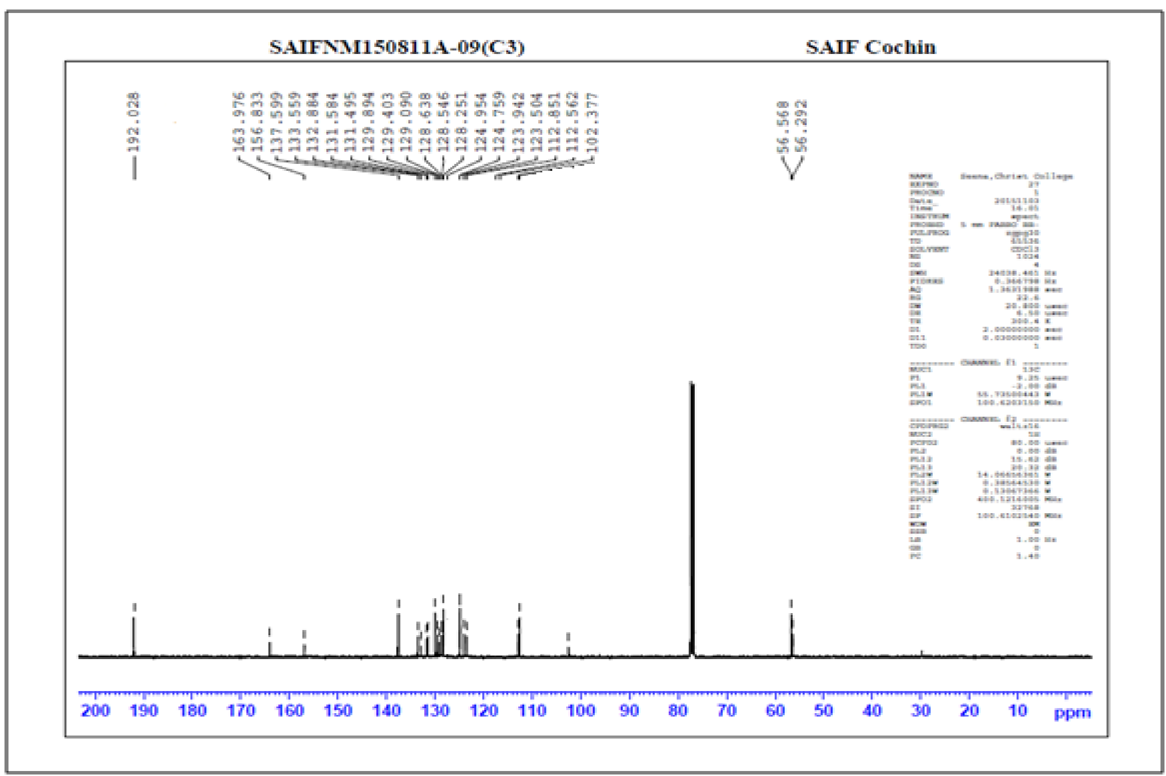


Fig.6.1.12 ^{13}C NMR spectra of 6b

The **DEPT(Distortionless Enhancement by Polarisation Transfer)** technique is a very useful adjunct to ^{13}C NMR Spectroscopy.The results of the DEPT experiment can tell us whether a given peak arises from a carbon on a methyl ,methylene or a methine group.Those carbons with no hydrogens will be missing in the DEPT Spectrum.In DEPT-135 spectrum methine and methyl carbons give rise to positive peaks,where as methylene carbons appear as inverse peak.The DEPT-135 spectrum of 6b is given in **Fig.6.1.13**.In the DEPT spectrum,the peaks due to C2(C=O group),C5,C6 ,C13 and C14 in the ^{13}C NMR spectrum are absent.These are carbons with no hydrogens attached to them.There are no inverse peaks in the spectrum suggesting the absence of methylene group.

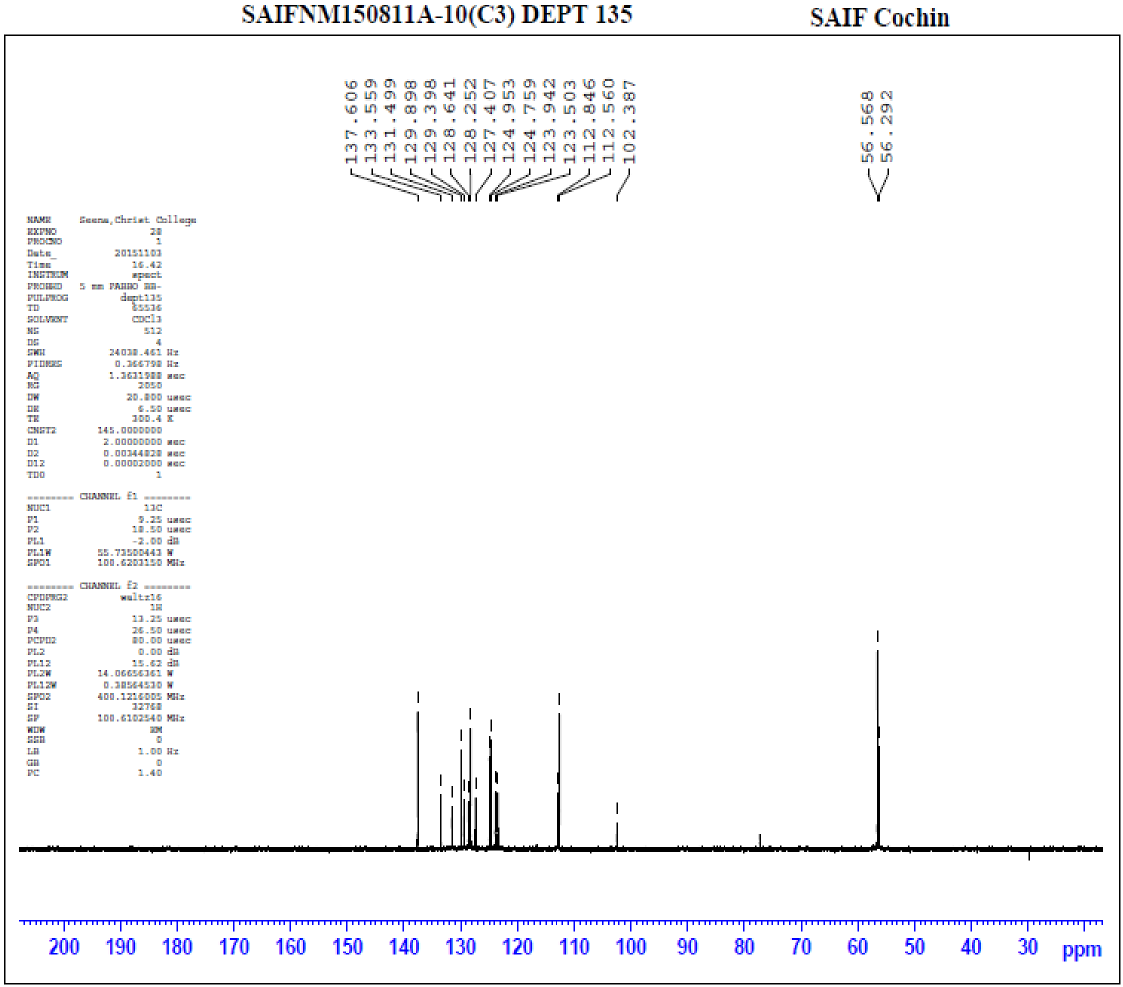


Fig.6.1.13 DEPT -135 Spectrum of 6b.

2 D COSY and HETCOR Spectra of 1,7-Bis(2-methoxy)naphthyl-1,6- heptadiene-3,5-dione(6b),were taken and are represented below.Fig 6.1.14 is the 2D H-H COSY NMR Spectrum of 6b.Fig.6.1.15 and 6.1.16 are the 2 D C-H NMR spectra of 6b.The 2D H-H COSYNMRSpectrum helps to identify the positions of methine,alkenyl,naphthyl,methoxy protons.This spectrum can be compared with ^{1}H NMR Spectrum.The 2D C-H HETCOR NMR spectra of 6bcan be compared with ^{13}C NMR Spectrum and the distint carbons can be identified.

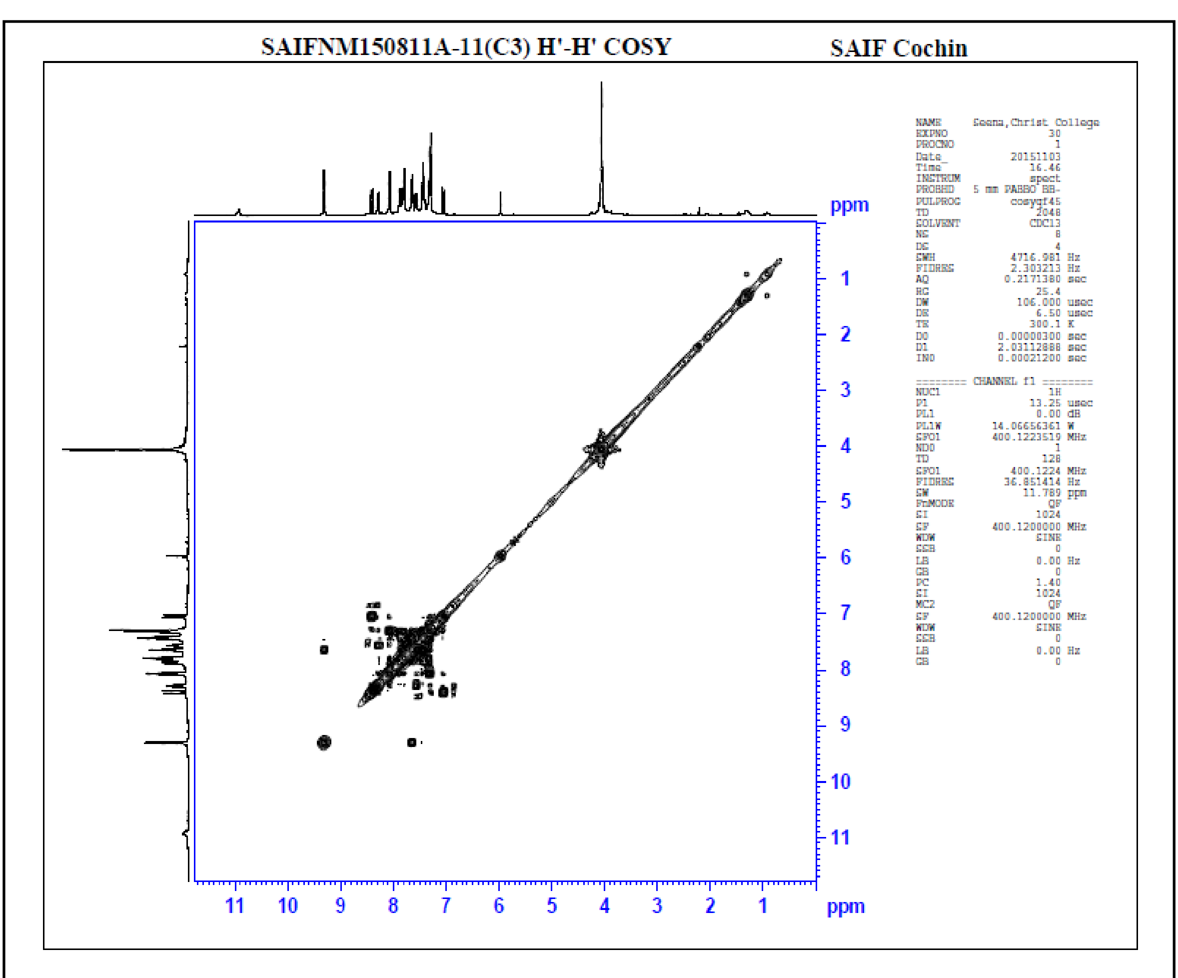


Fig.6.1.14 2D H-H COSY NMR Spectrum of 6b.

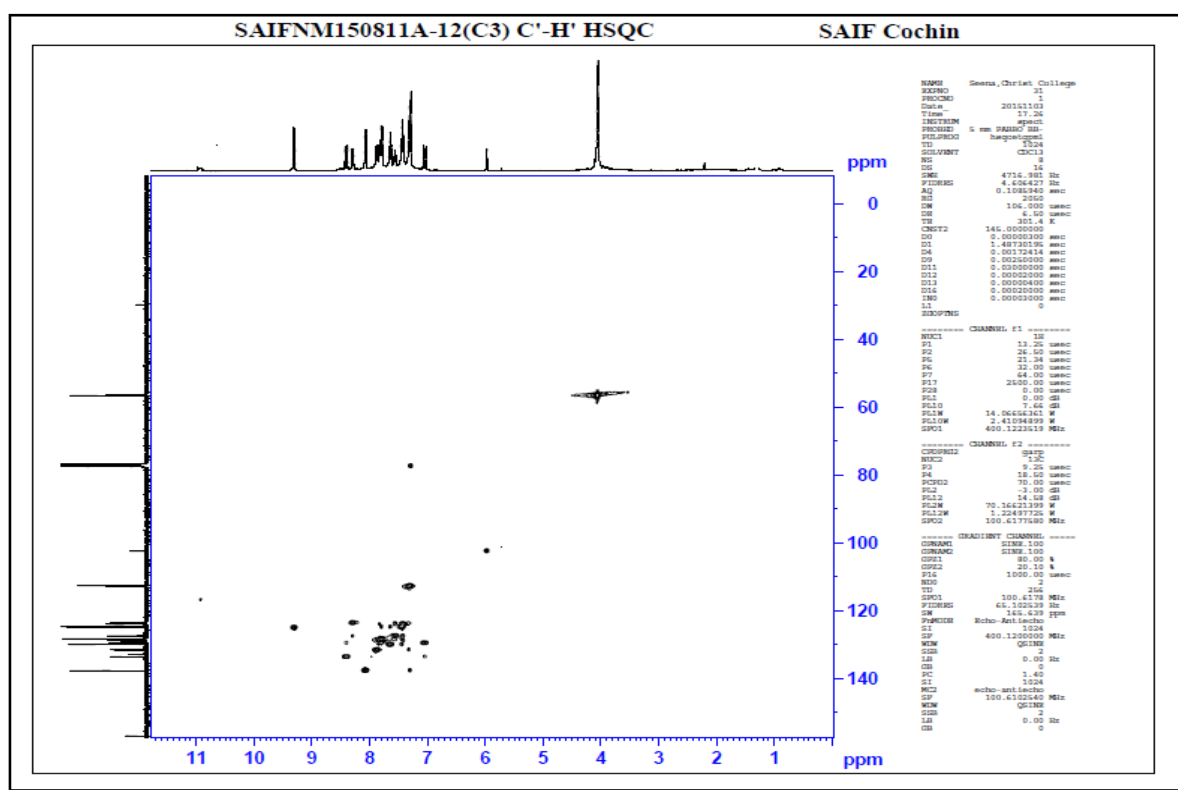


Fig .6.1.15 2D C-H NMR Spectrum of 6b.

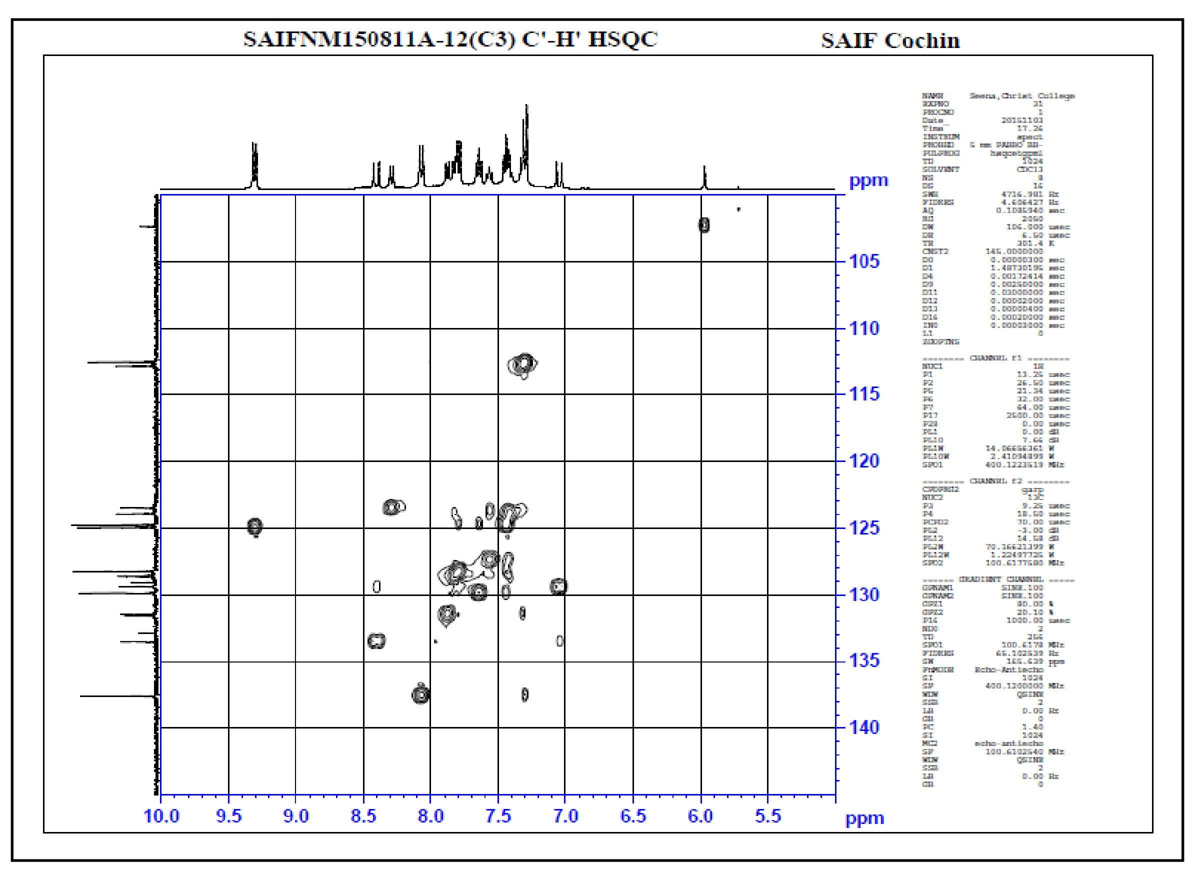


Fig .6.1.16 2D C-H NMR Spectrum of 6b.

In 1,7-bis(2-hydroxynaphthyl)1,6-heptadiene-3,5-dione(6c),the methine carbon C1 gave a peak at 102 ppm.The carbonyl carbon C2,C2'produced a peak at downfield, at 192 ppm.The olefinic carbons C3 & C4 gave peaks at 137 & 124ppm respectively.The carbon atoms in the aromatic ring ie C5 to C14 are observed in the range 122 ppm -158 ppm.The C6 carbon which is attached to the hydroxy group is seen downfield at 157.43ppm.The downfield position of this signal can be explained due to the electronegativity of oxygen attached to it. The carbon C5 which is attached to the alkenyl carbon atom and attached to the carbon with hydroxyl group is down shielded and present at a position ~ at 157 ppm.

^{13}C NMR spectral data of 6c is given below in **Table 6.1.7.**

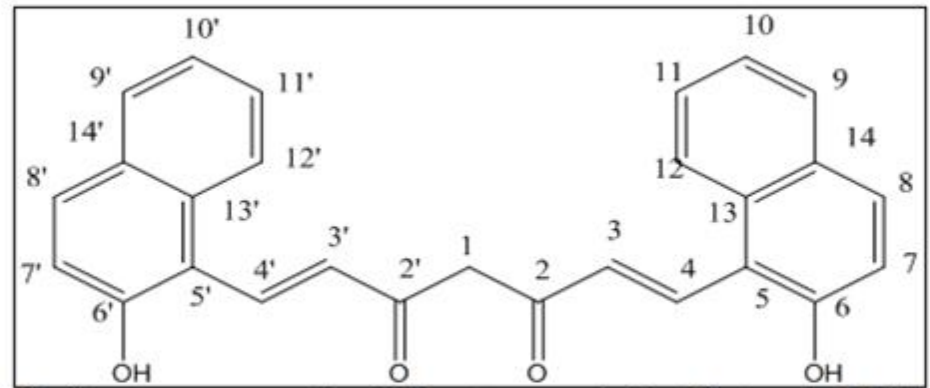


Fig 6.1.17 Structure representing different non equivalent C atoms in 1,7-bis(2-hydroxy)naphthyl-1,6- heptadiene-3,5-dione(6b)

Table 6.1.7 ^{13}C NMR spectral data of 1,7-bis(2-hydroxy)naphthyl-1,6- heptadiene-3,5-dione(6b) (chemical shift in ppm)

C1	**C2,C2'**	**C3,C3'**	**C4,C4'**	**C5,C5'**	**C6,C6'**	**C7,C7'**
102.13	192.16	137.46	124.32	139.51	157.43	122.65
C8,C8'	**C9,C9'**	**C10,C10'**	**C11,C11'**	**C12,C12'**	**C13,C13'**	**C14,C14'**
132.45	128.32	126.34	125.64	124.43	123.23	122.24

Mass spectra

The mass spectra of 6a shows molecular ion(M+2 ion) peak at 377.The base peak in the spectrum is observed at m/z=181 which is due to $[Ar-CH=CH-C=O]^+$ion.The next intense peak is observed at m/z=141 which is due to $[Ar-CH]^+$ ion,Ar= 1-naphthyl ring.Elimination of important groups like C2H2,-CH2=C=O,-CH2,-CH=C=O from the molecule gives different fragment ions .The formation of fragment ions is represented in Scheme 6.1.2 and the m/z values of the fragment ions are depicted in Table 6.1.8. Smaller fragments like O, OH, CH_2 etc. are removed from the molecular ion and are shown in the spectrum.The mass spectrum of 6a is given in Fig.6.1.18.

The (M+3) ion of 6b is observed at 439. Other important peaks of 6b are due to fragment ions which are represented in Table 6.1.8.The mass spectrum of 6b is given in Fig.6.1.19.

The mass spectrum of 6c showed an intense molecular ion peak at 408.The next intense peak is observed at m/z=154 which is due to $[Ar-CH]^+$ion,where Ar=2-hydroxynaphthyl group.The peaks at 237,198,167 are due to fragment ions.The formation of fragment ions can be explained from the fragmentation pattern given in Scheme below.The mass spectrum of 6c is given in Fig.6.1.20.

Table 6.1.8 Mass spectra of 6a, 6b, 6c

Fragments*	Ligands	M+/(M+1)/ (M+2)/(M+3) ion	A	B	C	D	E	F	G	H
Mass pattern	6a	377	197	181	141	153	127	152	252	223
	6b	439	225	207	170	189	163	188	281	253
	6c	408	210	198	157	167	144	166	265	237

*The alphabets corresponds to the fragments given in **Scheme 6.1.2**

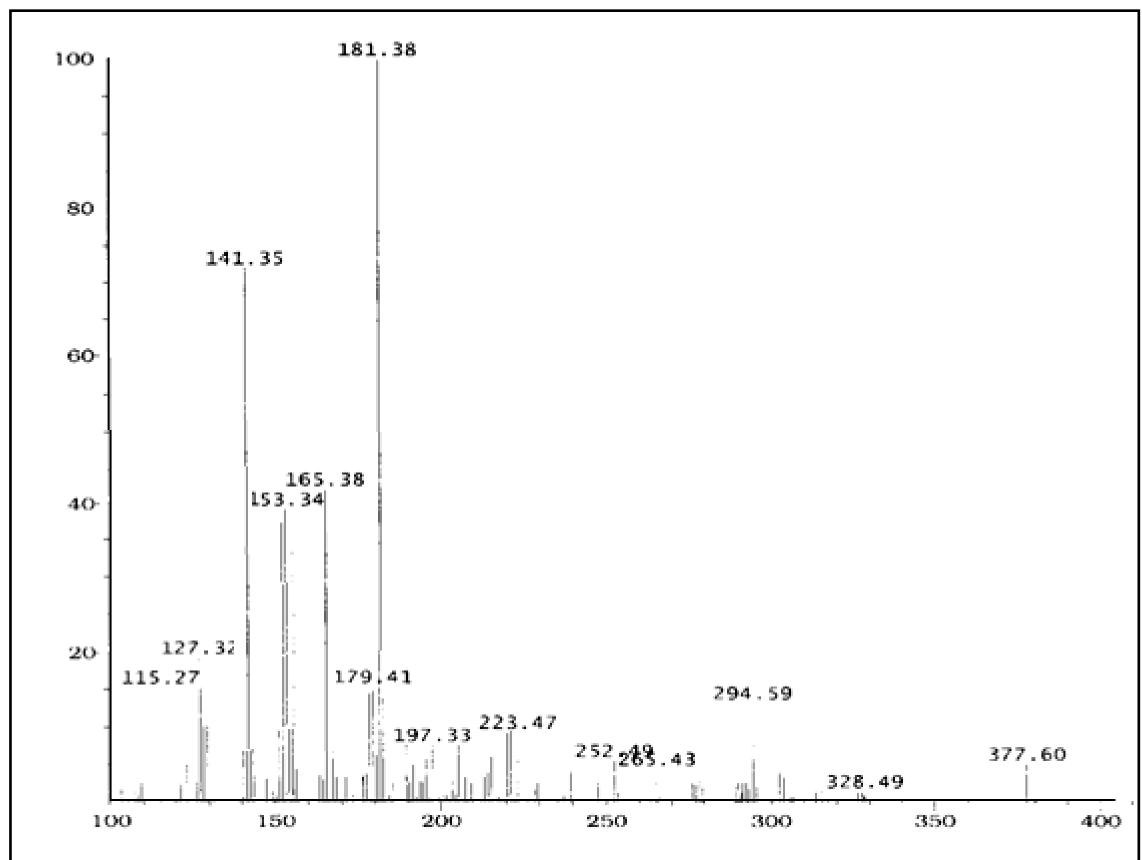


Fig.6.1.18 Mass spectrum of 6a

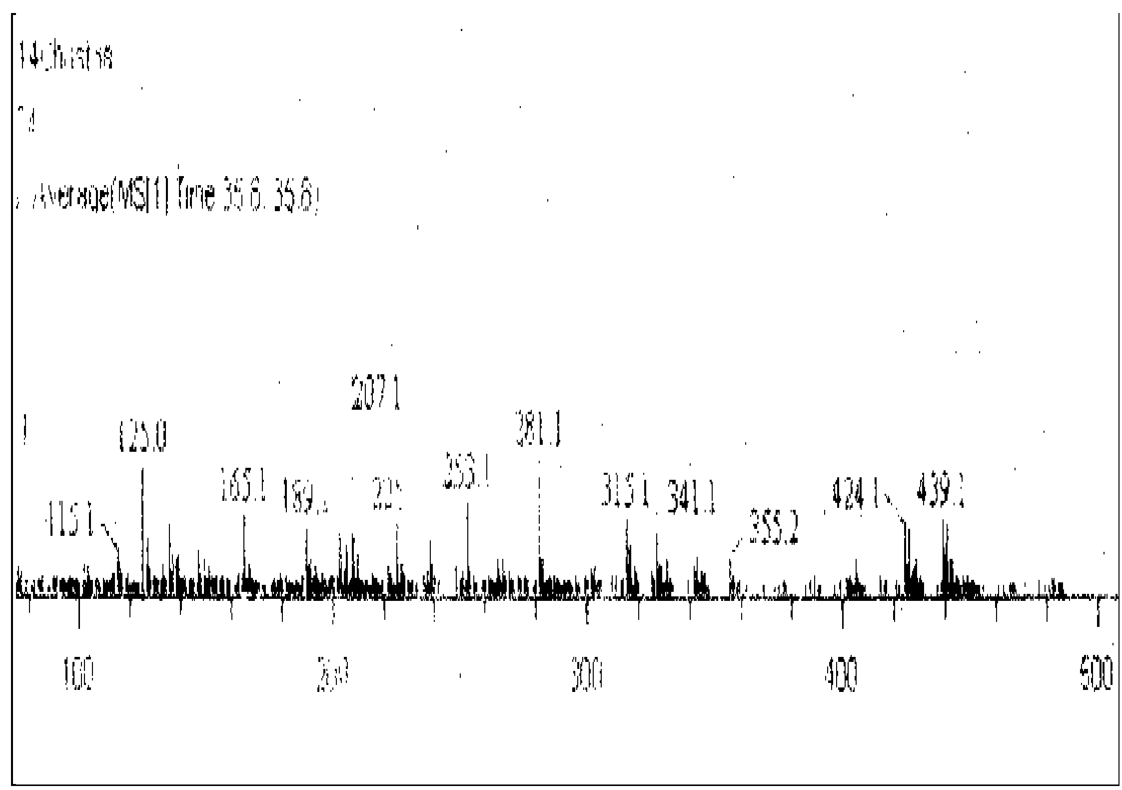


Fig.6.1.19 Mass spectrum of 6b

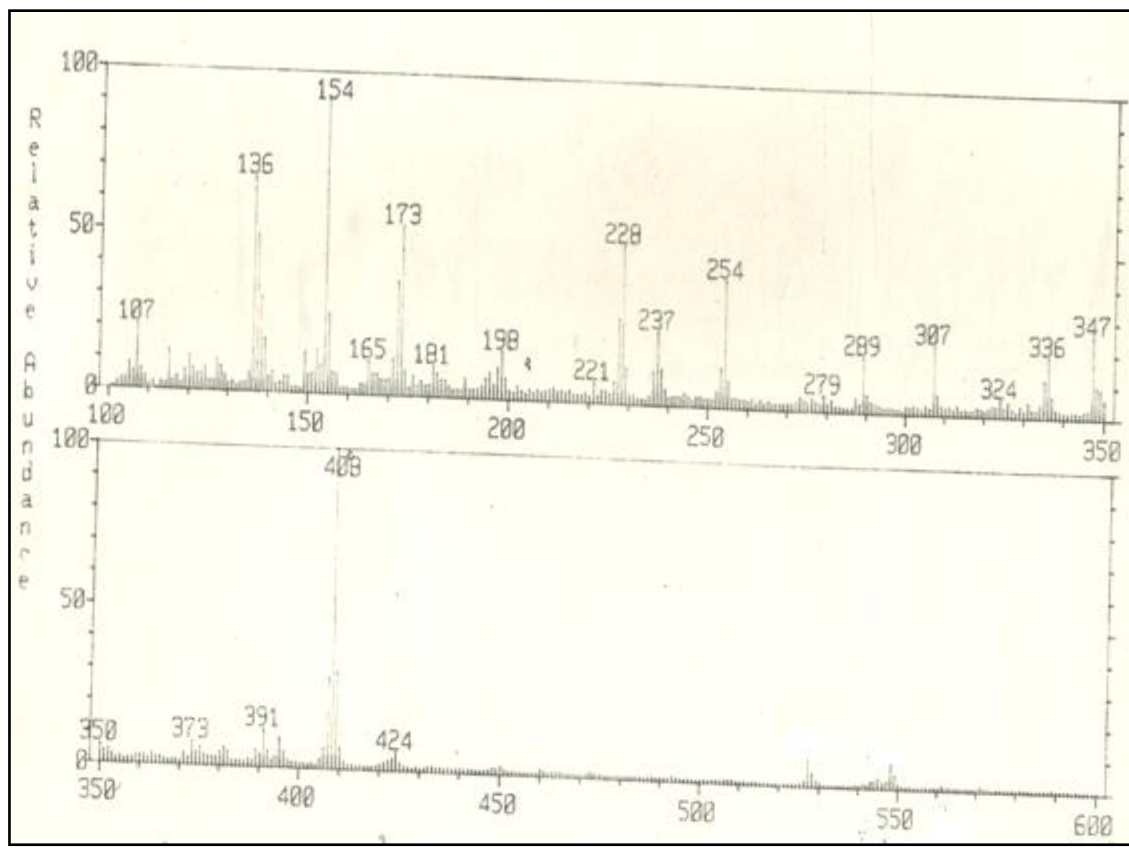


Fig.6.1.20 Mass spectrum of 6c

Scheme 6.1.2

Ar => 1-naphthyl,2-methoxy naphthyl, 2-hydroxy naphthyl

THERMOGRAVIMETRIC ANALYSIS OF 1,7-Bis(2-methoxy)naphthyl-1,6- heptadiene-3,5-dione

Thermogravimetric analysis of the compound was carried out in the range of 40° C to 740^{0}C.The thermogram obtained shows a two stage decomposition pattern .The compound is stable upto 175^{0}.The decomposition process begins slowly with a sharp drop in mass (35.76%) up to about 267.05^{0}.The % mass loss corresponds to the removal of the methoxy naphthyl part from the ligand.The second stage of decomposition continues with a mass loss of 70.862%.The mass loss corresponds to the removal of the next methoxy naphthyl part.The peak temperatures are observed at 267 and 385^{0}C.he final product of pyrolysis is found to be 2-methoxy naphthyl group . Thermogram of the ligand is given below in Fig.6.1.21.

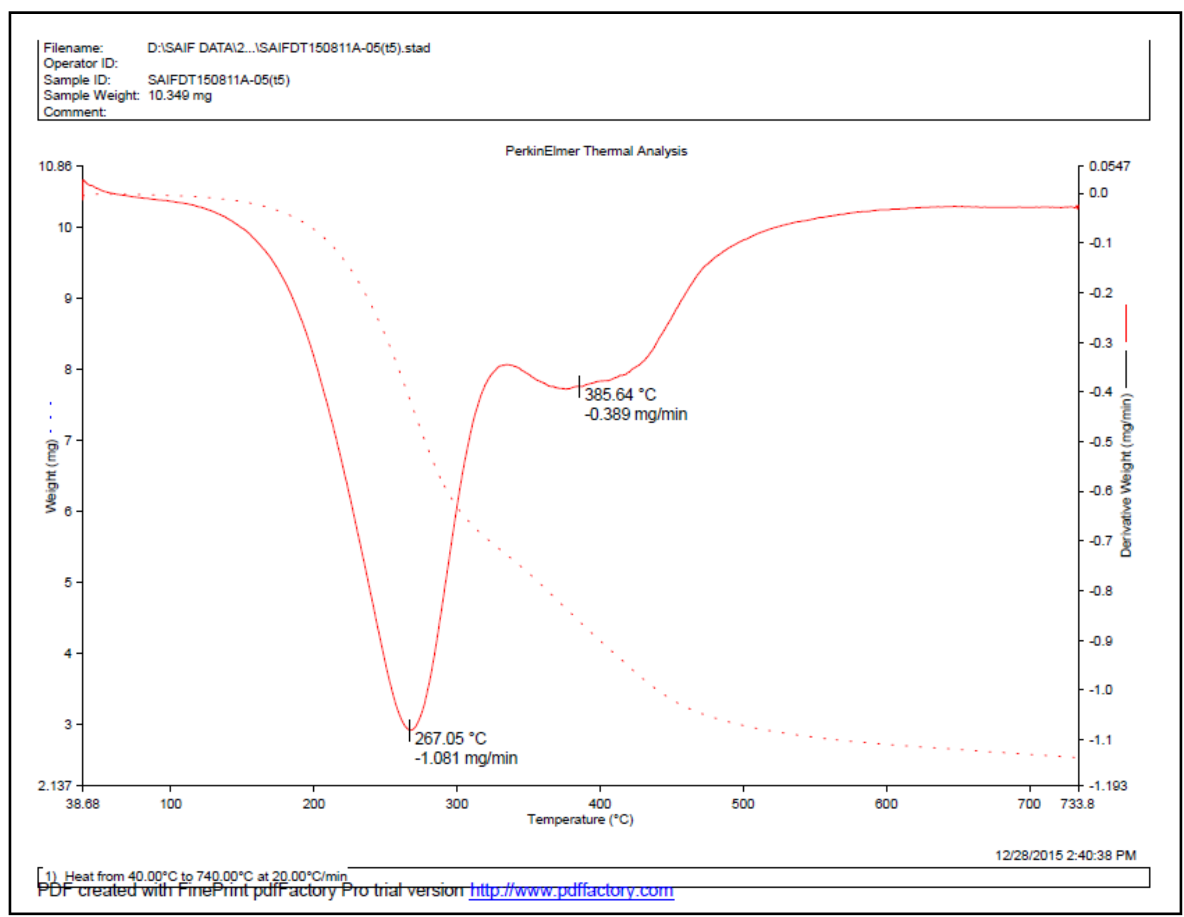


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Fig.6.1.21 Thermogram of 6b.

SECTION-II

SYNTHESIS AND CHARACTERISATION OF TRANSITION METAL CHELATES OF 1,7-DINAPHTHYL HEPTANOIDS WITH METHOXY &HYDROXY SUBSTITUTED NAPHTHYL RING

6.2.1 Synthesis of metal complexes of 1,7-dinaphthyl-1,6- heptadiene-3,5-dione(6a),1,7-bis(2-methoxynaphthyl)1,6-heptadiene-3,5-dione(6b),1,7-bis(2-hydroxynaphthyl)1,6-heptadiene-3,5-dione(6c)

Copper(II), Zinc(II), Nickel(II) and Oxovanadium(IV)complexes of curcuminoid analogues with hydroxy and methoxy substituted naphthyl rings were synthesized by the following general method.

To a refluxing solution of the ligand(0.002 mol) in methanol(25ml),a methanolic solution of metal salt (0.001mol)was added and the reaction mixture was refluxed for nearly 2 hrs and cooled to room temperature.The metal salts used were Copper acetate,Zinc acetate,Nickel acetate and Vanadium (IV)oxide sulphate for the preparation of Cu(II),Zn(II),Ni(II) &VO(IV) complexes respectively.The precipitated complex was filtered, washed with 1:1, methanol:water mixture and recrystallised from hot methanol.The general reaction involved in the formation of complexes is given below in **Scheme.6.2.1**

M (acetate)$_2$ M=(Cu,Zn,Ni)
Vanadium(IV) oxide sulphate M=(VO (IV))

Scheme 6.2.1

Ar = 1-Naphthyl, 2-Hydroxy naphthyl, 2-Methoxy naphthyl

6.2.2 Characterisation of transition metal complexes of methoxy &hydroxyl substituted 1,7-dinaphthyl heptanoids

Metal chelates (Cu,Zn,Ni,Vanadyl) of ligands 1,7-dinaphthyl-1,6-heptadiene-3,5-dione(6a),1,7-bis(2-methoxynaphthyl)1,6-heptadiene-3,5-dione(6b),1,7-bis(2-hydroxynaphthyl)1,6- heptadiene-3,5-dione(6c) were characterized using analytical and various spectral techniques like UV,IR,NMR and mass spectral data. Elemental analysis (C, H and metal percentages), physical data,UV and IR spectral data are given in Table 6.2.1,6.2.2 & 6.2.3. The analytical data together with mass spectral data suggest a ML_2 stoichiometry for all the synthesized complexes.

Table 6.2.1 Analytical and spectral data of metal complexes of 6a

Metal chelates	M.P (°C)	Elemental analysis (%) Found/(calculated)			μ_{eff} BM	UV λmax (nm)	Characteristic IR stretchingbands (cm^{-1})		
		C	H	Metal			(C=O)	(C-C-C)	(M-O)
Cu	209	78.10 (79.60)	4.85 (4.67)	7.70 (7.81)	1.75	262, 385	1581	1518	484, 428
Zn	201	79.01 (79.48)	4.26 (4.66)	7.96 (8.02)	…..	261, 385	1600	1523	472, 419
Ni	212	80.47 (80.14)	4.93 (4.69)	8.13 (7.26)	…..	263, 387	1598	1520	474, 437
VO(IV)	230	78.35 (79.31)	5.02 (4.65)	6.01 (6.24)	1.76	295, 316	1561	1493	479, 422

Table 6.2.2 Analytical and spectral data of metal complexes of 6b

Metal chelates	M.P. (°C)	Elemental analysis (%) Found/(calculated)			UV λmax nm	Characteristic IR stretching bands (cm^{-1})		
		C	H	Metal		(C=O)	(C-C-C)	(M-O)
Cu	183	73.54 (74.55)	4.85 (4.92)	6.70 (6.80)	265, 393	1624	1512	463, 420
Zn	185	73.92 (74.40)	4.01 (4.91)	6.59 (6.99)	270, 395	1602	1510	456, 417
Ni	191	74.68 (74.94)	4.69 (4.95)	6.05 (6.31)	273, 397	1590	1511	495, 425
VO(IV)	197	73.85 (74.28)	4.61 (4.90)	5.03 (5.43)	260, 398	1593	1522	465 , 420

Table 6.2.3 Analytical and spectral data of metal complexes of 6c

Metal chelates	M.P °C	Elemental analysis (%) Found/(calculated)			μ_{eff} BM	UV λmax nm	Characteristic IR stretching bands (cm^{-1})		
		C	H	Metal			(C=O)	(C-C-C)	(M-O)
Cu	133	74.27 (73.85)	4.96 (4.33)	6.49 (6.72)	1.80	263, 388	1595	1521	467, 409
Zn	131	74.06 (73.68)	4.31 (4.32)	7.09 (7.43)	…..	264, 390	1580	1510	455, 419
Ni	139	73.91 (74.15)	4.57 (4.28)	7.16 (6.69)	…..	262, 389	1585	1516	496, 427
VO(IV)	150	75.85 (74.91)	4.90 (4.39)	6.31 (5.89)	1.76	259, 392	1590	1512	468, 419

UV spectra

The UV spectra of metal complexes closely resembles the spectra of respective ligands. In certain cases there is a slight shift of absorption maxima to longer wavelength which indicate the involvement of the carbonyl oxygens in metal complexation. For comparison, the UV spectra of ligand 6b and its Cu(II) and Zn(II) complexes are shown in Fig.6.2.1.

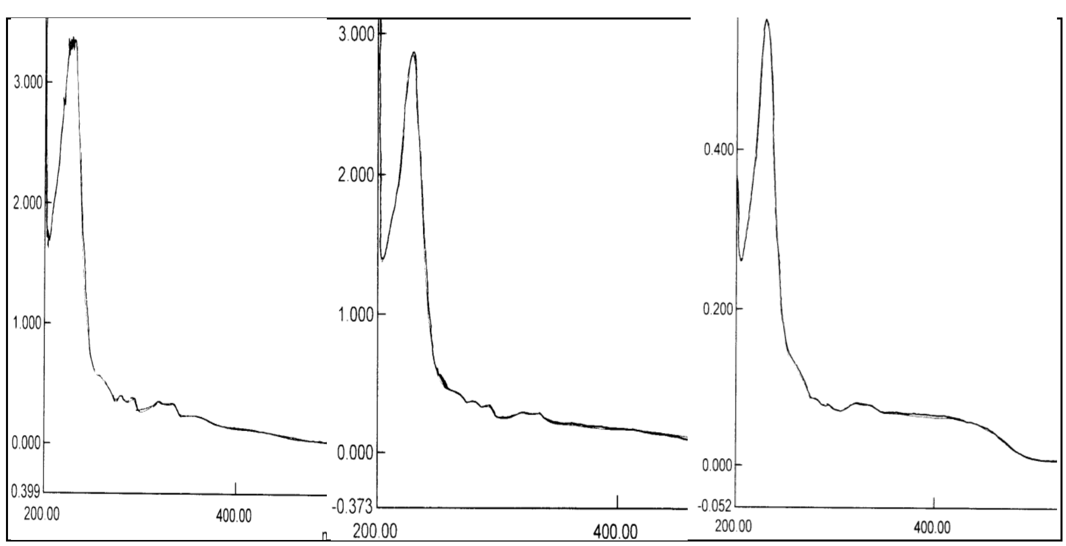


Fig.6.2.1 UV spectra of 6b and its Cu(II) & Zn(II) complexes

IR spectra

In the IR spectra of metal chelates, the bond due to intra molecularly hydrogen bonded carbonyl function of the ligand at ~1620 cm^{-1} disappeared and instead a strong band assigned to stretching of the coordinated carbonyl moiety appeared at~1600cm^{-1}. Additional bands appear at ~475 cm^{-1} and ~420 cm^{-1} assignable to ν (M–O) vibration. The broad band in the region of 2600 -3500 cm^{-1} present in the ligand is also reduced in the spectra of metal complexes. This is an indication of the replacement of the chelated proton by the metal ion during complexation. The IR spectrum of Cu(II) complex of 6b is depicted in Fig.6.2.2 & the IR spectrum of VO(IV) complex of 6c is depicted in Fig.6.2.3.

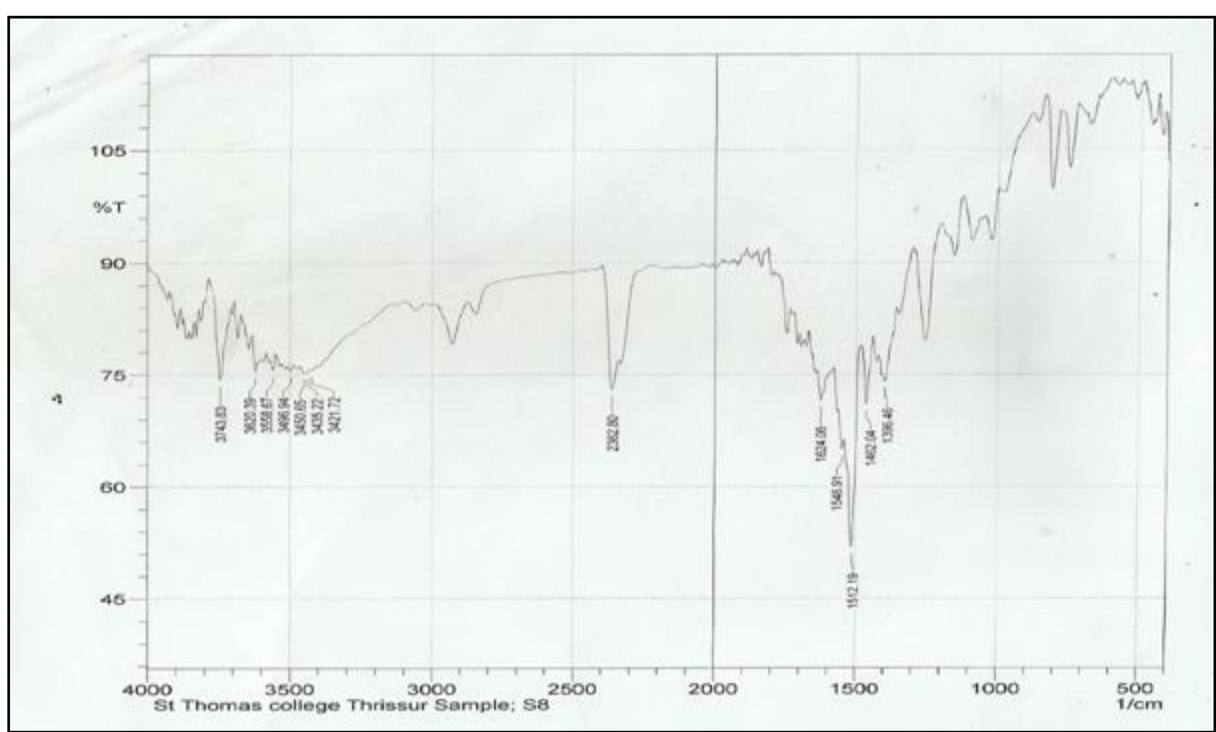


Fig.6.2.2 IR spectrum of Cu(II) complex of 6b

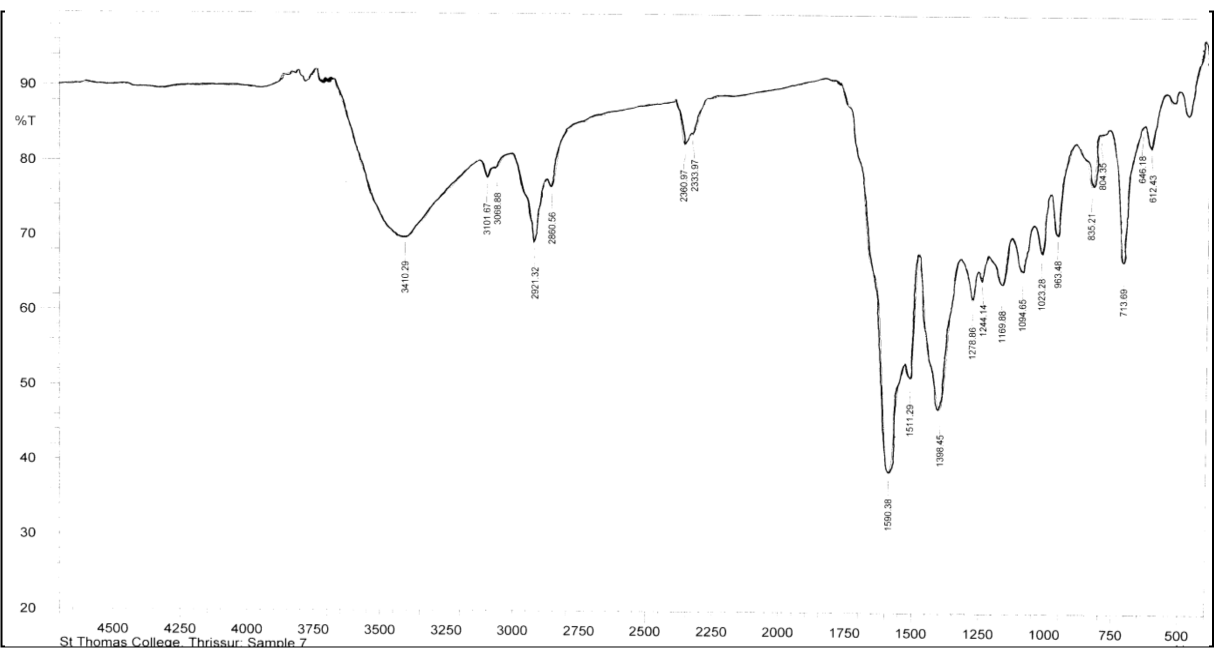


Fig.6.2.3 IR spectrum of VO(IV) complex of 6c

^{1}HNMR spectra

The enolic proton present in the ligands is replaced by metal atom in metal complexes. This is evident by the disappearance of the signal at $\delta \sim 16$ppm in metal complexes. The phenyl

and alkenyl protons are not altered much since they are not involved in metal complexation. There is a slight shift of methine signals to the downfield of the spectra. Thus the spectra of ligand and complexes are much similar except those of enolic proton. The NMR spectra of Zn(II) complex of 6b is given in Fig.6.2.4.

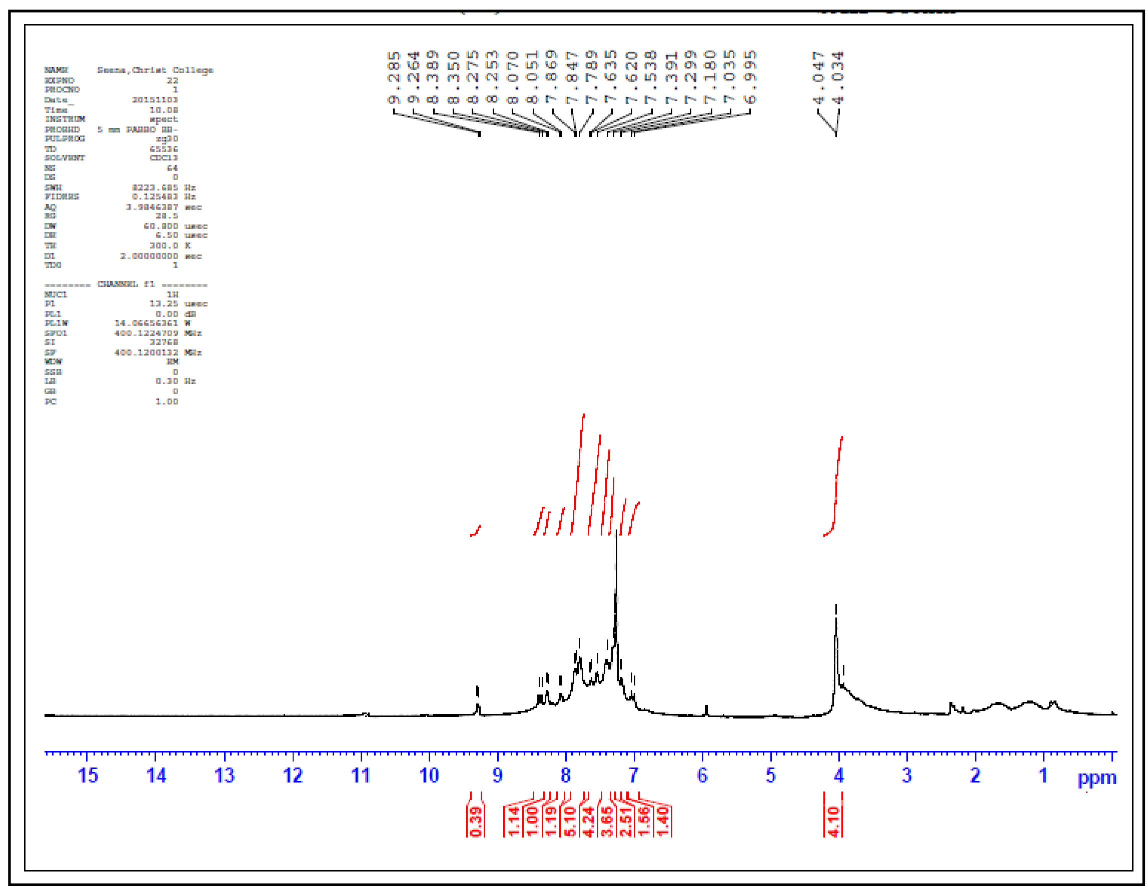


Fig.6.2.4. NMR spectra of Zn(II) complex of 6b

Mass spectra

The use of mass spectroscopy for the establishment of the stoichiometry and structure of metal 1,3-diketonates has been well rooted.It has been shown from the mass spectral analysis that stepwise removal of aryl group is a characteristic feature of all the complexes.Electronic and steric effect of the groups attached to the diketo function strongly influences the stability of various fragments formed under mass spectral condition.The suggested formulation and structure of complexes are clearly in agreement with the observed spectra of complexes. The

fragmental patterns of the metal chelates 6a, 6b & 6c follow the **Scheme 6.2.2.** Mass spectral pattern of metal complexes of 6a, 6b & 6c are given in Table **6.2.4 , 6.2.5 &** Table **6.2.6.**

Table 6.2.4 Mass spectral fragmental pattern of metal chelates of 1,7-dinaphthyl-1,6-heptadiene-3,5-dione(6a)

Fragments	Metal chelates	M+/ (M+1)/ (M+2) ion	A	B	C	D	E
Mass Pattern	Cu	814	559	439	120	307	186
	Zn	816	561	441	120	309	183
	Ni	809	554	434	120	302	181
	VO(IV)	817	563	442	120	308	188

*The alphabets corresponds to the fragments given in **Scheme 6.2.2.**

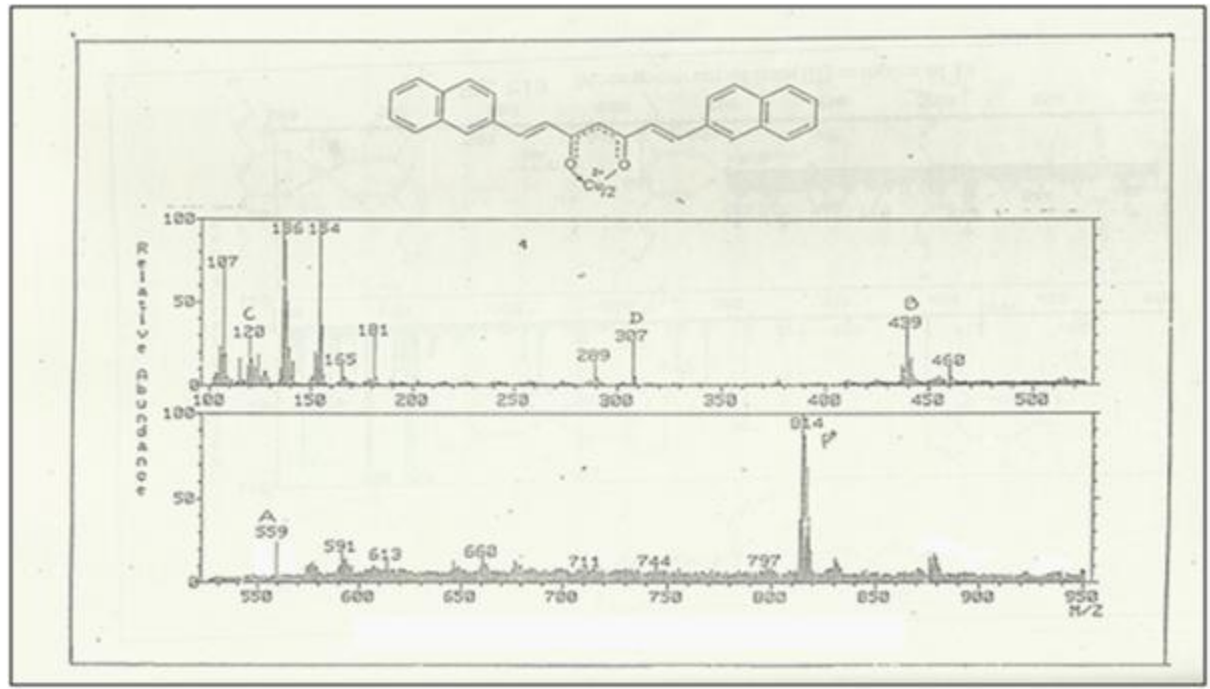


Fig 6.2.5 Mass Spectrum of Cu(II) complex of 1,7-dinaphthyl-1,6- heptadiene-3,5-dione(6a)

The mass spectrum of Cu(II) complex of 6a has an intense molecular ion peak at 814.The peak at 559 and 307 is due to the removal of 2 Ar and 4 Ar groups from the molecular ion where Ar=1-naphthyl .The peak at 439 is due to the [ML]+ fragment where M is metal and L is ligand.The peaks at 181,165,154,136 are all due to fragment ions of the ligand and are observed in the spectrum of ligand.

Table 6.2.5 Mass spectral fragmental pattern of metal chelates of 1,7-bis(2-methoxynaphthyl)1,6-heptadiene-3,5-dione(6b)

Fragments	Metal chelates	M+/ (M+1)/ (M+2) ion	A	B	C	D	E
Mass Pattern	Cu	934	620	499	121	306	185
	Zn	936	622	501	121	308	187
	Ni	929	615	494	121	301	180
	VO(IV)	937	623	502	121	309	188

The alphabets corresponds to the fragments given in **Scheme 6.2.2.**

The mass spectrum of Zn(II) complex of 6b is given below.The molecular ion peak is less intense and observed at 936.The intense peak in the spectrum at 437 is due to the ligand peak.The base peak is observed at 187 which is assigned to $[C_7H_5O_2Zn]^+$.The peaks at 622 and 308 are due to the removal of 2Ar and 4 Ar groups respectively from the molecular ion where Ar=2-methoxy naphthyl.

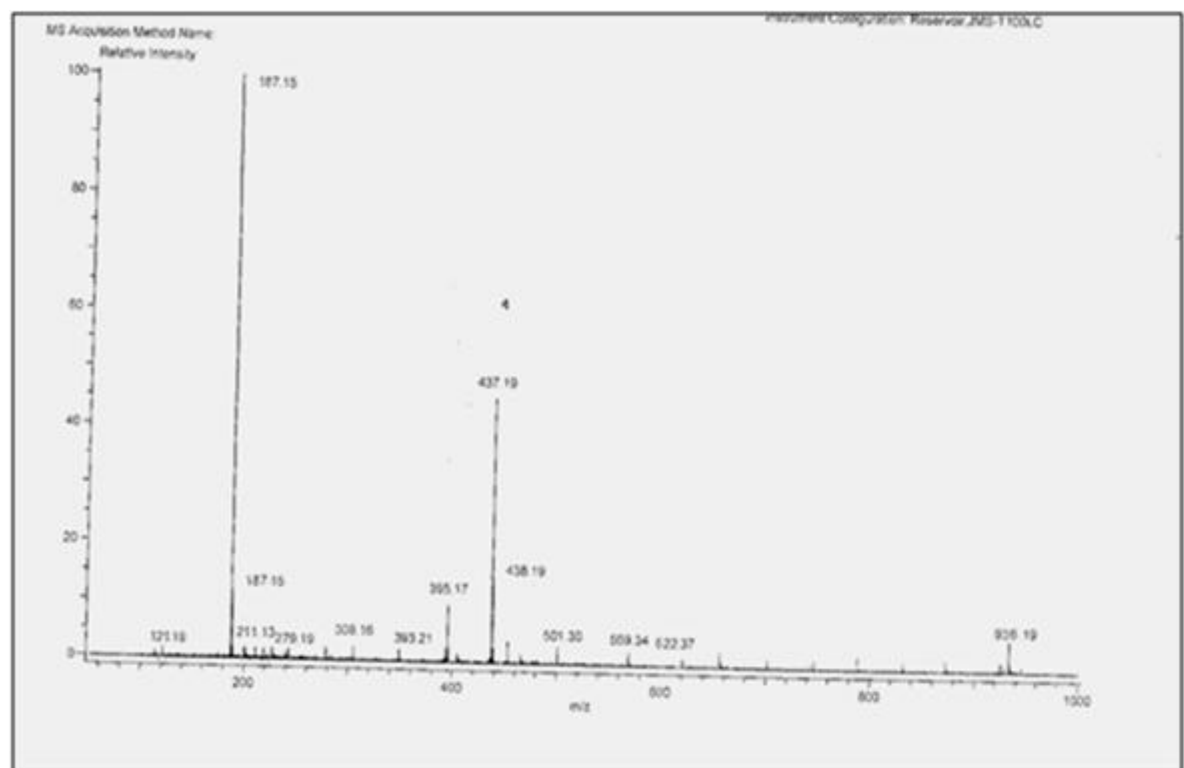


Fig.6.2.6 Mass Spectrum of Zn(II) complex of 6b

Table 6.2.6. Mass spectral fragmental pattern of metal chelates of 1,7-bis(2-hydroxynaphthyl)1,6-heptadiene-3,5-dione(6c)

Fragments	Metal chelates	M+/ (M+1)/ (M+2) ion	A	B	C	D	E
Mass Pattern	Cu	878	593	471	120	307	187
	Zn	880	595	473	120	309	189
	Ni	873	588	466	120	302	182
	VO(IV)	881	596	474	120	311	191

*The alphabets corresponds to the fragments given in **Scheme 6.2.2.**

Scheme 6.2.2

The observed UV, IR, ^{1}H NMR and Mass spectral data clearly reveals that metal chelates of Cu,Zn,Ni &Vanadyl are having ML_2 stoichiometry(metal ligand ratio is 1:2).The confirmed structure of metal chelates is given below.

n = 2 for Cu(II),Zn(II),Ni(II),VO(IV)
Ar = methoxy and hydroxy substituted naphthyl

ESR SPECTRAL STUDIES OF COPPER(II) COMPLEX OF 1,7-bis(2-methoxynaphthyl) 1,6-heptadiene-3,5-dione(6b)

The ESR Spectrum of copper(II) complex of 6b is measured at 77 ^{0} K in DMF solution.The observed g// and g ⊥ values are comparable to that reported for copper acetyl acetonates for which g// =2.264 and g ⊥ =2.036.The g// and g⊥ values for Cu(II) complex of the compound are 2.2791 and 2.0715 respectively.This suggests extensive delocalization in the chelate ring and significant covalent character for the metal ligand bonds.The ESR Spectra of Cu (II) complex of 6b is reproduced in fig.6.2.7.

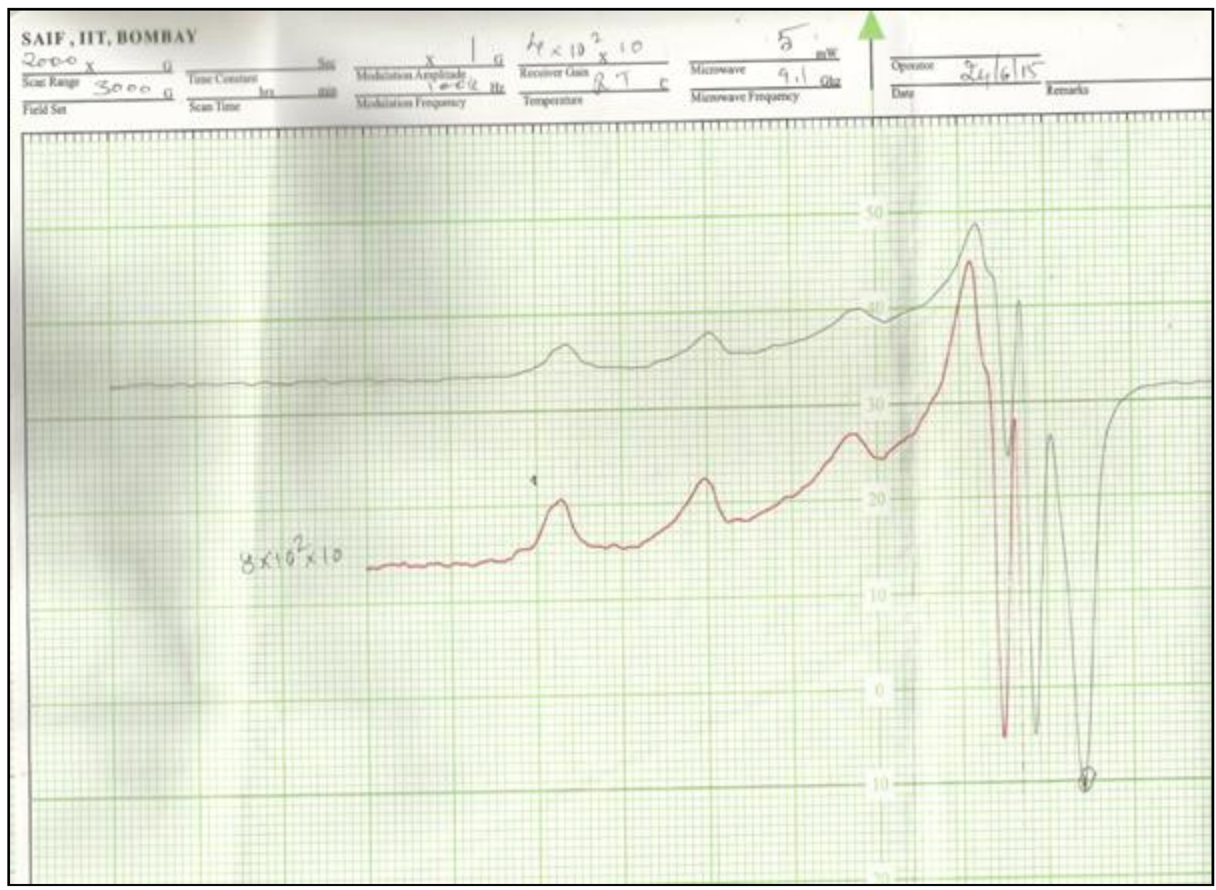


Fig.6.2.7 Esr spectrum of Cu(II) complex of 6b

SECTION III

INVITRO CYTOTOXIC STUDIES OF CURCUMINOID ANALOGUES WITH SUBSTITUTED NAPHTHYL RING AND THEIR TRANSITION METAL COMPLEXES

In this chapter,the cytotoxic activity of curcuminoid analogues namely 1,7-di(naphthalen-1-yl) hepta-1,6-diene-3,5-dione (**6a**), 1,7-bis(2-methoxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(**6b**), 1,7-bis(2-hydroxy naphthalen-1-yl) hepta-1,6-diene-3,5-dione(**6c**)were evaluated in vitro.Also their metal chelates with Cu (II), Zn(II), Ni(II) and VO(IV) were also subjected to *in vitro* cytotoxicity studies .The compounds were tested for their invitro cytotoxic activity against Daltons Lymphoma Ascites (DLA) and Ehrlich Ascites Carcinoma (EAC) cancer cells.The results of the study are discussed here.

6.3.1. *In vitro* Cytotoxic studies of 1,7-di(naphthalen-1-yl) hepta-1,6-diene-3,5-dione(6a) and their metal complexes Cu (II), Zn(II), Ni(II) and VO(IV)

Cytotoxic activities of 1,7-di(naphthalen-1-yl) hepta-1,6-diene-3,5-dione(**6a**) and their metal complexes Cu (II), Zn(II), Ni(II) and VO(IV) were determined by finding the percentage of viability of DLA and EAC cells using Trypan blue dye exclusion technique. Results of the studies with EAC & DLA are given in **Table 6.3.1 & Table 6.3.2.**respectively.They are also represented diagrammatically in **Fig. 6.3.1 & Fig. 6.3.2**

Table.6.3.1. ***In vitro*** **Cytotoxic studies of 1,7-di(naphthalen-1-yl) hepta-1,6-diene-3,5-dione (L_1) and their metal complexes towards EAC**

Drug Con. µg/ml	% Cell death				
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$	$VO(L_1)_2$
200	30	72	54	40	68
100	20	45	38	32	44
50	9	30	23	18	29
20	5	15	13	8	15
10	3	8	6	4	8

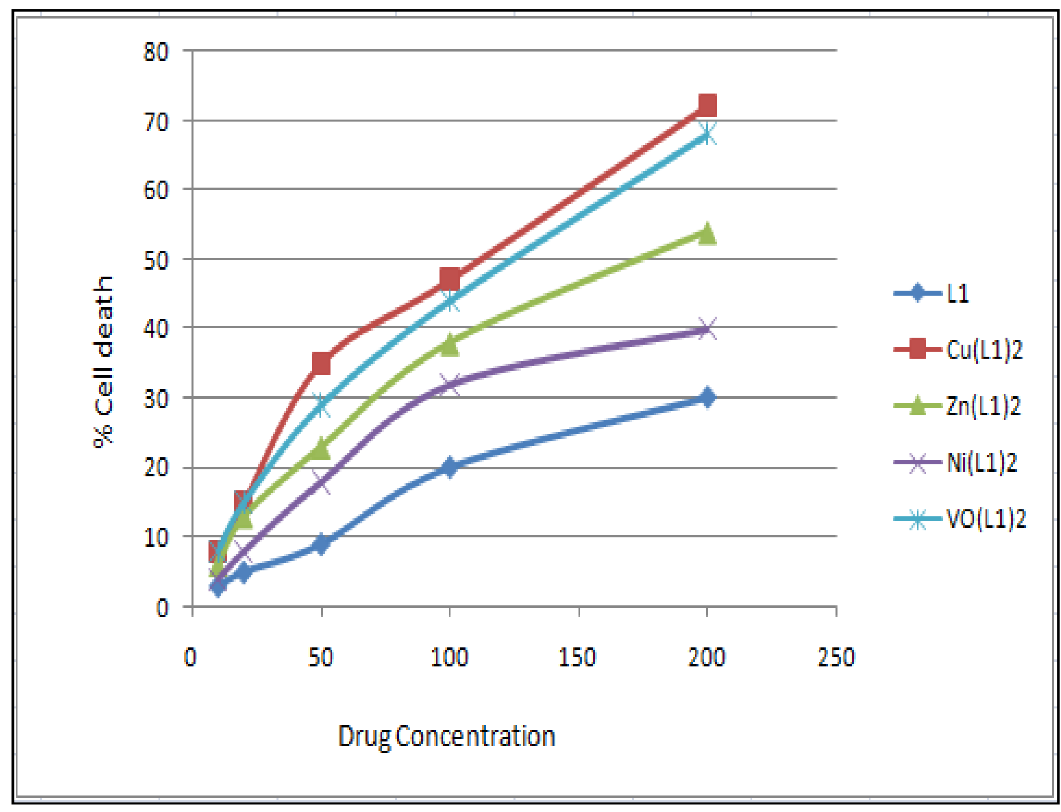


Fig.6.3.1. ***In vitro*** **Cytotoxic studies of 1,7-di(naphthalen-1-yl) hepta-1,6-diene-3,5 dione(L_1)and their metal complexes towards EAC**

The cytotoxic nature of all the compounds increased with increase in concentration. All were quite active at higher concentrations ie 200 µg/ml. All the metal complexes produced greater % cell death than the ligand.The maximum activity was observed for Cu(II) complex(72%)and least activity for Ni(II)complex(40%).Both Cu(II) and VO(IV) complexes showed comparable activity.The metal complexes presented activity in the order Cu(II)>VO(IV)>Zn(II)>Ni(II).

Table 6.3.2. *In vitro* Cytotoxic studies of 1,7-di(naphthalen-1-yl) hepta-1,6-diene-3,5-dione (L_1) and their metal complexes towards DLA

Drug Con. µg/ml	% Cell death				
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$Ni(L_1)_2$	$VO(L_1)_2$
200	30	70	50	40	65
100	16	43	34	32	41
50	10	28	18	18	26
20	5	13	8	6	12
10	3	6	4	3	6

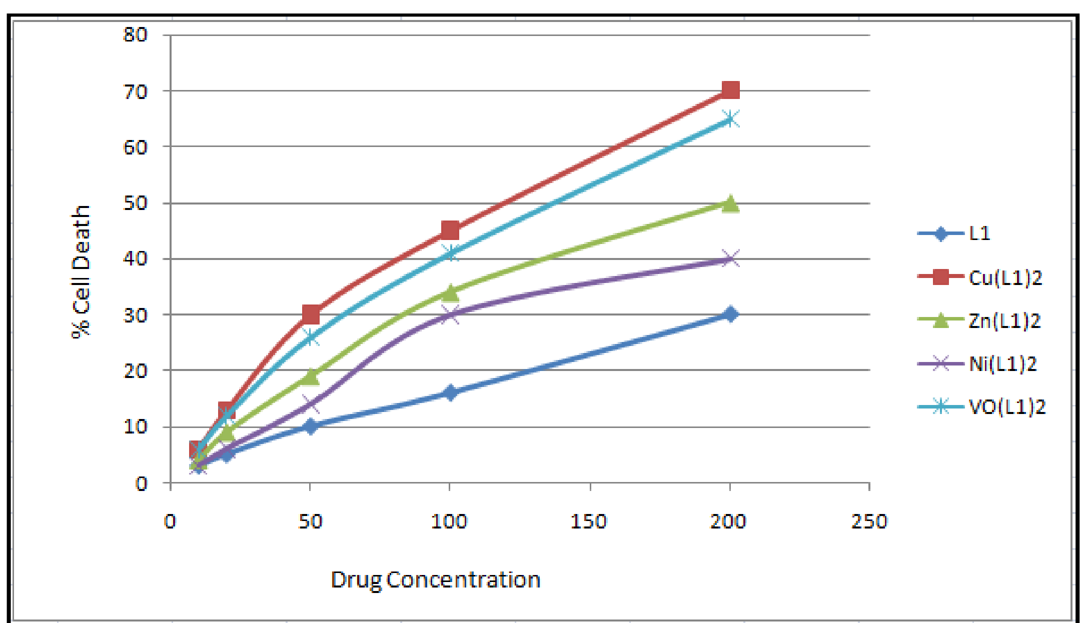


Fig.6.3.2. In vitro Cytotoxic studies of 1,7-di(naphthalen-1-yl) hepta-1,6-diene-3,5-dione (L_1) and their metal complexes towards DLA

6.3.2. *In vitro* Cytotoxic studies of 1,7-di(2-methoxy naphthalen-1-yl) hepta-1,6-diene-3,5-Dione(6b) and their metal complexes Cu (II), Zn(II), Ni(II) and VO(IV)

In vitro Cytotoxic studies of 1,7-di(2-methoxy naphthalen-1-yl) hepta-1,6-diene-3,5-dione(**6b**) and their transition metal chelates were evaluated and the results with DLA and EAC cells are represented in **Tables** below.

Table 6.3.3. ***In vitro*** **Cytotoxic studies of 1,7-bis(2-methoxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione (L_2) and their metal complexes towards EAC**

Drug Con. µg/ml	% Cell death				
	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$	$VO(L_2)_2$
200	36	80	58	44	76
100	22	53	42	36	47
50	11	38	27	22	30
20	6	23	17	12	17
10	4	16	10	7	12

The ligand with a methoxy group on naphthyl ring was a little more active against cancer cells than the ligand with no substituent on naphthyl ring.At lower concentrations,the activity of the ligand as well as the metal complexes were minimum.The ligand gave 36% cell death at 200 µg/ml concentration.The Cu(II) complex of the ligand exhibited significant cytotoxic effect to both DLA and EAC cells.It possessed the maximum activity(80%).The VO(IV) complex was also effective in producing cell death.The activities of different metal complexes and the ligand are as follows: Cu(II) > VO(IV) > Zn(II) > Ni(II) > ligand.

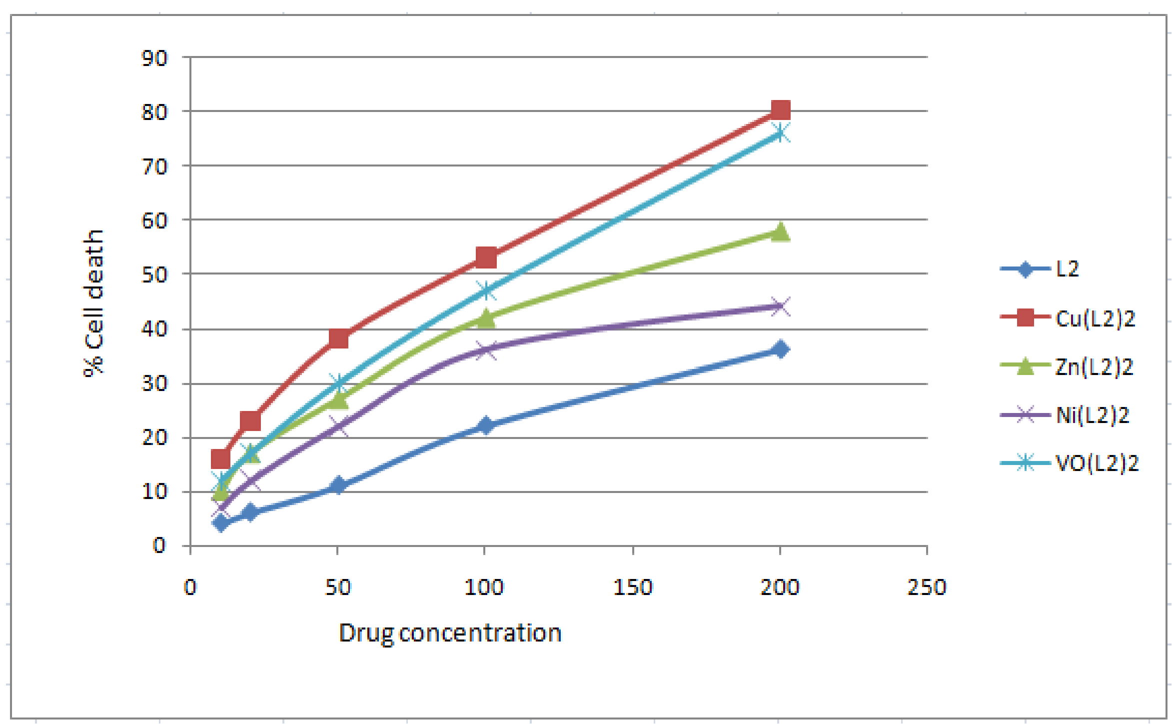


Fig.6.3.3. In vitro Cytotoxic studies of 1,7-bis(2-methoxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(L_2) and their metal complexes towards EAC

Table 6.3.4. In vitro Cytotoxic studies of 1,7-bis(2-methoxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(L_2) and their metal complexes towards DLA

Drug Con. µg/ml	% Cell death				
	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$Ni(L_2)_2$	$VO(L_2)_2$
200	34	78	58	42	70
100	18	51	39	34	44
50	11	36	25	20	29
20	6	21	15	10	18
10	3	14	8	5	10

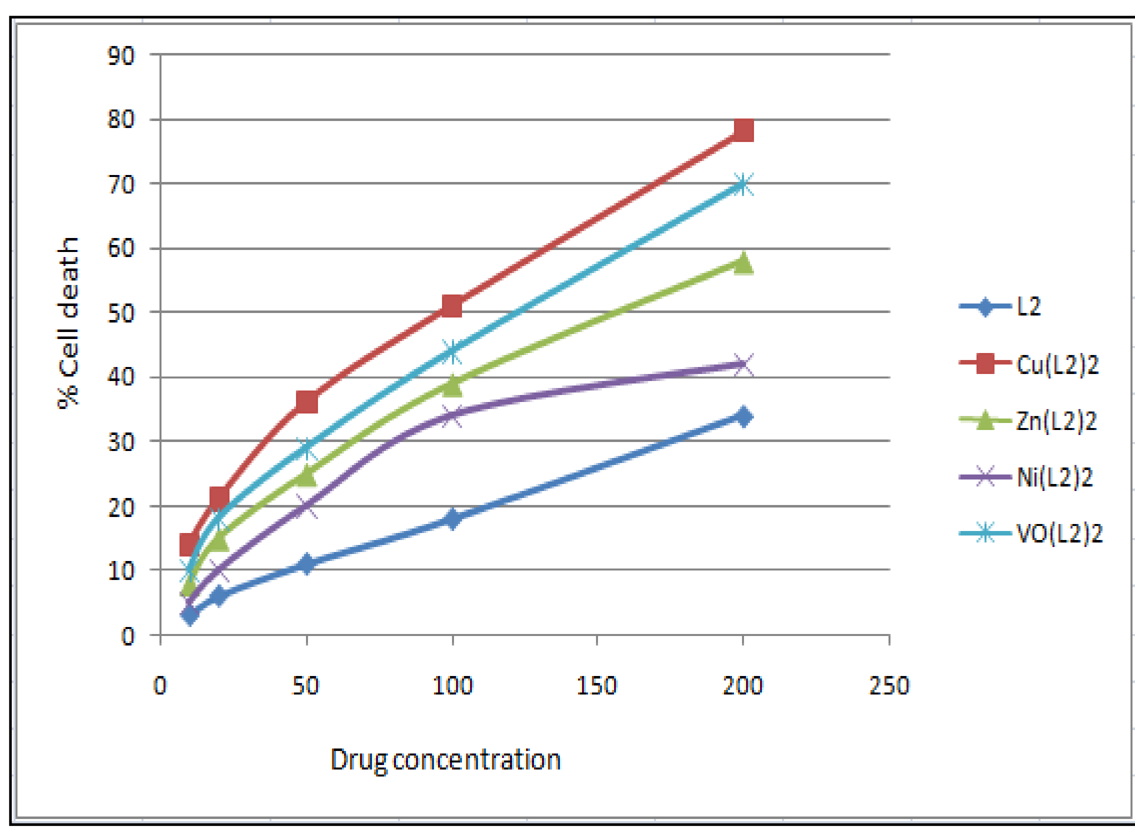


Fig.6.3.4. In vitro Cytotoxic studies of 1,7-bis(2-methoxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(L_2) and their metal complexes towards DLA

Almost similar results were obtained for the cytotoxic activity of the compounds towards DLA cells.The % cell death produced by them is comparable with that towards EAC cells.All the compounds were less cytotoxic with DLA cells than EAC cells. The activity of ligands and their metal complexes reveals that metal complexation increases activity.

6.3.3 *In vitro* Cytotoxic studies of 1,7-di(2-hydroxy naphthalen-1-yl) hepta-1,6-diene-3,5-Dione(6c) and their metal complexes Cu (II), Zn(II), Ni(II) and VO(IV)

1,7-di(2-hydroxy naphthalen-1-yl) hepta-1,6-diene-3,5-dione(**6c**) and their metal complexes were subjected to *in vitro* Cytotoxic studies.The observations are presented in **Table 6.3.5 and Table 6.3.6.**

Table 6.3.5. ***In vitro*** **Cytotoxic studies of 1,7-bis(2-hydroxy naphthalen-1-yl) hepta-1,6-diene-3,5-dione(L_3) and their metal complexes towards EAC**

Drug Con. µg/ml	% Cell death				
	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$Ni(L_3)_2$	$VO(L_3)_2$
200	42	85	71	49	76
100	27	76	51	32	55
50	16	50	36	22	38
20	10	28	20	16	23
10	7	15	13	10	12

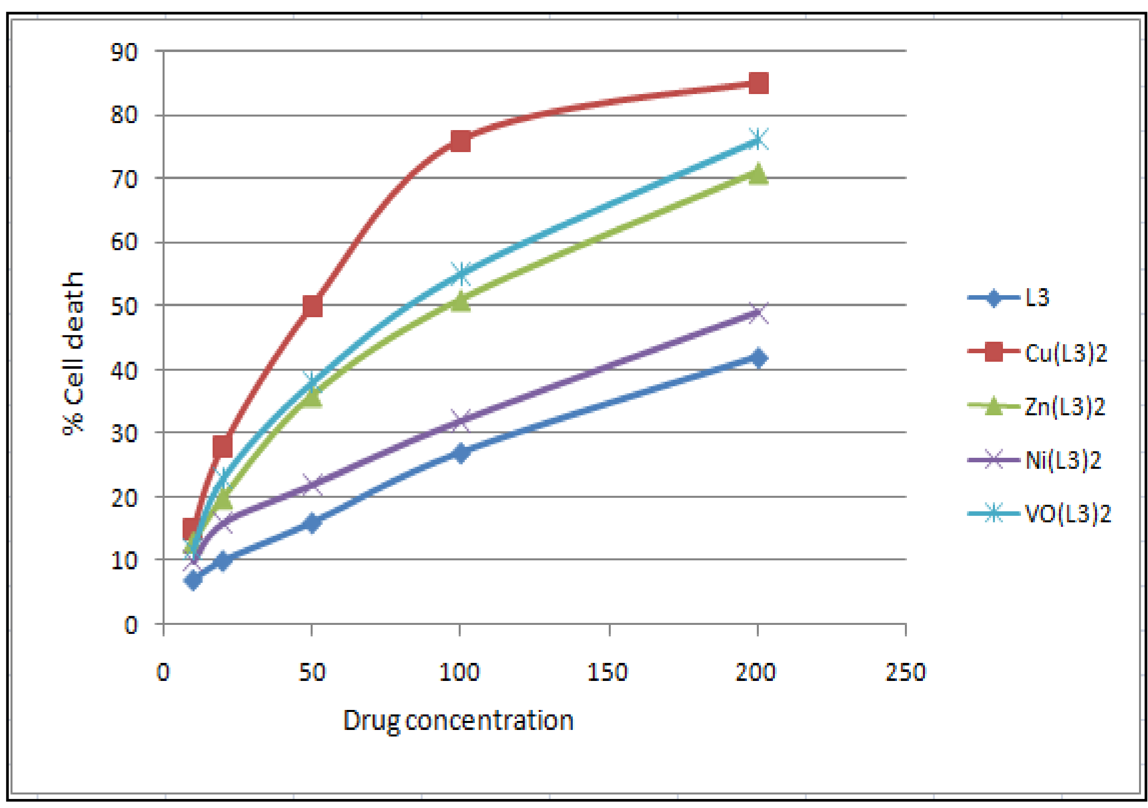


Fig.6.3.5. ***In vitro*** **Cytotoxic studies of 1,7-bis(2-hydroxy naphthalen-1-yl) hepta-1,6-diene-3,5-dione(L_3) and their metal complexes towards EAC**

Table 6.3.6. In vitro Cytotoxic studies of 1,7-bis(2-hydroxy naphthalen-1-yl) hepta-1,6-diene-3,5-dione (L_3) and their metal complexes towards DLA

Drug Con. µg/ml	% Cell death				
	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$Ni(L_3)_2$	$VO(L_3)_2$
200	38	83	65	45	70
100	23	72	48	30	50
50	14	44	29	19	32
20	8	20	14	10	18
10	4	13	9	6	11

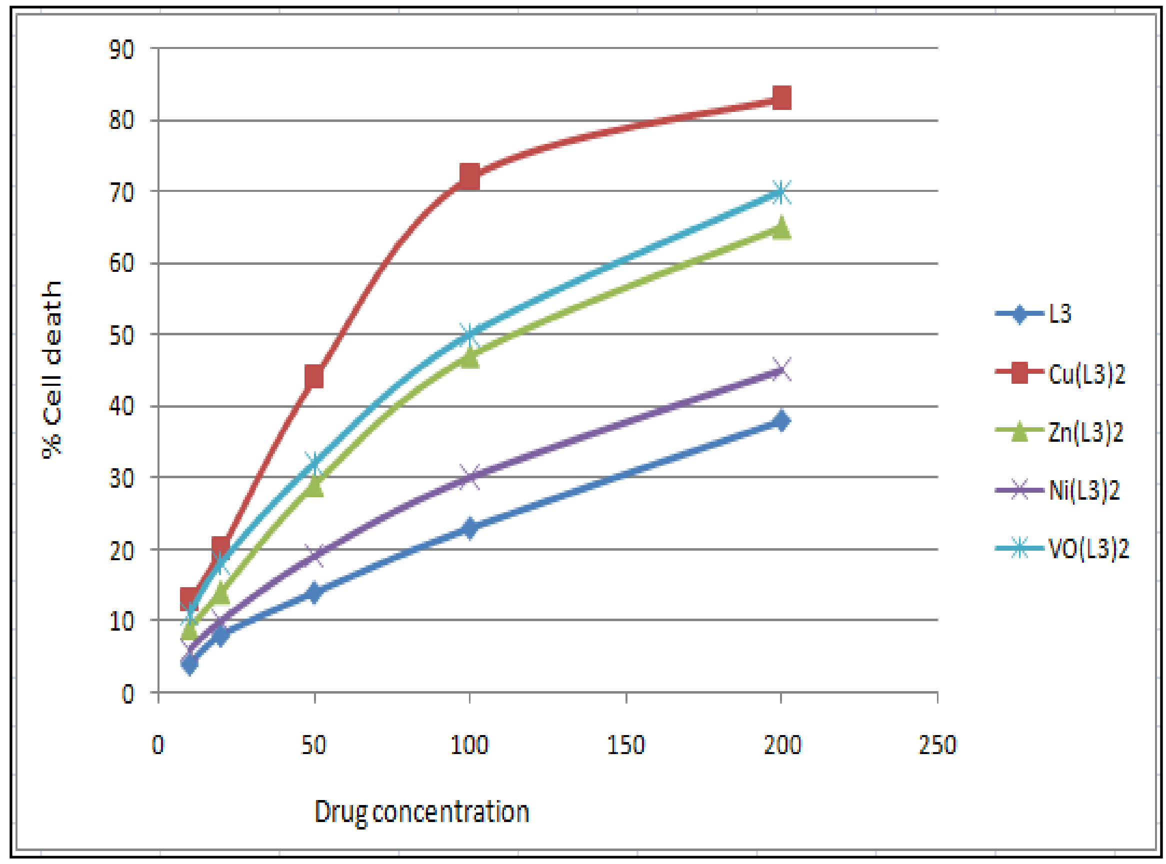


Fig.6.3.6. ***In vitro*** **Cytotoxic studies of 1,7-bis(2-hydroxy naphthalen-1-yl) hepta-1,6-diene-3,5-dione(L_3) and their metal complexes towards DLA**

The ligand with hydroxyl group on naphthyl ring was most effective in exhibiting cytotoxic effect among the ligands discussed in this chapter.The ligand showed 42% cell death at 200 µg/ml.The present study indicated that Cu(II)complex of the ligand could significantly inhibit both DLA and EAC cells.It produced a % cell death of nearly 85%.The IC50 value(the concentration which produces 50% cell death) of Cu(II) complex is 50 µg/ml where as for VO(IV) and Zn(II)complex it is 100 µg/ml.The Cu complex was effective even at lower concentrations.The Vanadyl and Zinc complexes were also effective against cancer cells and they gave nearly 70% cell death.The Ni(II) complex was slightly more active than the ligand but among the metal complexes it possessed minimum cytotoxicity.Both the ligand as well as the metal complexes showed lesser activity towards DLA cells as compared with EAC cells.

THE *IN VIVO* ANTITUMOUR STUDIES OF CURCUMINOID ANALOGUES WITH SUBSTITUTED NAPHTHYL RING AND THEIR Cu(II) COMPLEXES

Compounds exhibiting cytotoxicity towards tumour cells may also show antitumour activity in experimental animals (Ruby *et al* ,1995).So in this study the ligands 1,7-bis(2-methoxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(**6b**) & 1,7-bis(2-hydroxy naphthalen-1-yl) hepta-1,6-diene-3,5-dione(**6c**)and their Cu(II) complexes which were found to be quite cytotoxic in the in vitro studies conducted were selected.The present study was undertaken to evaluate the in vivo antitumour activity of the compounds against EAC cancer cell lines using ascites tumour model.Viable EAC cells were injected into the peritoneal cavity of mice so that they develop tumours in their body. Drugs (6b & 6c and their Cu(II) complexes)were administered as intraperitoneal injection at different concentrations namely 20,10,5 µg/ml for 10 days after tumour injection. The death pattern of animals due to tumour burden was noted and the percentage increase in life span calculated.The values of No. of days survived are means of five determinations $\pm SD$ (standard deviation).The results of the study with 1,7-bis(2-

methoxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(**6b**) and its Cu(II) complex are given in **Table 6.3.7.**

Table 6.3.7 Effect of 1,7-bis(2-methoxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione (L) and its Cu(II)complex 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	30.6±3.1	76.88
3.L	20	5/5	25.6±1.1	47.97
4.L	10	5/5	24.3±2.4	40.46
5.L	5	5/5	18.8±2.50	8.67
6. $Cu(L)_2$	20	5/5	28.1±3.2	62.42
7. $Cu(L)_2$	10	5/5	26.0±2.4	50.26
8. $Cu(L)_2$	5	5/5	19.7±2.55	13.87

The mice with EAC induced ascites tumour survived for a period of 17.3±1.1 days.The administration of standard drug cyclophosphamide increased the life span to 30.6±3.1 days.The ligand at 20,10 and 5 µg/ml increased the average life span of animals to 25.6±1.1, 24.3±2.4, 18.8±2.50 days respectively. Compared with the std.drug the % ILS produced by the ligand L was 47% at concentration 20µg/ml and that produced by its Cu(II) complex was 62% at concentration 20µg/ml. Both the ligand and metal complex were more active at higher concentrations.The Cu(II) complex was more active than the ligand in increasing the life span of tumour burden animals.

THE *IN VIVO* ANTITUMOUR STUDIES OF 1,7-BIS(2-HYDROXY NAPHTHYL)-1,6-HEPTADIENE-3,5-DIONE AND Cu(II) COMPLEX

The compound 1,7-bis(2-hydroxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(**L1**) and its Cu(II) complex was given by intraperitoneal injection from the first day of tumour induction as drug to mice groups. The death pattern of animals due to tumor burden was noted and the percentage increase in life span was found.The results are discussed in **Table 6.3.8**

Table 6.3.8 Effect of 1,7-bis(2-hydroxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione (L1) 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			17.3±1.1	
2.Std.drug			30.6±3.1	76.88
3. L1	20	5/5	26.6±2.5	53.75
4. L1	10	5/5	25.3±2.6	46.24
5. L1	5	5/5	19.8±1.60	14.45
6. Cu(L1)2	20	5/5	29.2±2.9	68.78
7. Cu(L1)2	10	5/5	28.0±2.5	56.06
8. Cu(L1)2	5	5/5	20.7±3.65	19.65

The compound and its Cu(II) complex when administered intraperitoneally could produce significant increase in the life span of mice bearing ascites tumour. The animals of the control group survived for a period of 17.3±1.1 days and those treated with std.drug cyclophosphamide for a period of 30.6±3.1days. Cu(II) complex of the compound produced an increase in life span of tumour bearing mice compared with that of the ligand . The percentage increase in life span(%ILS) of tumour bearing mice were 53.75, 46.24 and 14.45 for the ligand at diffferent concentrations namely 20,10,5μg/ml respectively. For the Cu(II) complex the % increase was 68.78 % at a concentration of 20μg/ml. There was an increase

in the average life span of animals for both the ligand and the metal complex.But the studies reveal that Cu(II) complex is very effective in reducing tumour development in mice and increasing the life span of the animal.The Cu(II) complex exhibits significant antitumour activity in invivo studies.

SECTION-IV

ANTIBACTERIAL STUDY OF 1,7-DINAPHTHYL HEPTANOIDS WITH METHOXY &HYDROXY SUBSTITUTED RING AND THEIR Cu(II),Zn(II)&VO(IV)METAL COMPLEXES

6.4.1 Antibacterial studies of 1,7-di(naphthalen-1-yl) hepta-1,6-diene-3,5-dione(6a), 1,7-bis(2-methoxynaphthalen-1-yl)hepta-1,6-diene-3,5-dione(6b),1,7-bis(2-hydroxynaphthalen-1-yl)hepta-1,6-diene-3,5-dione(6c) and their Cu(II),Zn(II)&VO(IV) metal complexes

The compounds and their complexes were evaluated for their antibacterial activities.The invitro antibacterial studies were performed on three types of bacterial strains namely Escherichia Coli, Klebsiella Pneumoniae & Bacillus Subtilis using agar well diffusion method.The test compounds showed varying degree of inhibition against different bacterial strains.All synthesized compounds have shown to be susceptible to excellent potency against the different bacterial strains. The results of the antibacterial activity of methoxy and hydroxy substituted 1,7-dinaphthyl heptanoids and their complexes revealed that the ligands and their complexes possess comparable antibacterial activity to that of standard drug streptomycin.The activity is expressed in terms of diameter of zone of inhibition in mm. In all the cases metal complexes possess better antibacterial activity than that of ligands, which means that metal complexation enhance the activity . The results of the antibacterial studies are given below.

Table 6.4.1 Antibacterial studies of 6a (L_1) and their Cu(II),Zn(II) & VO(IV) complexes

Bacteria	Diameter zone of inhibition in mm			
	L_1	$Cu(L_1)_2$	$Zn(L_1)_2$	$VO(L_1)_2$
E Coli	15	19	16.5	18.5
Klebsiella	12	15	13.5	14.5
Bacillus	8	10	8.5	9.5
Standard	20	20	20	20

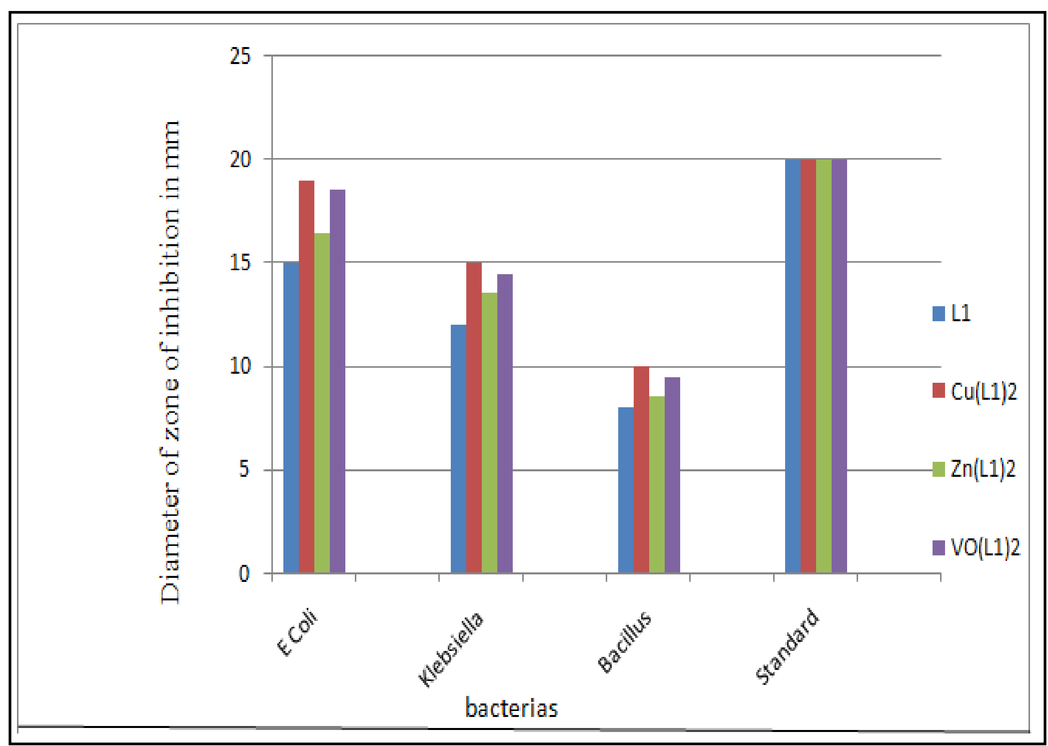


Fig. 6.4.1 Antibacterial studies of 1,7-di(naphthalen-1-yl) hepta-1,6-diene-3,5-dione(L_1) and their Cu(II),Zn(II) & VO(IV) complexes

Table 6.4.2 Antibacterial studies of 6b (L_2) and their Cu(II),Zn(II) & VO(IV) complexes

Bacteria	Diameter zone of inhibition in mm			
	L_2	$Cu(L_2)_2$	$Zn(L_2)_2$	$VO(L_2)_2$
E Coli	19	21	18.5	19.5
Klebsiella	16	18	16.5	17
Bacillus	12	14	13	13.5
Standard	20	20	20	20

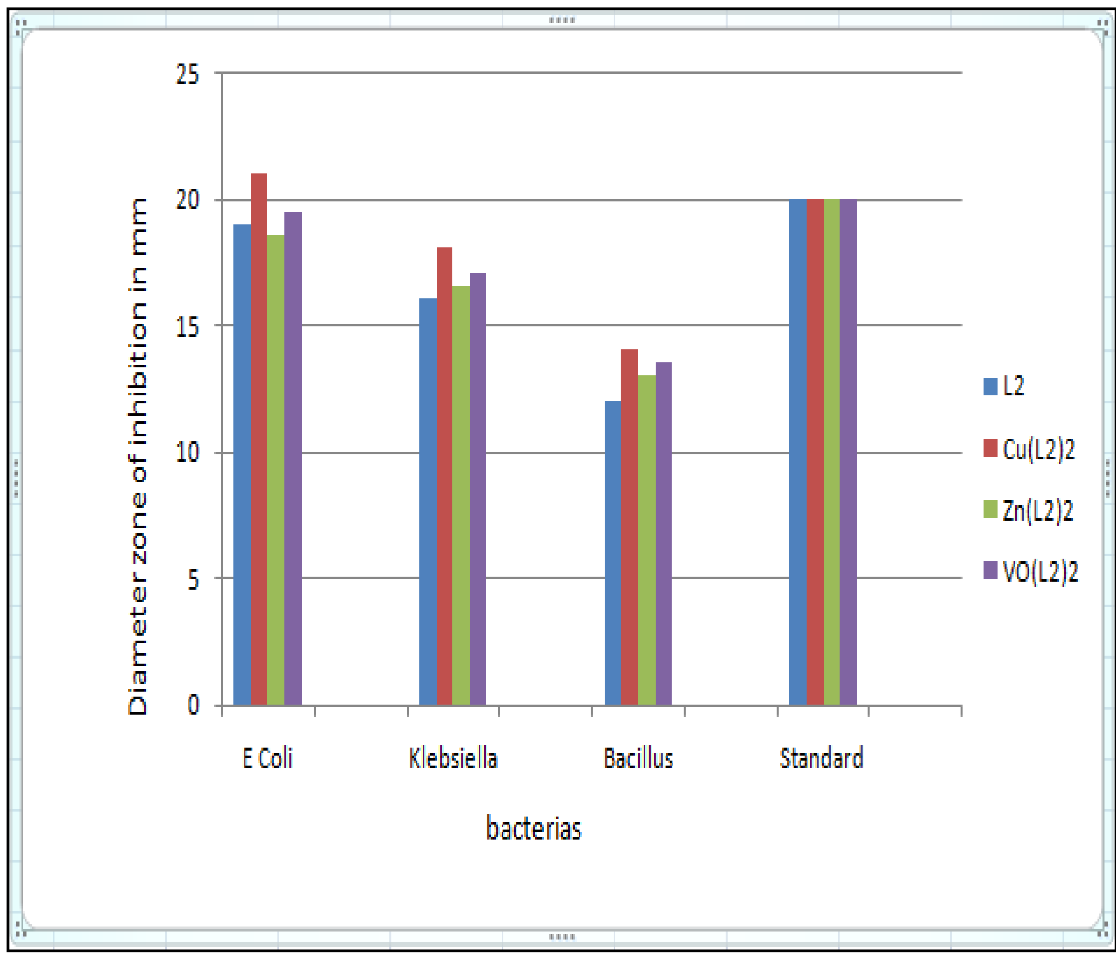


Fig. 6.4.2 Antibacterial studies of 1,7-bis(2-methoxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(L_2) and their Cu(II),Zn(II) & VO(IV) complexes

Table 6.4.3 Antibacterial studies of 6c (L_3) and their Cu(II),Zn(II) & VO(IV) complexes

Bacteria	Diameter of zone of inhibition in mm			
	L_3	$Cu(L_3)_2$	$Zn(L_3)_2$	$VO(L_3)_2$
E Coli	17	20	17	18.5
Klebsiella	14	17	14	16
Bacillus	10	13	12	13
Standard	20	20	20	20

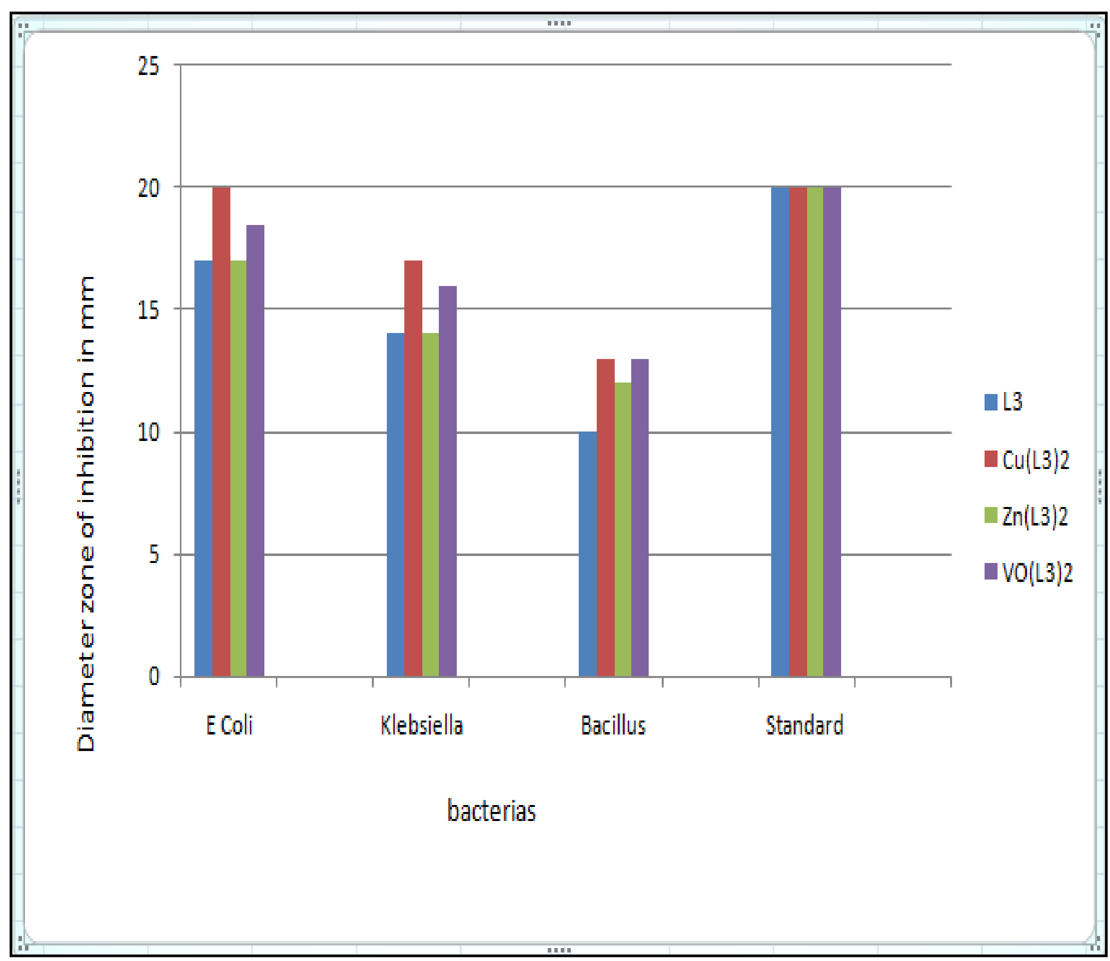


Fig. 6.4.3 Antibacterial studies of 1,7-bis(2-hydroxy naphthalen-1-yl) hepta-1,6-diene-3,5-dione(L_3) and their Cu(II),Zn(II) & VO(IV) complexes

All the ligands 1,7-di (naphthalen-1-yl) hepta-1,6-diene-3,5-dione(L_1),1,7-bis (2-methoxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(L_2),1,7-bis(2-hydroxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(L_3),were quite effective against E.Coli bacterial strains producing a zone of inhibition of 15mm,19mm and 17 mm respectively.Among the ligands the activity is in the order $L_2 > L_3 > L_1$ ie the ligand with methoxy substituent on naphthyl ring is more active than the one with hydroxyl substituent on naphthyl ring.The ligand with no substituent on naphthyl ring showed least activity.

The activity of ligands with different bacterial strains followed the order E.Coli>Klebsiella> Bacillus.For all the three ligands,Cu(II) complexes possessed maximum antibacterial activity .They produced a zone of inhibition in the range 20mm against E.Coli which is same as that of standard drug.The Cu(II) complex of 1,7-bis(2-methoxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(L_2) produced a zone of inhibition of 21mm against E.Coli which is more active than the standard drug.The Cu (II) complex of L_2 & L_3 was also very active against Klebsiella species.

The VO(IV) complexes also exhibited significant activity next to Cu(II)complexes.The VO(IV) complexes of all ligands were quite active against E.Coli species producing a zone of inhibition in the range 19mm.Among the metal complexes ,Zn(II) exhibited least activity.The metal complexes of L_2 exhibited greater activity towards all bacterial strains comparing with metal complexes of other ligands.So it is concluded that methoxy substituent on naphthyl ring plays an important role in antibacterial activity.

SECTION-V

ANTIFUNGAL STUDY OF 1,7-DINAPHTHYL HEPTANOIDS WITH METHOXY &HYDROXY SUBSTITUTED RING AND THEIR VO(IV)METAL COMPLEXES

6.5.1 Antifungal studies of curcuminoid analogues with naphthyl ring

Invitro antifungal activities of methoxy and hydroxy substituted 1,7-dinaphthyl heptanoids and their metal complexes with VO(IV)were investigated against three fungal cultures namely Aspergillus Niger, Penicillium Chrysogenum and Alternaria Alternate by Kirby Baurer method.The test compounds were prepared in different concentrations [100, 250, 500µg/ml] by dissolving in 2% DMSO solvent.This method was standardized using flucanazole drug. A control disc was used with DMSO only and without drug. For compounds effective against fungal cultures,the growth of fungus is inhibited as zone.The antifungal activities are measured in terms of zone of inhibition in mm.The results of the studies are given in Table **6.5.1,6.5.2 and 6. 5.3.**

Table 6.5.1 Antifungal studies of 6a (L_1) and its VO(IV) complexes

Fungi	Diameter of zone of inhibition in mm					
	L_1			$VO(L_1)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	13	16	17	15	19	21
Penicillium	12	15.5	16	14	17.5	20.5
Alternaria	12.5	16.5	16.5	13	18	21

Table 6.5.2 Antifungal studies of 6b (L_2) and its VO(IV) complexes

Fungi	Diameter of zone of inhibition in mm					
	L_2			$VO(L_2)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	14.5	17	19	15.5	19	23.5
Penicillium	13	15	18	14	19	22
Alternaria	12.5	15.5	18	14	17	22.5

Table 6.5.3 Antifungal studies of 6c(L_3) and its VO(IV) complexes

Fungi	Diameter of zone of inhibition in mm					
	L_3			$VO(L_3)_2$		
	100µg	250µg	500µg	100µg	250µg	500µg
Aspergillus	12	15.5	16	16	19	20
Penicillium	11	14.5	15	14	17	19
Alternaria	11.5	13.5	16	14	17.5	19.5

It has been observed that all the ligands with naphthyl rings exhibited significant antifungal behavior.Their antifungal activity varied with the substituents present on the naphthyl ring.All the ligands as well as VO(IV) complexes presented greater activity at higher concentrations.The curcuminoid analogues 1,7-di(naphthalen-1-yl) hepta-1,6-diene-3,5-dione(L_1), 1,7-bis(2-methoxynaphthalen-1-yl)hepta-1,6-diene-3,5-dione(L_2), 1,7-bis(2-hydroxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(L_3) exhibited comparable antifungal activities against all the three fungi species.Among the three ligands the one with methoxy substituent on naphthyl ring was most active against all fungal cultures.It exhibited a zone of inhibition of 19mm,18mm and 18mm respectively against Aspergillus,Penicillium and

Alternaria.The ligand presented greater activity against Aspergillus where as its activity against Penicillium and Alternaria were the same.

The ligand with unsubstituted naphthyl ring was not as active as ligand (L_2)with methoxy substituted naphthyl ring against the fungi species.The compound with hydroxy substituted naphthyl ring exhibited least activity among the three compounds.It produced a zone of inhibition in the range 16mm against the fungi species.

The VO(IV) complexes of all the ligands exhibited highly significant activity.The Vanadyl complex of 1,7-bis(2-methoxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(L_2) was very effective against Aspergillus species producing a zone of inhibition of 23.5mm which is greater than that produced by the standard drug flucanazole.The Vanadyl complex of 1,7-di(naphthalen-1-yl) hepta-1,6-diene-3,5-dione(L_1) had also shown pronounced activity against all fungi species.Its activity is comparable with that of standard drug which produces a zone of inhibition of 21mm.The VO(IV) complexes of 1,7-bis(2-hydroxy naphthalen-1-yl)hepta-1,6-diene-3,5-dione(L_3) showed least activity among the complexes of all the ligands .

A comparative study of the antifungal nature of Ligands and their Vanadyl Complexes at 500 μg concentration is given below in Figure **6.5.1**.

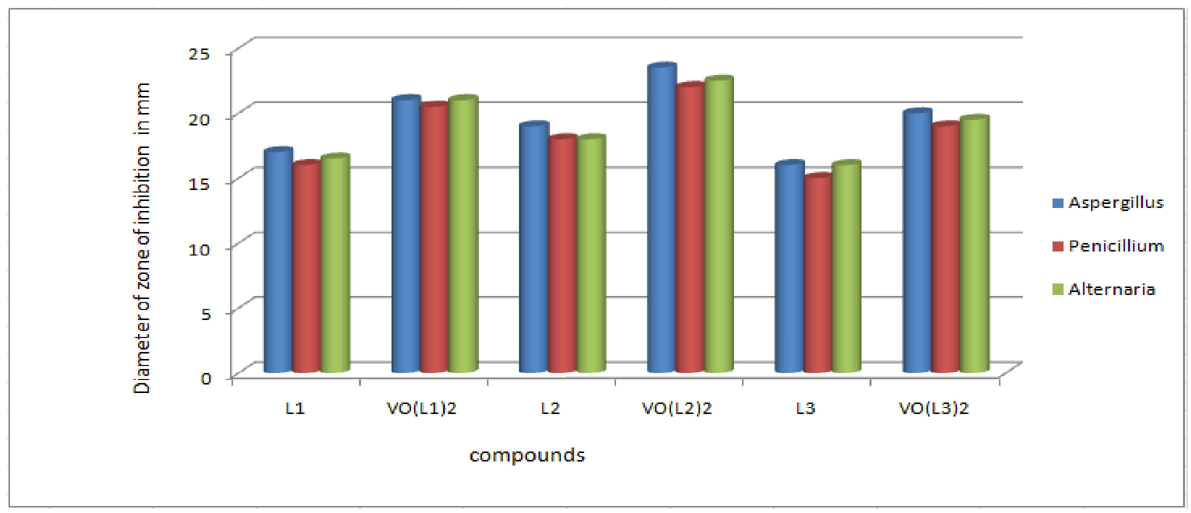


Figure 6.5.1 Comparative study of Ligands and Vanadyl Complexes at 500μg conc.

CONCLUSION

Fourteen 1,7-diaryl heptanoids (curcuminoid analogues) have been synthesized and were characterized. They were classified into the following three structural types (1,2 &3) based on the nature of aryl groups. Type 1 with heterocyclic thiophenyl ring,Type 2 with mono,di &trisubstituted phenyl ring and Type 3 with polynuclear naphthyl and anthracenyl ring.

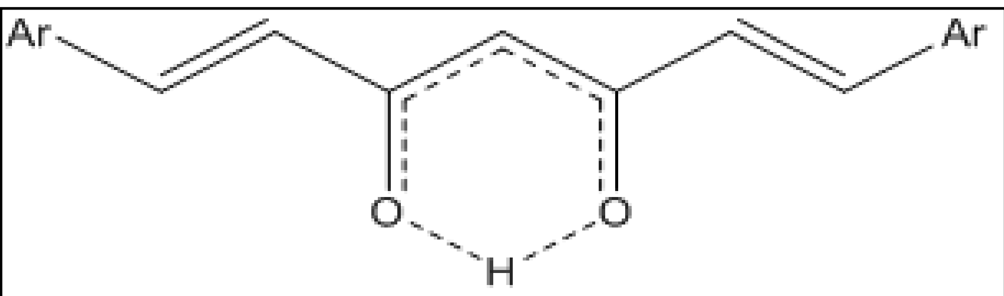
Type 1

2a - $R_1 = H$ **2b -** $R_1 = CH_3$

Type 2

Compounds	R_1	R_2	R_3	R_4
1a	CH_3	H	H	H
Ib	CH_3	H	H	CH_3
3a	H	H	Cl	H
3b	Cl	H	H	H
3c	H	Cl	Cl	H
4a	H	OCH_2CH_3	OH	H
4b	OH	H	OH	H
4c	H	OCH_3	OCH_3	OCH_3

Type 3 (Ar=polynuclear ring)



6a – Ar = 1-Naphthyl
6b – Ar = 2-Methoxy naphthyl
6c – Ar = 2-Hydroxy naphthyl
5a=Anthracenyl

Analytical and spectral data of all the compounds clearly support the existence of intramolecularly hydrogen bonded enol tautomeric form. All these compounds form stable metal chelates with Cu(II), Zn(II), Ni(II) and VO(IV) having ML_2 stoichiometry. These unsaturated diketones behave as monobasic bidentate chelating agent. In all the metal complexes, only the oxygen atoms of the diketo group are involved in bonding with the formation of a stable six membered chelate ring involving the metal ion. The analytical and spectral data clearly suggest that metal binding groups such as –OH, sulphur of thiophenyl ring etc. present in the ligands are not involved in bonding with metal ions.

All the 1,7-diaryl heptanoids and their metal complexes show significant antibacterial, antifungal activity. They also show significant *in vitro* and *in vivo* anticancer activity. All these results are summarised below.

Antibacterial studies

The antibacterial studies of 1,7-diaryl heptanoids and their metal complexes were carried out using bacterial strains Escherichia Coli, Klebsiella Pneumoniae & Bacillus Subtilis. Good results were obtained for the antibacterial activity of 1,7-diaryl heptanoids and their complexes. The results were compared with that of standard drug streptomycin. In all the cases metal complexes possess better antibacterial activity than that of ligands.They have shown greater antibacterial activity with Escherichia Coli species and moderate activity with Klebsiella Pneumoniae & Bacillus Subtilis. Type 1 ligands and their metal complexes show lesser antibacterial activity than other two types. A comparative study of antibacterial activity of 1,7-diaryl heptanoids of Type 2 & 3 towards the bacteria Escherichia Coli was done and it was found that the maximum activity was obtained with ligand 6b. This may be due to the presence of methoxy group and naphthyl ring present in it. The ligand 4b was also equally active with a dihydroxy substituent phenyl ring. The results are summarized in Fig.7.1.

A comparative study of antibacterial activity of metal chelates have shown that Cu(II)complexes are the most active among the metal complexes. VO(IV)complexes of the ligands have also exhibited significant antibacterial nature. The most active compound among the metal complexes is Cu(II)complex of 1,7-bis(2-methoxynaphthyl)hepta-1,6-diene-3,5-dione(6b) with a zone of inhibition of 21mm.

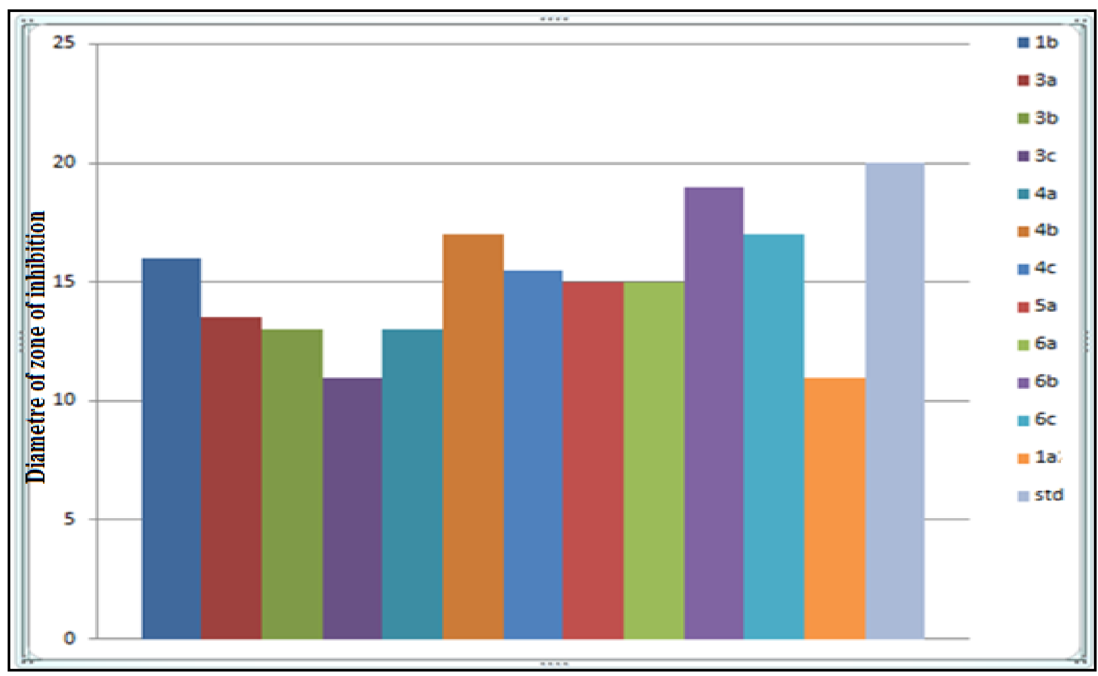


Fig.7.1 Antibacterial activity of Type 2 & Type 3 ligands towards E Coli

A comparative study of antibacterial activity of Cu(II) & VO(IV) complexes of 1,7-diaryl heptanoids towards the bacteria Escherichia Coli were also carried out. Cu(II) complex of 6b show maximum results among Cu complexes where as VO(IV) complex of 6b show maximum activity among Vanadyl complexes (Fig.7.2 & 7.3).

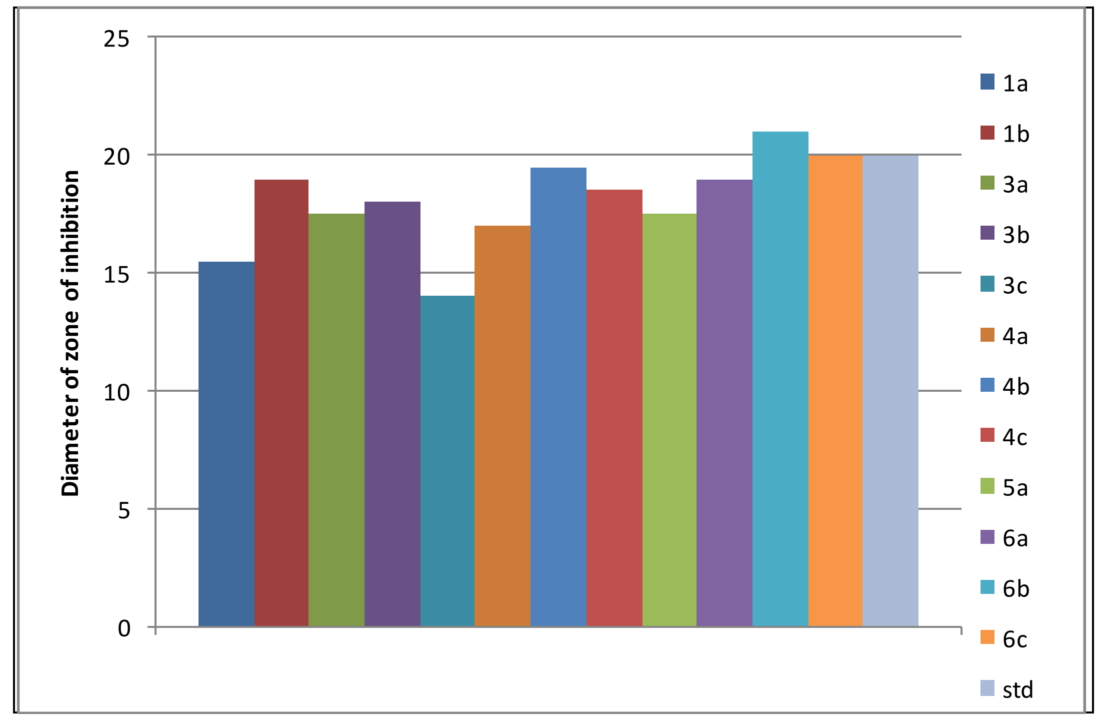


Fig.7.2 Antibacterial activity of Cu(II) complexes of Type 2 & Type 3 ligands

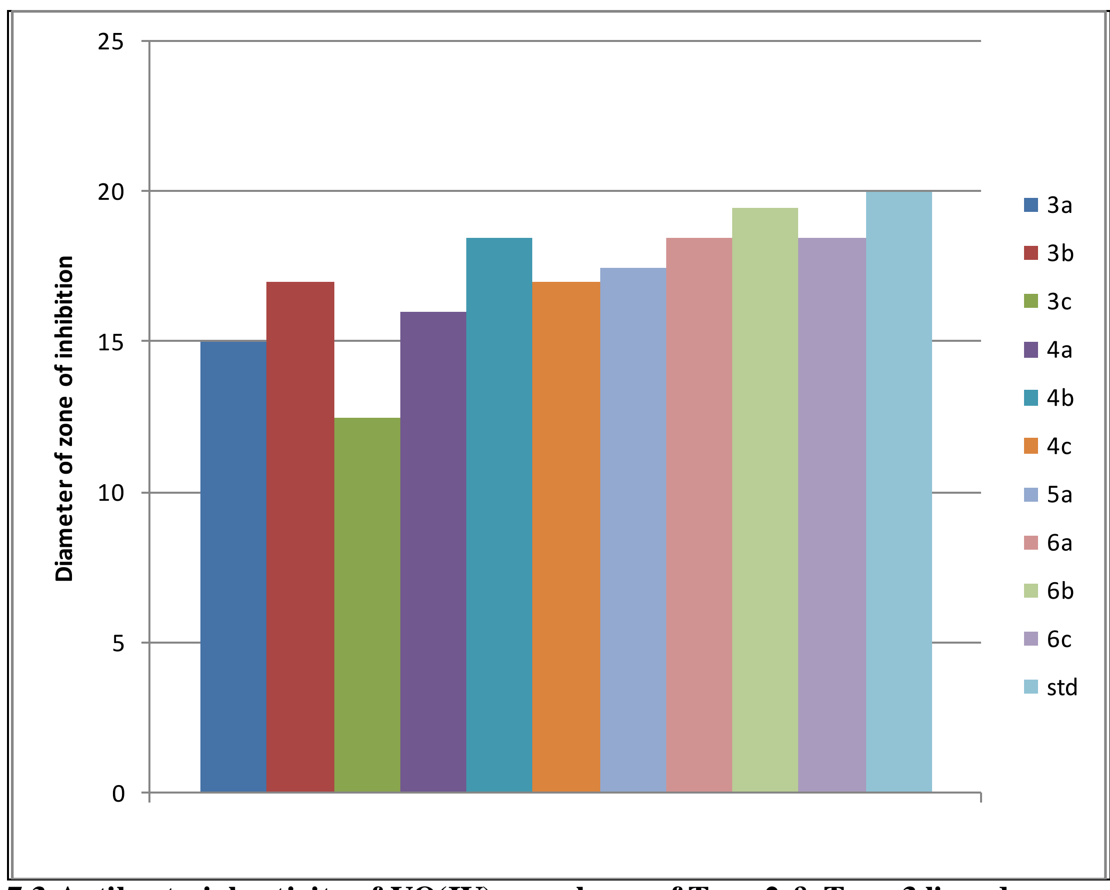


Fig.7.3 Antibacterial activity of VO(IV) complexes of Type 2 & Type 3 ligands

Antifungal activity

The antifungal studies of 1,7-diheteroaryl heptanoids and their metal complexes were carried out against the fungi species Aspergillus niger, Penicillium chrysogenum and Alternaria alternate. Ligands and their metal complexes with different concentrations [100, 250, 500µg/ml] were used as drugs. Maximum activity was found with a con. 500µg/ml. Generally low values were obtained with type 2 ligands with methyl substituted phenyl ring . Type 1 ligands and their metal complexes were moderately active against all fungi species. The VO(IV) complexes of the ligands exhibited appreciable antifungal nature. A comparison of their activities is given below in Fig.7.4.

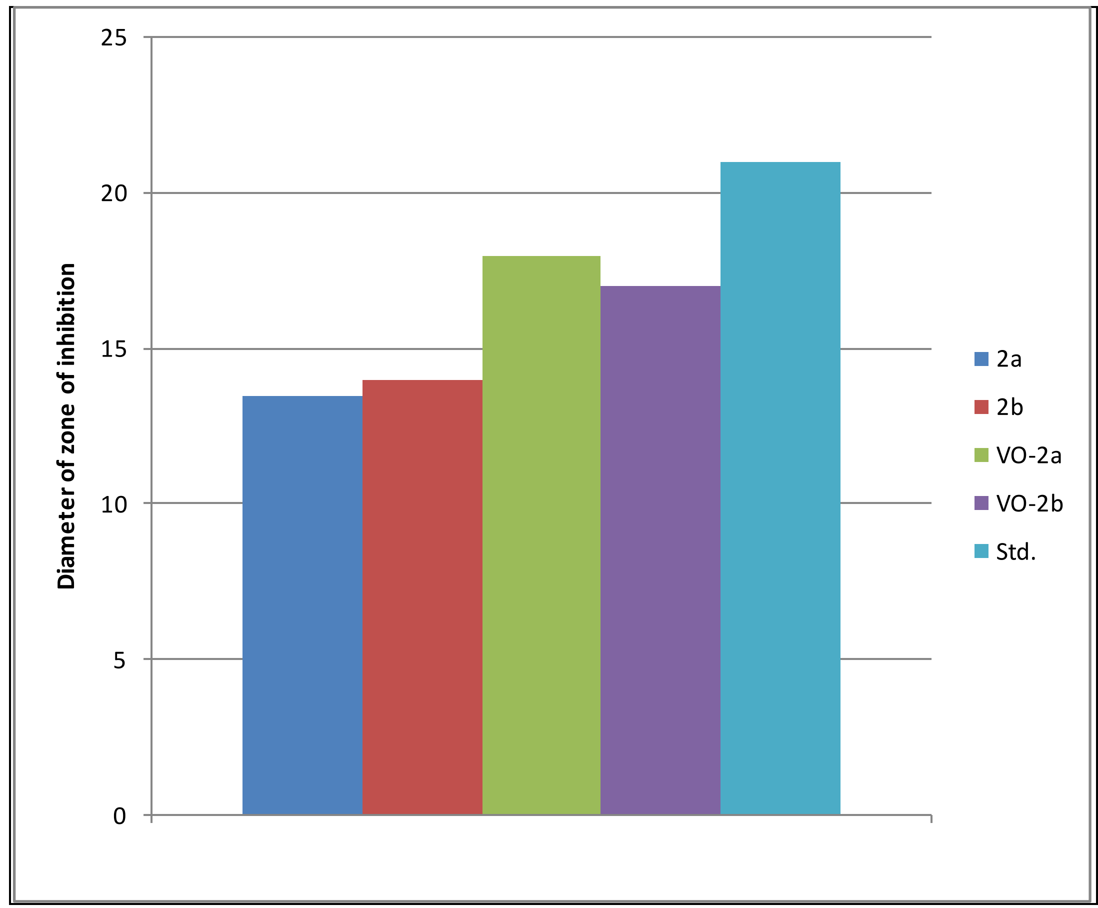


Fig.7.4 Antifungal studies of Type 1 ligands and their Vanadyl complexes towards Aspergillus

Type 2 & 3 ligands and their complexes generally gave very good activity towards the fungi Aspergillus. Zone of inhibition of 21mm was found with the ligand 5a and 19 mm with ligands 4c and 6b.The ligand 5a has a polynuclear anthracenyl ring,4c has a trimethoxy substituted phenyl ring and 6b has a methoxy substituted naphthyl ring. The presence of methoxy group enhances antifungal activity.

A comparative study of VO(IV) complexes of type 2 & 3 ligands show maximum activity. Maximum value was obtained with complex of 5a. This may be due to the polynuclear nature of the ligand (Fig.7.5 & 7.6). The zone of inhibition value of 24mm was found with 5a, which is higher than the standard value.

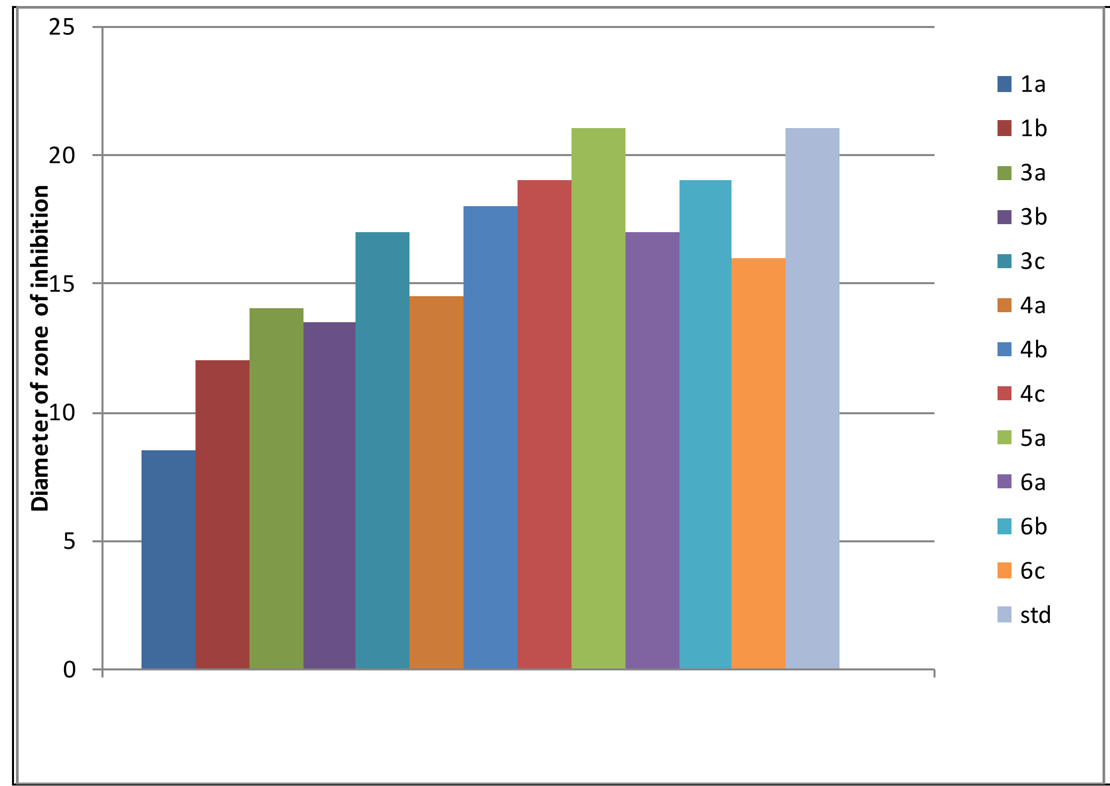


Fig.7.5 Antifungal studies of Type 2 & 3 ligands towards Aspergillus

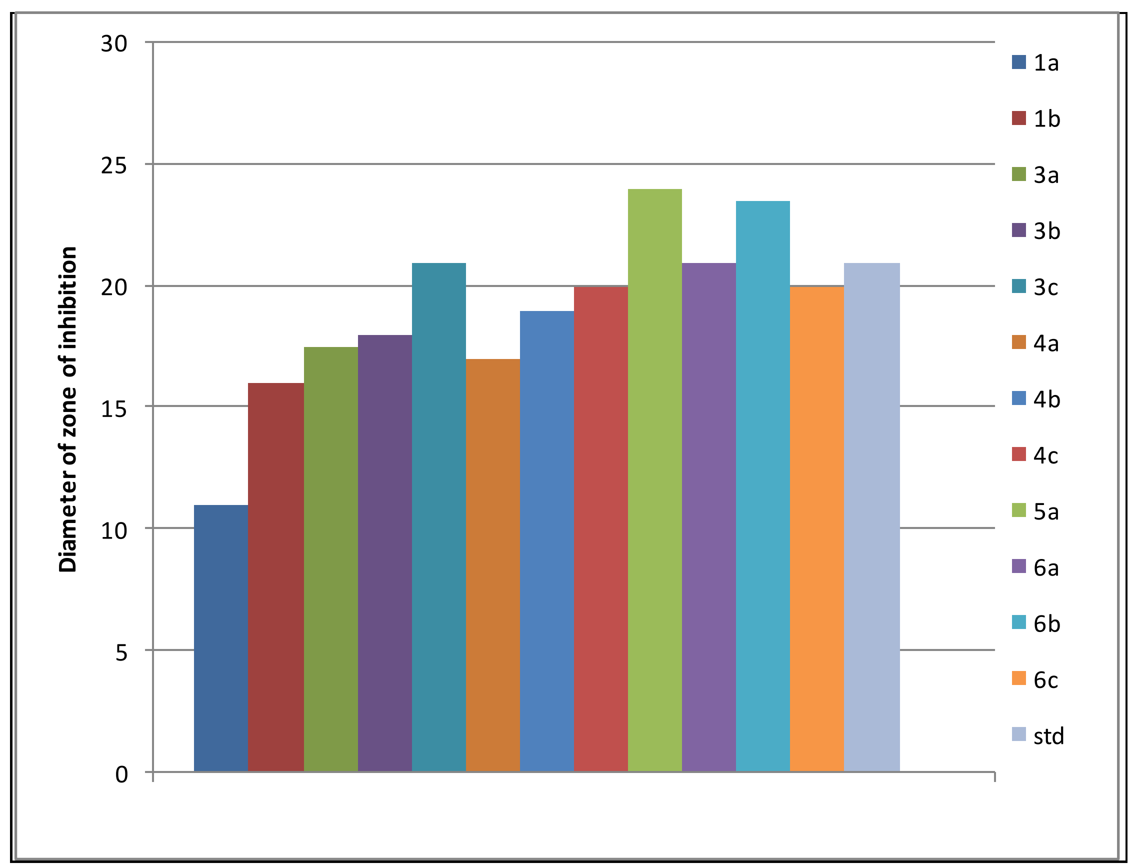


Fig.7.6 Antifungal studies of VO(IV) complexes of Type 2 & 3 ligands towards Aspergillus

In vitro cytotoxicity study

In vitro cytotoxicity studies were carried out using 1,7-diaryl heptanoids & metal complexes with concentrations 200, 100, 50, 20 & 10 µg/ml against the tumour cells (DLA & EAC). Cytotoxic nature of the compounds were found in terms of % cell death produced by it. All the compounds produced greater % cell death at a concentration of 200 µg/ml.Ligands with methyl and chloro substituted phenyl rings exhibited nominal activity.A comparison of cytotoxic nature of the ligands have shown that the most active compound is 5a with anthracenyl ring which gave 70 % cell death. All the ligands except 5a require more than 200 µg/ml concentration for 50% cell death.The ligand with heterocyclic thiophenyl ring,hydroxyl substituted naphthyl ring and trimethoxy substituted phenyl ring were also very active against cancer cells producing 40% cell death. It may be concluded that both 2a & 5a are better candidates for in vitro cytotoxic studies towards EAC & DLA.The activities of the ligands are compared and presented in Fig.7.7

In vitro cytotoxicity studies were carried out using metal chelates with Cu(II), Zn(II), Ni(II) & VO(IV).Out of the metal complexes,Cu(II) chelates possess maximum activity.The most active compound is Cu(II) complex of 5a with 98% cell death.The Cu(II) complex of 2a with thiophenyl ring also gave 92% cell death.The Vanadyl complexes of 5a,2a & 4c were also very effective against cancer cells.It is observed that metal chelation enhances cytotoxic nature considerably.The activities of Cu(II) complexes and Vanadyl complexes are compared and presented in Fig.7.8 &7.9 respectively.

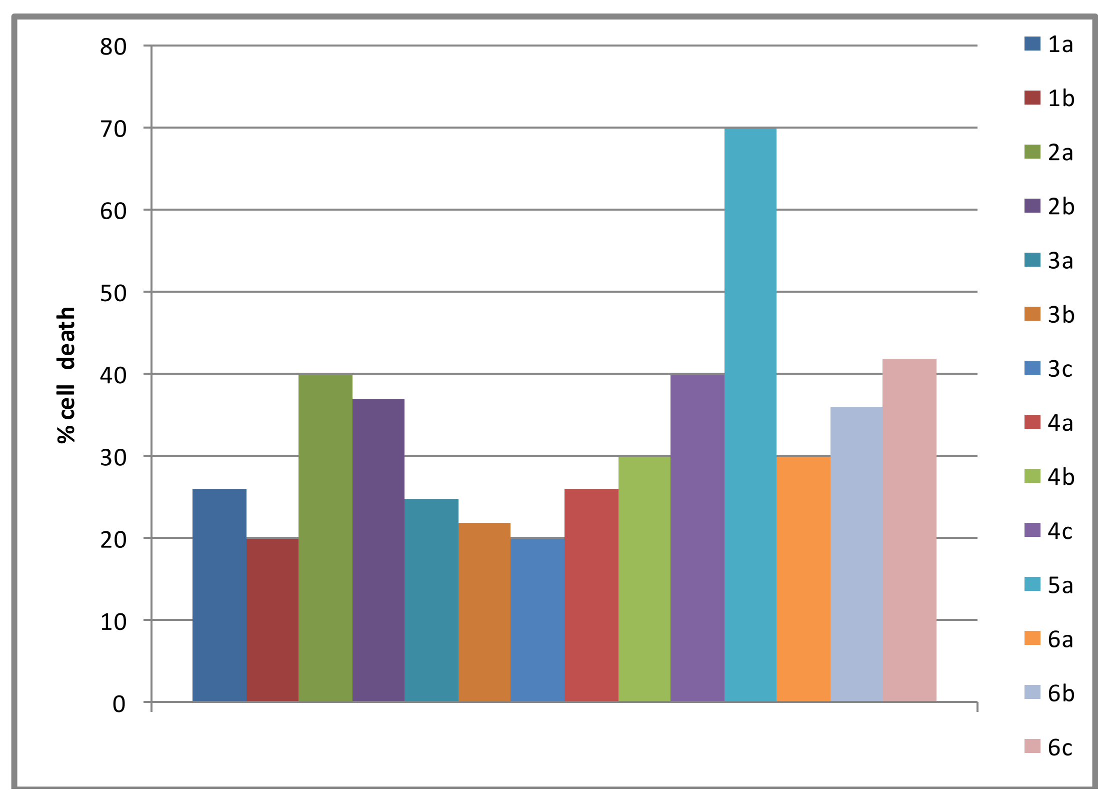


Fig.7.7 % cell death for ligands towards EAC at concentration 200µg/ml

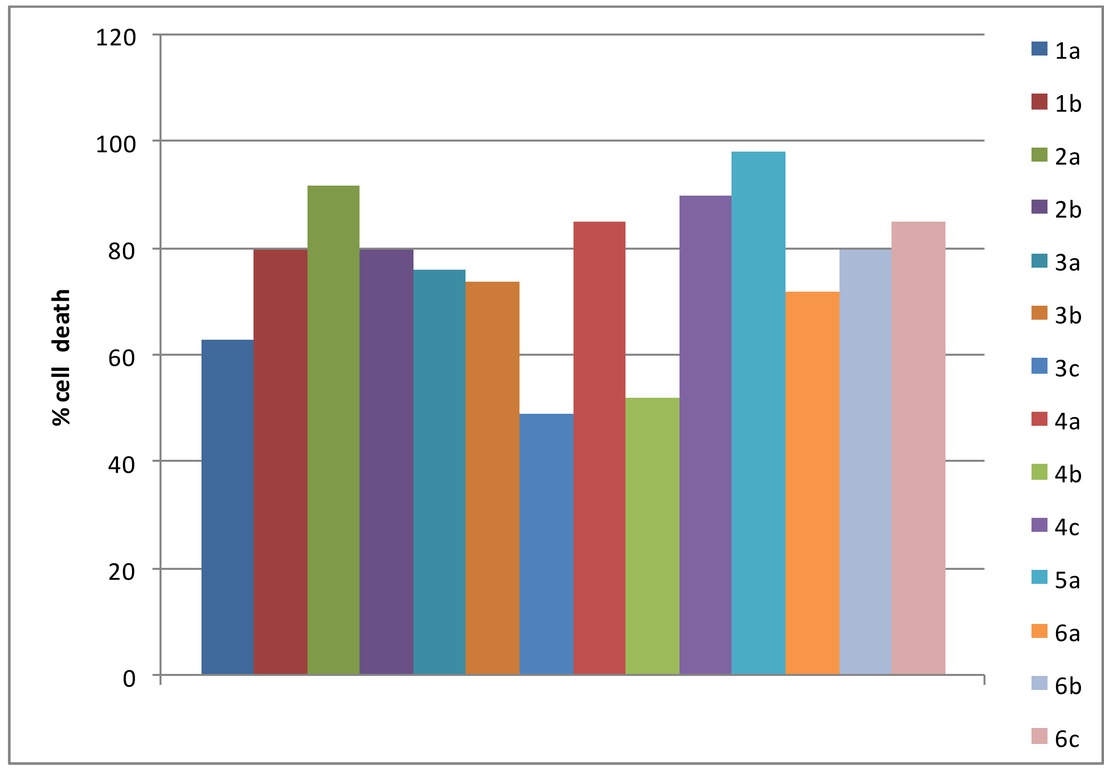


Fig.7.8 % cell death for Cu(II) complexes of ligands towards EAC at concentration 200µg/ml

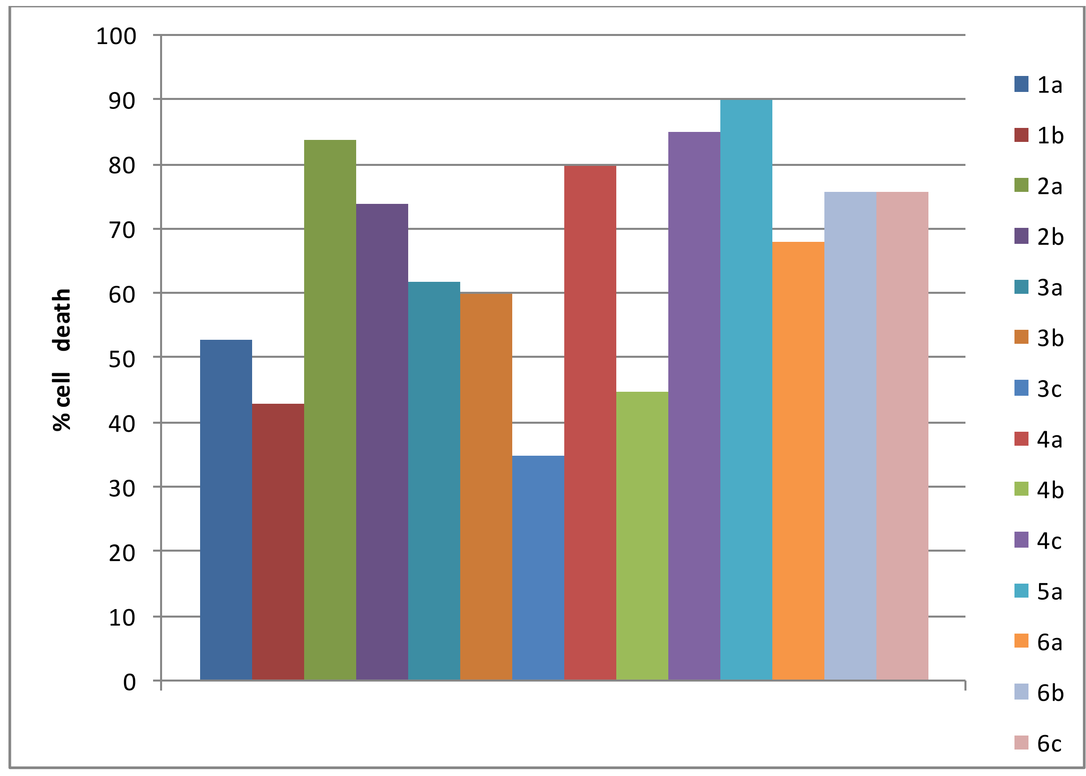


Fig.7.9 % cell death for VO(IV) complexes of ligands towards EAC at concentration 200µg/ml

In vivo antitumour studies

In vivo antitumour studies were carried with tumour bearing mice with EAC cells. Selected ligands and their metal complexes were given as drugs with concentrations 20µg/ml, 10µg/ml & 5µg/ml. The percentage increase in life span of tumour bearing mice were noted and compared with that of std. drug. Ligands which were active in invitro studies conducted were selected for in vivo studies. Maximum results were obtained with a con. 20µg/ml. Out of the ligands, 5a gave maximum results. The % ILS shown was 66.4% against 76.8% for standard drug. The % ILS shown was 54.9% for the ligand with thiophenyl ring(2a).

Metal complexes especially Cu(II) and VO(IV) gave maximum value of % ILS(Increase in life span) compared to that of ligands. The metal complexes were administered as drugs with different concentrations and the maximum result was obtained with con.20 µg/ml. The %ILS for Cu(II) complex of 5a is 78.6% and of 2a is 74.6% which is comparable with that of

std.drug.A comparative study of %ILS for ligands and their Copper complexes are given in Fig.7.10 &7.11 respectively.

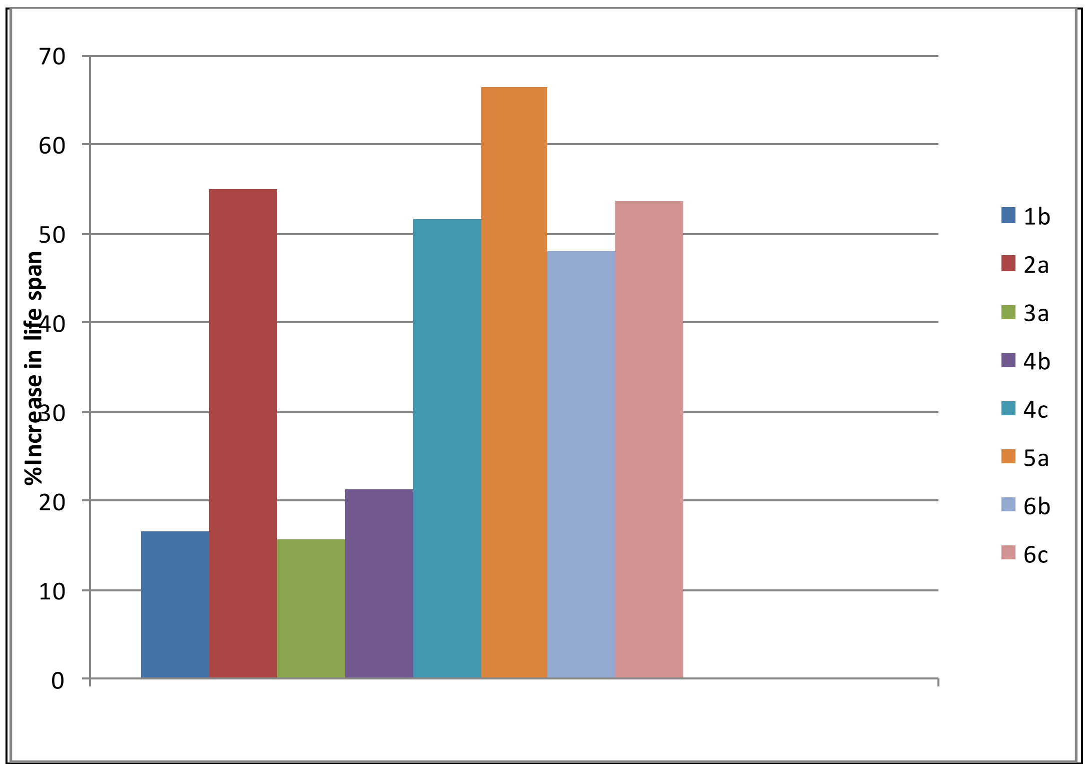


Fig.7.10 % ILS of tumour bearing mice by the administration of ligands.

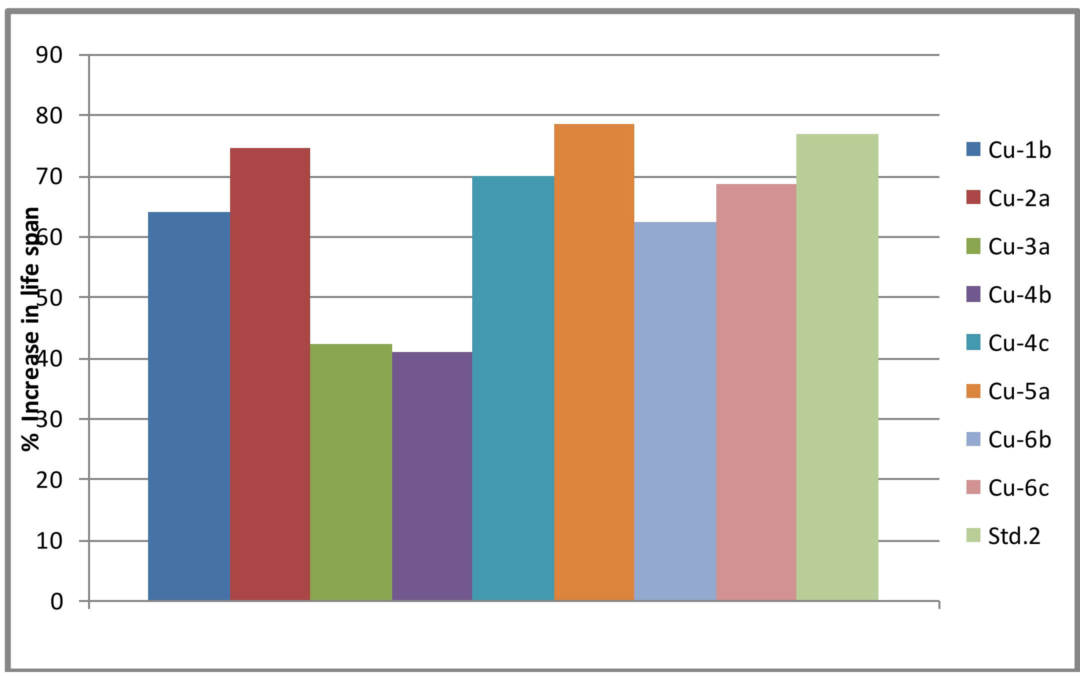


Fig.7.11 % ILS of tumour bearing mice by the administration of Cu(II) complexes of ligands.

Effect of compounds on solid tumour development

The effect of compounds on solid tumour development was studied with ligands 5a and 2a and their Cu(II) complexes .The results are comparable with the results of the invivo studies conducted in tumour bearing mice.The ligand 5a and its Cu(II) complex was very effective in reducing the volume of the solid tumor developed.The ligand treated group and metal complex treated group significantly decreased the tumour volume to 2.25 and 1.985 cm^3 .The std.drug treated mice showed the reduced tumour volume 1.982cm^3 when compared with the control group with tumor volume 5.042 cm^3 .For ligand 2a treated group the tumour volume was 3.08 cm^3 and for complex treated group the volume was 2.25 cm^3.So the ligand 2a with thiophenyl ring and its Cu(II) complex was also effective in reducing solid tumour volume.

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