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Synthesis, Characterization and Biochemical assessment of 1,7 diphenyl heptanoids

Lovely Jacob A[1], Tom Cherian[2], Jesy E J [3], Seena Thomachan[4], Deepthy Varghese[5]

[1]Assistant Professor, Department of Chemistry, Little Flower College, Guruvayoor

[2] Assistant Professor, Christ College, Irinjalakkuda, University of Calicut

[3]Little Flower College, Guruvayoor

[4]SRGPTC,Thriprayar,University of Calicut

[5]Christ College, Irinjalakkuda, University of Calicut

Abstract: In recent years, there has been a surge in interest in using natural products to cure diseases. Turmeric's bioactive pigment, curcumin, has a wide range of applications in this sense. Curcumin, its analogues, and metal complexes have all been investigated extensively for biological activities such as antimicrobial, anti-inflammatory, antioxidant, and anticarcinogenic. A variety of compounds with structural similarities to curcuminoids have recently been synthesised, and their biological activities have been investigated. The synthesis and characterization of curcuminoid analogues with non substituted phenyl rings, dimethoxy substituted phenyl rings, and 1,2,3 trihydroxy substituted phenyl rings were discussed in this article. IR, ^{13}C NMR, ^{1}H NMR, and mass spectrum are used to characterise the samples. In vitro cytotoxic experiments against DLA cells (Daltons Lymphoma Ascites) were carried out using the Trypan blue exclusion process, and antimicrobial studies were carried out using the Kirby Bauer method. Antioxidant tests have also been conducted.

Keywords: Curcumin analogues, antioxidant, antimicrobial, cytotoxicity, 1,7 diphenyl heptanoids

1. INTRODUCTION

Turmeric contains curcuminoids, a bioactive yellow-orange pigment found in the plant's rhizomes (Curcuma longa Linn). Turmeric's anti-inflammatory, antifungal, antimicrobial, and antitumor properties have been used in Ayurvedic medicine for centuries. Synthetic curcuminoids have also been shown to be effective as chemopreventive agents [1].(Azuine MA et al 1992). Curcuminoids are linear diarylheptanoids with tautomeric forms such as the unsaturated 1,3-diketo form and the enol form. Curcumin has numerous biological effects, including anti-inflammatory[2] (Holt PR.et al 2005), antioxidant [3](Iqbal M et al 2003)anticarcinogenic [4](R Wilken et al 2011), and antibacterial properties[5] (N.Raman et al 2003). Curcuminoids and metal chelates of curcuminoids have a lot of biological actions (Krishnankutty K et al 1998 and Pabon HJJ 1964). Curcuminoid analogues synthesised in this study kept the unsaturated 1,3-diketo moiety, but the aryl ring in genuine curcumin is changed[6,7](Sreejayan N et al 1994 and Subramanian M et al 1994).

2. MATERIALS AND METHODS

The Amala Cancer Research Centre in Keraka, India, provided Daltons Lymphoma Ascites (DLA). Bacterial stains, including Escherichia coli, Staphylococcus aureus, and fungal cultures, were obtained from the Institute of Microbial Technology's Culture Collection in Chandigarh, India.

2.1 Preparation and Characterization of Curcumine analogues

Aldehydes (benzaldehyde, 3,4-dimethoxy benzaldehyde, and 2,3,4 tri hydroxy Benzaldehyde) were combined with an acetylacetone-boric oxide complex in an ethyl acetate medium with tributyl borate and n-butyl amine to produce curcuminoid analogues. To acquire pure crystalline content, the products were purified using a 5:1 (v/v) chloroform:acetone mixture as the eluent in column chromatography over silica gel (60–120 mesh) and recrystallized twice from hot benzene.[8]

IR, ^{13}C NMR, ^{1}H NMR, and mass spectrum methods are used to characterise the ligands and their metal coplexes.The structures of the synthesized analogues are shown in Table 1.

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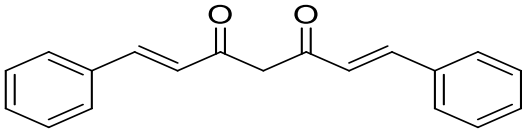
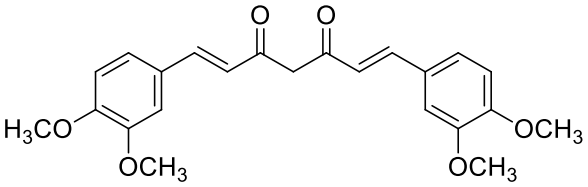
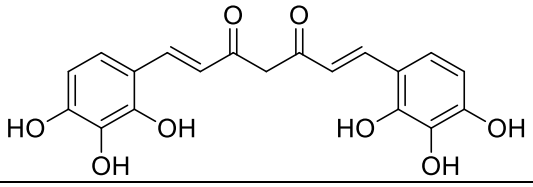
Table 1.Name and Structure of compounds

Sample Code	Copound (Systematic Name)	Structure
L_1	(1E,6E)-1,7-diphenylhepta-1,6-diene-3,5-dione	
L_2	(1E,6E)-1,7-bis(3,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione	
L_3	(1E,6E)-1,7-bis(2,3,4-trihydroxyphenyl)hepta-1,6-diene-3,5-dione	

2.2 Antioxidant assay

Here used Ferric reducing power (FRAP) Assay. This process is based on the reduction of ferric (Fe3+) to ferrous (Fe2+) in the presence of antioxidants. Potassium ferricyanide forms potassium ferrocyanide, which subsequently combines with FeCl3 to generate an extreme prussian blue complex with a maximum absorbance of 700 nm [9].

2.3 Antimicrobial Studies

The antimicrobial test was performed using a modified Kirby Bauer method. The microbiological cultures (Escherichia coli, Staphylococcus aureus, and Saccharomyces cerevisiae) were kept alive using Sabouraud's Dextrose broth. Each culture was equally dispersed on SDA plates using sterile swabs. 3mm diameter filter paper discs were put at a distance of 2cm on the surface of SD agar plates using sterile forceps. The chemical was dissolved in 2% DMSO, which exhibited no deleterious effects on the microbial cultures. AMP (5mg/ml) was the positive regulator. The plates were then incubated at room temperature for 2-3 days. After incubation, the diameter of the zone was measured in millimeters.

2.4 In vitro cytotoxicity studies against DLA cancer cell

Using the trypan blue exclusion method, in vitro cytotoxic investigations on the curcumin analogues and metal chelates were performed (A Fadda et al 2010).

For in vitro cytotoxicity testing, the samples were dissolved in a tiny amount of DMSO. Tumor cells were collected from tumor-bearing animals' peritoneal cavity and centrifuged in PBS for 15 minutes at 1500 rpm. The trypan blue exclusion method was used to assess the cells' viability. Viable cells (1106 cells in 0.1 ml) were added to tubes containing various concentrations of the test chemicals, and the volume was then raised to 1 ml using PBS. The cell suspension is the sole item in the control tube. These combinations were incubated for 3 hours at 37°C. After that, the cell suspension was mixed with 0.1ml of 1% trypan blue for 2-3 minutes before being fed into a haemocytometer.The percentage cytotoxicity was determined using the trypan blue exclusion process, which counted the number of stained (dead) and unstained (live) cells. % Cytotoxicity=(No:of dead cells/No:of dead cells+No:of live cells)x100

3. RESULTS AND DISCUSSION

3.1.Spectral Details of Curcumine analogues

The curcuminoid analogues (L1,L2, and L3) produced were crystalline in nature with acute melting points. Different spectrum approaches, including as IR, 1H NMR, 13C NMR, and mass spectra, were used to characterize them.

3.1.1 IR Data

The importance of IR spectra in establishing the keto-enol tautomers of β- diketones has been well established. The IR spectral data of compounds L1,L2 and L3 are given in Table 2.

The appearance of prominent bands at 1622,1620 and 1630cm-1 in the IR spectra of curcuminoid analogues are attributable to the enolised conjugated 1,3- diketo group.Usually free carbonyl group gives stretching frequency at

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~1710cm-1.There is no peak in that region indicating that the C = O group is not in the keto form but in the enol form. The shift is a result of internal hydrogen bnding.The resonance also contributes to the lowering of carbonyl group frequency in the enol form. A weak broad O-H stretch is observed for the enol form at 3200-2400cm^{-1}.Other IR peaks due to ν(C-C)Alkenyl, ν $_{as}$(C-C-C)Chelate ring, ν $_{s}$(C-C-C)Chelate ring,and ν 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 968,958 and 975 respectively for L1,L2 and L3.

Table 2. IR Data

L1	L2	L3	Probable IR Assaignments
3040	2929	2927	ν Enolic
1622	1620	1630	ν (C=O)Chelated
1581	1585	1594	ν (C=C)Phenyl
1512	1507	1507	ν (C-C)Alkenyl
1456	1466	1456	ν $_{as}$(C-C-C)Chelate ring
1426	1423	1426	ν $_{s}$(C-C-C)Chelate ring
1145	1121	1157	ν β(C-H)Chelate ring
968	958	975	ν (CH=CH)trans

3.1.2 ^{1}H NMR and Mass Spectral Data

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 the clue regarding the type of proton originating the signal. The derivatives of different 1,7 diphenyl heptanoids show specific peaks corresponding to enolic,methine,and alkenyl protons(Table 3).The compounds L1, L2 and L3 displayed a one proton singlet at ~10ppm assignable to strong intra molecularly hydrogen bonded enolic proton. Another one proton singlet at ~6.7 ppm corresponds to the strong intra molecularly hydrogen bonded methine proton.

The mass spectra also give an idea about the various fragmentation modes of the substance. The mass spectra of the compound showed an intense molecular ion peak. Elimination of important groups like CH_2, C_2H_2, C_2H_2O, CH_2=C=O from the molecule gives different fragments. Important fragment ion peaks that appeared in the spectra of the compound can be conveniently accounted for by the fragmentation pattern. The **^{1}H NMR** and mass spectral data of the ligands are given in Table 3.

Table 3. ^{1}H NMR and Mass Spectral Data

Ligands	Chemical shift(δ) in ppm					Mass spectral data (m/z)
	Enolic	Methine	Alkenyl	Phenyl	Substituent	
L1	10.014	6.78	6.98-7.79	7.31-7.60	---	276,199,173,145,131,103,90,77
L2	9.84	6.75	6.81-7.61	7.06-7.14	3.85 (methoxy)	396,259,233,205,191,137,163
L3	10.24	6.84	6.46-8.72	6.38-7.07	8.72-10.66 (Hydroxy)	372,247,221,193,179,151,125

3.1.3 ^{13}C NMR data

The ^{13}C NMR spectral data of (1E,6E)-1,7-diphenylhepta-1,6-diene-3,5-dione(L1), (1E,6E)-1,7-bis(3,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione(L2) and (1E,6E)-1,7-bis(2,3,4-trihydroxyphenyl)hepta-1,6-diene-3,5-dione(L3) are given in Table 4, Table 5 and Table 6.

The peak corresponding to methine carbon(C1) of L1,L2 and L3are present at positions 98.63ppm, 101.38ppm and 108.9ppm respectively.Here also there is a possibility of keto-enol tautomerism which makes the shift of C1 carbon to ~100ppm.The C2and C2' carbons of carbonyl of all the ligands are appeared at a position ~200ppm.The alkenyl carbons are present nearer to the phenyl ring system.Out of the two alkenyl carbons,C3/ **C3'** are down shielded and are presented at ~130ppm.The aromatic carbon atoms are present between ~110-150ppm. The methoxy carbons C11/C12 in the aryl rings of L2 are down shielded and present at a position ~55ppm.

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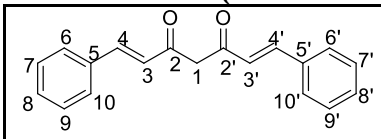
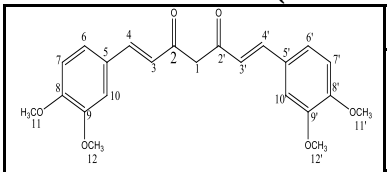
Table 4. ^{13}C NMR data of L1(Chemical shift(δ) in ppm)



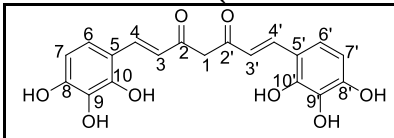
C1	C2,C2'	C3,C	C4,C4'	C5,C5'
98.63	200.99	130.26	130.26	133.76
C6,C6'	**C7,C7'**	**C8,C8'**	**C9,C9'**	**C10,C10'**
128.57	128.57	128.57	128.57	128.57

Table 5. ^{13}C NMR data of L2(Chemical shift(δ) in ppm)



C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'	C6,C6'
101.38	191.06	128.75	140.51	122.74	122.11
C7,C7'	**C8,C8'**	**C9,C9'**	**C10,C10'**	**C11,C11'**	**C12,C12'**
111.21	149.31	151.13	109.81	56.07	55.99

Table 6. ^{13}C NMR data of L3(Chemical shift(δ) in ppm)



C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'
108.9	193.66	132.76	151.51	116.01
C6,C6'	**C7,C7'**	**C8,C8'**	**C9,C9'**	**C10,C10'**
124.26	108.9	153.81	132.76	151.51

3.2 Antioxidant Activity

The capacity to reduce the oxidation potential of oxidants is determined using the reducing power method for determining antioxidant activity. The hydrogen donating capacities of the samples account for their reducing power. Ascorbic acid was utilized as the standard in this test. The reaction mixture's increased absorbance showed an increase in reducing power. Ligands (L1,L2,&L3) had antioxidant activity comparable to that of conventional ascorbic acid Fig.1.

The ability of an antioxidant to trap free radicals is its most important feature. Antioxidant compounds including phenolic acids, polyphenols, and flavonoids scavenge free radicals like peroxide, hydroperoxide, and lipid peroxyl, blocking the oxidative pathways that cause degenerative diseases. As a result, our phenolic-group-containing curcumin mimics have therapeutic efficacy.

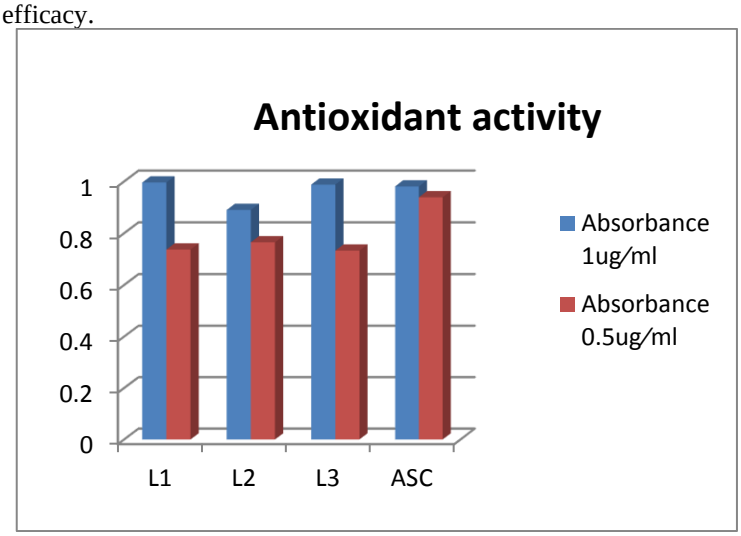


Fig.1. Antioxidant Activity

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3.3 Antimicrobial Activity

The result of the antimicrobial study revealed that all the compounds have potent antimicrobial activities.Here we used gram negative and positive bacteriae(Escherichia coli, Staphylococcus aureus)and fungal culture(Saccharomyces cerevisiae). ,L1,L2and L3 showed comparable results towards antibacterial property.Among those L3 possessed the hieghest activity.But the atifungal property of L3 is very low compared to the others.

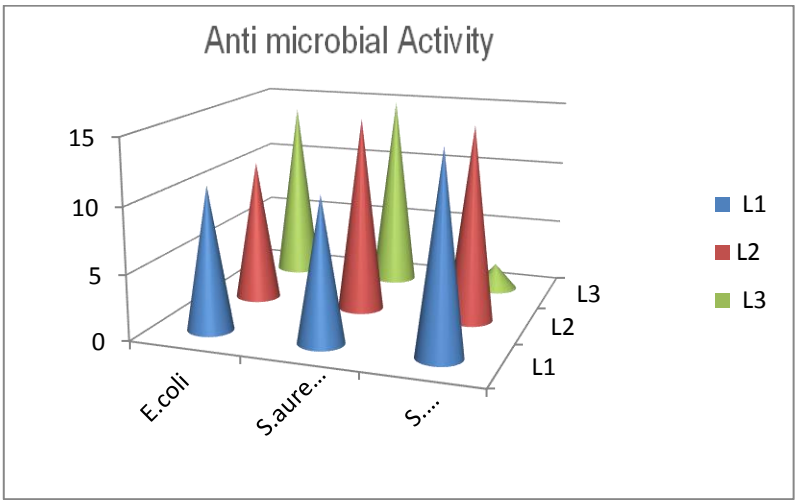

Fig. 2. Antimicrobial Activity

3.4 Invitrocytotoxic Activity

According to a SAR study of curcumin derivatives, the presence of a coplanar hydrogen donor group and a -diketone moiety is essential for antiandrogenic activity in the treatment of prostate cancer[10]. Furthermore, it has been demonstrated to have a potent antiproliferative effect on estrogen-dependent breast cancer cells[11]. In vitro cytotoxicity tests on DLA cells found that at higher doses, such as 200g/ml, ligands caused more cell death. As the concentration of the medicinal ingredient rises, so does the percentage of cells that die. Among the three analogues, L1 and L3 showed significant invitro cytotoxic effect.

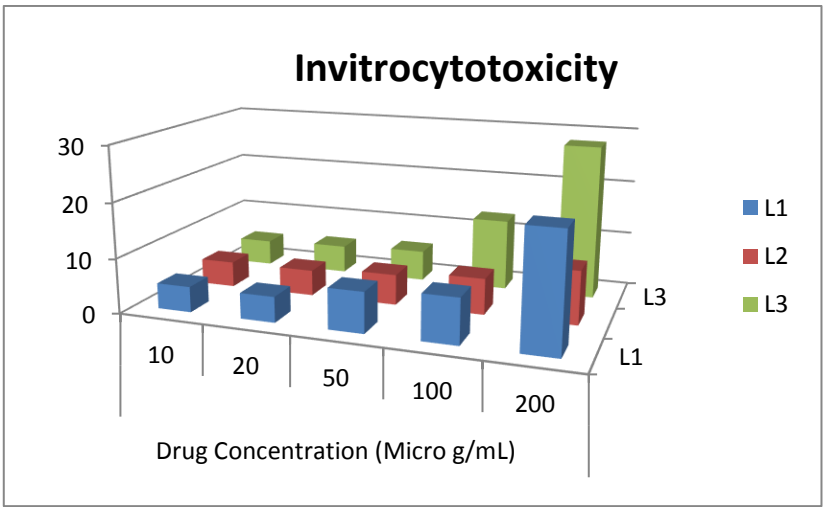

Fig.3. Invitrocytotoxicity

CONCLUSION

The ligands (1E,6E)-1,7-diphenylhepta-1,6-diene-3,5-dione(L1), (1E,6E)-1,7-bis(3,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione(L2)and(1E,6E)-1,7-bis(2,3,4-trihydroxyphenyl)hepta-1,6-diene-3,5-dione were synthesized and characterized by various spectral techniques.

The reason for the enhanced biological activity of curcuminoids are known to be the hydrogen donor group, the β-diketone moiety, the phenyl rings, and the substituent groups on them. Chemical modifications of these moieties has lead to curcumin derivatives with higher efficacy and/or enhanced water solubility or stability. The present study

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revealed that the synthesized curcumine analogues possessed enhanced antioxidant and cytotoxic activity. The antimicrobial studies clearly showed the significant antimicrobial properties of the analogues.

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