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Antibacterial and Antifungal Activities of Curcuminoid Analogue with Chloro Substituted Phenyl Ring and the Transition Metal Chelates

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Abstract: ***Natural plant products have been used throughout human history as they possess biological activity that can be exploited in pharmaceutical drug design. In recent years there has been an increased enthusiasm in treating the diseases by natural products. Curcumin, the bioactive pigment in Turmeric has a huge therapeutic value. Curcumin , its analogues and metal complexes have been extensively studied for their biological activities including antimicrobial, anti-inflammatory, antioxidant, anticarcinogenic etc. Recently a number of compounds structurally related to curcuminoids were synthesized and their biological activities have been studied. In the present study, the synthesis and characterization of a curcuminoid analogue with chloro substituted phenyl ring and its metal chelates Cu(II) & Oxovanadium(IV) are discussed here. The curcuminoid analogue 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione and the metal chelates were synthesized and characterized using UV, IR, ^{1}H NMR and mass spectral data. Antifungal study of the compounds were done using Kirby Bauer method and Antibacterial study was conducted by Agar well diffusion method. The present investigation reveals that the Cu(II) complex show enhanced antibacterial activity and Vanadyl complex exhibited better antifungal activity than the curcuminoid analogue.***

Keywords: 1,7–diarylheptanoids, mass spectra,NMR, antifungal, antibacterial

1. Introduction

Curcuma longa Linn (turmeric) is a medicinal plant botanically related to Zingaberaceae family.Turmeric powder derived from the rhizome of Curcuma longa is commonly used as a spice,food preservative and food colouring agent.It also has a long history of therapeutic uses.The compounds showing yellow colour in Turmeric are three curcuminoids namely curcumin, demethoxycurcumin and bisdemethoxy curcumin . Curcumin [1, 7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], a yellow bioactive pigment is the major component of Turmeric. Curcumin has a wide spectrum of biological activities exhibiting anti inflammatory[1-5], antifungal, anticarcinogenic, antibacterial [16,17], antiprotozoal and antioxidant activities[6-9]. It has also been studied extensively as a chemo preventive agent in several cancer cells[10-15]. Structurally curcuminoids are linear 1,7-diaryl-1,6-heptadiene-3,5-diones which exist in tautomeric forms as α,β unsaturated 1,3-diketo form and enol form. Curcuminoid analogues prepared by synthesis retain the α,β unsaturated 1,3-diketo moiety but the aryl ring in natural curcumin is modified and here in the present study it is replaced with a chloro substituted phenyl ring .

Curcuminoids are expected to form metal complexes similar to other 1,3-diketones.They are powerful chelating agents[18]. Inorganic chemists have used its metal chelating abilities through the β diketo group to form new structural entities with modified biochemical activities. Complexation with transition metals has attracted much interest over the past years as one of the useful requirement to treat deadly diseases.Curcuminoids and their metal chelates possess remarkable biochemical activity[19-21,23].HereCu(II) & Oxovanadium(IV)complexes of curcuminoid analogues are synthesized and characterized.

In the present study, aldehyde namely 4-chloro benzaldehyde was condensed with acetylacetone in presence of B2O3 using tri-secondary butyl borate and n-butylamine as the condensing agent[22]. The ligand prepared [Fig.1]was complexed with Cu(II),and VO(IV) to form metal chelates[Fig.2] .The ligand and the metal complexes were also subjected to antibacterial activity against the test organisms *Escherichia coli, Klebsiella pneumoniae and Bacillus subtilis*[16,18].The synthesized compounds were also evaluated for antifungal activity.

2. Materials and Methods

The chemicals required were obtained from Sigma Aldrich chemical suppliers and are of analar grade. Fungi species namely *Aspergillus niger,Penicillium Chrysogenum and Alternaria alternata* and bacterial strains were obtained from the culture collection of Institute of Microbial Technology (IMTECH), Chandigarh, India.

2.1 Analytical instruments

UV spectra were recorded on a Schimadzu UV-VIS-1601 spectrophotometer. IR spectra (KBr pellets) were recorded on 8101 Schimadzu FTIR spectrophotometer. The ^{1}H NMR spectra were recorded on a Varian 300 NMR spectrophotometer. The FAB mass spectra were recorded on a Joel SX–102 mass spectrophotometer from CDRI, Lucknow, India.

2.2. Synthesis of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione

The curcuminoid analogue was prepared by the condensation of 4-chloro benzaldehyde 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.

Figure 1: Structure of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione

2.3 Synthesis of Metal Complexes

The Cu(II)&VO(IV) complexes were prepared respectively by adding a methanolic solution of copper(II) acetate, vanadium(IV)oxide sulphate(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 Figure below represents the structure of Cu(II) complex of the ligand.

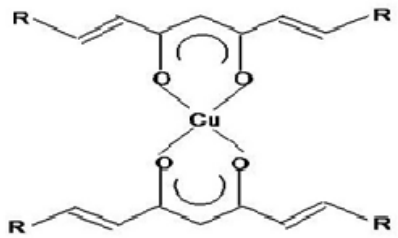

Figure 2: Structure of Cu(II) chelate of ligand.(R=4-chloro phenyl)

2.4. Characterisation of ligand and metal complexes.

The ligand prepared and the transition metal chelates with Cu(II) &VO(IV) are characterized by various spectral techniques like UV,IR, ^{1}H NMR and mass
Spectral data.

2.5 Antibacterial Activity

The antibacterial assay was performed using agar – well diffusion method with the test compounds . Agar plates were prepared using sterile agar medium . Selected bacterial strains of 24 hr culture were evenly spread on the surface of the agar plates using sterile swab sticks. Wells were cut in the plates with sterile gel puncture. The test compounds in the concentration 5 mg/ml were added in the wells. DMSO solvent served as negative control and streptomycin served as positive control . The plates were incubated at 37°C for 24 hrs and observed for zones of inhibition which was then measured and the activity was expressed in terms of the mean diameter of the zone of inhibition in millimeters.

2.6. Antifungal activity (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.The acivity was compared with that of a std.drug flucanazole.

3. Results

3.1. Structural characterization of synthesized ligand

The curcuminoid analogue namely 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione(L1) was synthesized and characterized. Analytical and UV spectral data are given in **TABLE 1**.The IR spectral data of the compound is given in **TABLE 2.**The IR spectrum of the ligand is represented in **Fig.3**.The NMR spectral details are given in **TABLE 3** and the spectrum is given in **Fig.4.**The Mass spectral details are given in **TABLE 4** and the mass spectrum is given in **Fig.5.**The ligand has been characterized by various spectral techniques like UV,IR,^{1}H NMR& Mass spectrum.

Table 1: Analytical & UV Spectral data of 1,7-bis(4-chloro phenyl) hepta-1,6-diene-3,5-dione (L1)

Ligand	Elemental analysis (%)		Molecular weight	UV λmax (nm)
	C	H		
	Found/(Calculated)			
L1	65.31 (66.07)	3.72 (4.05)	342	225, 307

Table 2: IR spectral data of chloro substituted 1,7-diaryl heptanoids

Compound L1	Probable IR assignments
1639	V(C=O) chelated
1593	V(C=C) phenyl
1513	V(C-C) alkenyl
1489	V_{as}(C-C-C) chelate ring
1412	V_s(C-C-C) chelate ring
1091,1014	β(C-H) chelate ring
983	V(CH=CH) trans

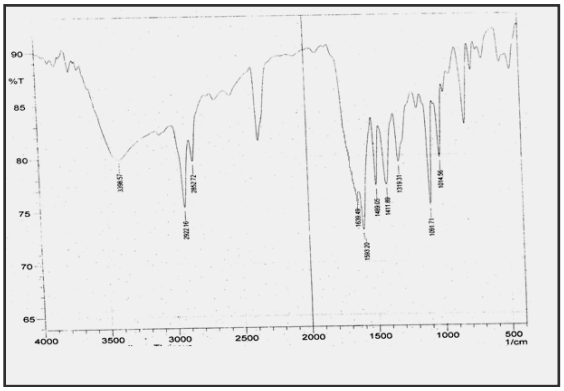

Figure 3: IR spectrum of 1,7-bis(4-chloro phenyl)hepta-1,6-diene-3,5-dione(L1)

Table 3: ^{1}HNMR spectral data of chloro substituted 1,7-diaryl heptanoids

Ligand	Chemical shifts (δ ppm)			
	Enolic	Methine	Alkenyl	Phenyl
L1	16.024	5.82	6.57-8.04	7.3-7.63

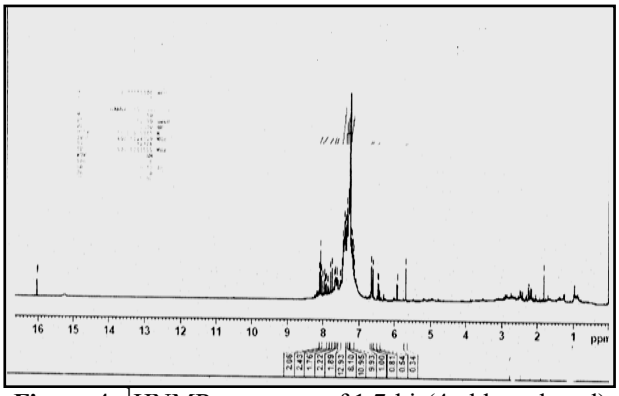

Figure 4: ^{1}HNMR spectrum of 1,7-bis(4-chlorophenyl) hepta-1,6-diene-3,5-dione(L1)

Table 4: Mass spectral data of of 1,7-bis(4-chlorophenyl) hepta-1,6-diene-3,5-dione(L1)

Ligand	*M+ /M+1 ion*	*Fragment ions*
L1	342	175, 169 ,125 ,140 ,115, 141, 230 ,204

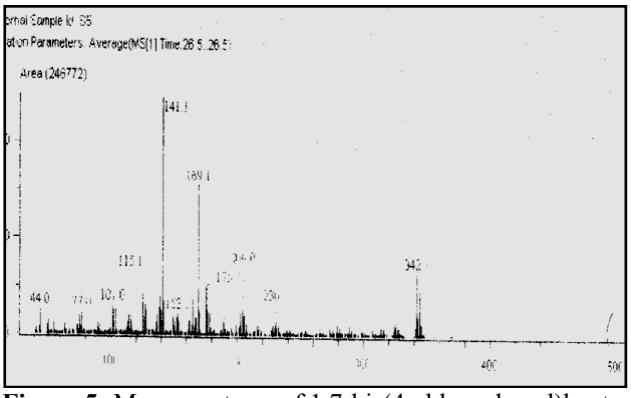

Figure 5: Mass spectrum of 1,7-bis(4-chlorophenyl)hepta-1,6-diene-3,5-dione(L1)

3.2. Structural characterization of metal complexes with Cu(II)&VO(IV)ions

The ligand L1 form well defined crystalline complexes with Cu(II) and VO(IV) ions. UV and IR spectral data of synthesized metal complexes are given in **Table 5.** The approximate formulae of the metal complexes has been found to be as expected ie two ligands are coordinated with the metal ion.(ML2).

Table5: Spectral data of Cu(II) & Oxovanadium(IV)complexes of ligands

Metal chelates	UV Λmax (nm)	Characteristic IR stretching bands (cm^{-1})		
		(C=O)	(C-C-C)	(M-O)
Cu(II)	232,312	1592	1495	465,420
VO(IV)	237,315	1598	1511	463,420

Table 6: Mass spectral data of metal complexes

Metal Chelates	M+ (Molecular ion)	Fragment ions
Cu(II)	749	527 ,408,121,307,185,345,223
VO(IV)	755	532,411,121,309,188,345,223

3.3. Antibacterial activity

The results of antibacterial activity of curcuminoid analogues and their metal complexes are represented in **Table 7**.All the compounds were taken in the concentration 5mg/ml in DMSO.Results are compared with a std.drug.The activity is expressed as diameter of zone of inhibition in mm.

Table 7: Shows antibacterial activity for curcuminoid analogue and Cu(II)&VO(IV) chelates

Bacteria	Diameter zone of inhibition in mm		
	L_1	$Cu(L_1)_2$	$VO(L_1)_2$
E Coli	13.5	17.5	15
Klebsiella	11	15	13
Bacillus	4.5	8.5	7
Standard	20	20	20

3.4. Antifungal Activity

The results of the antifungal activity of **1,7-bis(4-chloro phenyl)hepta-1,6-diene-3,5-dione(L1)** and its complexes with VO(IV)are given in **Table 8.**For comparison the diameter of zone of inhibition in mm given by the standard.drug has also been included .The diameter of zone of inhibition in mm observed for the standard drug is 21mm.

Table 8: Antifungal studies of 1,7-bis(4-chlorophenyl) hepta-1,6-diene-3,5-dione (L_1) and its Cu(II) & Vanadyl complexes

Fungi	Diameter of zone of inhibition in mm					
	L_1 (µg)			$VO(L_1)_2$ (µg)		
	100	250	500	100	250	500
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

4. Discussion

4.1 Characterization of chloro substituted analogue of 1,7-diaryl-1,6-heptadiene-3,5-diones

The synthesized chloro substituted derivative of 1,7-diaryl heptanoids were characterized by various analytical techniques . The UV spectra of the compound in methanol show two absorption maxima corresponding to n→ π* and π→ π* transitions The UV spectra of the compound L1 in methanol show two absorption maxima at 307nm & 225nm respectively due to the n→ $\pi*$ and $\pi \rightarrow \pi*$ transitions.

IR spectra of L1 is characterized by the presence of a strong band at 1639 cm^{-1} due to the enolised conjugated C=O group. The C=O frequency decreases from the value of the free C=O group due to hydrogen bonding and increased conjugation. 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 1570-1400 cm^{-1} due to various stretching vibrations of the phenyl group, alkenyl & chelate ring. The band in the region 983 cm^{-1} is assigned to the trans CH=CH vibration.

The ^{1}H NMR spectra of chloro substituted 1,7-diaryl heptanoids also supports the enolic structure of the compound.The peaks corresponding to enolic, methine, alkenyl and phenyl groups can be observed in the spectrum.. Ligand **L1** 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 aryl protons show signals in the region 7.3 – 7.6ppm and the alkenyl protons show signals in the region of 6.5 – 8.0ppm.

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 substance. The mass spectra of compound showed intense molecular ion peak. Elimination of important groups like CH2,C2H2,C2H2O,CH2=C=O from the molecule gives different fragments.Important fragment ion peaks that appeared in the spectra of compound can be conveniently accounted by the fragmentation pattern . The M+ ion of L1 is observed at 342.The remaining important peaks are that due to the fragment ions.

4.2 Characterization of metal complexes

Analytical and mass spectral data clearly suggests a stochiometry ML2 for complexes. The UV absorption bands of the ligand were almost unaffected by complexation with metal ions. The spectra of complexes closely resembles the spectra of respective ligand. In the IR spectra of metal chelates,additional bands appear at ~ 475 cm^{-1} and~420cm^{-1}assignable to ν (M–O) vibrations. 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 ~ 1622 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.

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. It was found that some fragments rearrange to form stable cyclic species . The mass spectral analysis shows that stepwise removal of aryl groups is a characteristic feature of all the complexes. Smaller molecules like O, OH, CH etc. are also eliminated.Peaks due to $[ML_2]+$,L+ and fragments of L+ are also detected in the spectrum.

4.3 Antibacterial activity

The results reveals that the curcuminoid analogue with chloro substituted phenyl ring was more active against E.Coli species producing a zone of inhibition of 13.5mm.The ligand was less active against Bacillus species.Both the metal complexes possessed better antibacterial activity than that of ligands.Cu(II) complexes show maximum activity against all bacterial strains. The Cu(II) complexes of the ligand exhibited significant antibacterial effect to E.Coli &Klebsiella species producing a zone of inhibition of 17.5 mm and 15 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.

4.4 Antifungal activity

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. 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 VO(IV) complex of the ligand is a potent antifungal compound.

5. Acknowledgement

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