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Spectral and Biological investigations of Nd(III) Juglonates

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Abstract: *Metal chelates of Nd(III) with Lawsone , juglone, Phthiocol and Plumbagin are synthesized and are characterized with the help of elemental analysis, Infrared spectroscopy, UV visible spectrosco and TGA. Morphological changes as a result of chelation are studied from their SEM photographs. The ligands and metal chelates are screened against four bacterial strains; B. subtilis, S. aureus, E. coli and P. aeruginosa. All the properties of chelates investigated are compared to explore effect of chelation and ring isomerism.*

Keywords: *Nd(III) chelates, Juglones, spectroscopic properties, antimicrobial activity*

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I. INTRODUCTION

A group of ligands consisted of 1,4 hydroxy naphthoquinones is known as Juglones. Juglones are powerful donors and undergo chelation forming five or six membered rings with a number of metals in the periodic table. They exhibit attractive analytical, biological properties in a variety of fields. Antimicrobial and anticancer properties of these ligands are well known[1-5]. Lawsone(LW), Juglone(JU), Phthiocol(PH) and Plumbagin(PL) are important members of Juglones. Fig-1

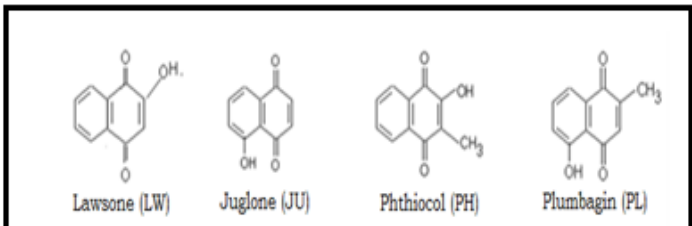

Fig-1 Important members of 1,4 hydroxy naphthoquinones i.e juglones

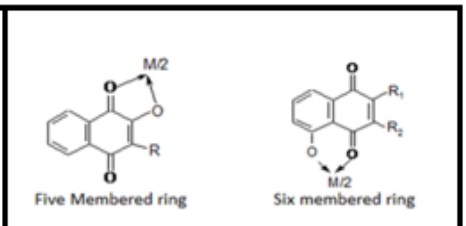

Fig-2 Ring Isomerism in Metal Juglonates

Due to difference in position of –OH group on napthoquinone ring lawsone and phthiocol can form five membered ring while juglone and plumbagin can form six membered ring chelates with metals Fig-2, resulting in isomeric pairs of metal chelates. Difference in ring size gives rise to Ring isomerism in these pairs of chelates.Therefore comparative structural and biological study of isomeric metal chelates of juglones is an interesting aspect of juglone chemistry.

We have reported the structural and biological investigations on Samarium, Erbium and Terbium juglonates previously [6-9].The present studies on nyodymium juglonates is a continuation of these studies.

II. MATERIALS AND METHOD

Synthesis of ligands:

The ligand Lawsone and Plumbagin which are available commercially, were purchased from Aldrich and Hi media respectively. The Juglone and Phthiocol were prepared by using standard procedures reported by Radt [10] and Fiser [11] respectively.

Synthesis of Chelates:

The metal chelates were precipitated by mixing the methanolic solution of the ligand and aqueous solution of Lanthanide metal salt in distilled water which are taken in the ratio 3:1 proportion. The mixture was then refluxed for 45 min at 60̊ C. Then pH of the mixture was adjusted between 5 to 6 by 10 % aq. ammonia solution and refluxation was continued for 3 hours. After cooling the precipitate was kept in refrigerator overnight and the product was filtered and it was dried in vacuum desiccators.

II. INSTRUMENTAL TECHNIQUES

The ligands and the metal chelates were subjected to microanalysis for finding out the percentage of Carbon, Hydrogen and residue (as metal oxide) using Thermo Finnigan CHNS and O analyzer.The thermal behavior of the chelates was studied in the temperature range RT to 10000C using simultaneous thermal analyzer (Shimadzu DTG-60) in nitrogen atmosphere. The SEM photographs were obtained using scanning electron microscope JEOL-3SM-5200. The IR spectra were recorded in the region 4000-450 cm-1 on Thermo Scientific (Nicolet) spectrophotometer. The UV-Visible spectra were recorded in solid state (1mg sample in 100 mg KBr) making pellet, on U.V-300 double spectrophotometer.

IV. RESULTS AND DISCUSSION

Table-1 Elemental analysis of Juglones and its Nd(III) Chelates

Sr. No.	Compounds. Ligands/	Empirical Formula of Complexes	% C		% H		% O		% Metal	
			Cal.	Expt.	Cal.	Expt.	Cal.	Expt.	Cal.	Expt.
1.	LW	C10H6O3	68.96	68.17	3.472	3.77	27.56	27.46	-	-
2.	Nd-LW	C30H19O11	51.48	47.363	2.71	2.191	25.17	25.08	20.62	20.11
3.	JU	C10H6O3	68.96	71.99	3.47	3.23	27.56	27.86	-	-
4.	Nd-JU	C30H19O11	51.48	46.323	2.71	2.145	25.17	24.10	20.62	19.45
5.	PH	C11H8O3	70.20	73.26	4.28	4.03	25.50	25.55	-	-
6.	Nd-PH	C33H25O11	53.42	57.104	3.37	2.819	23.47	23.17	19.45	19.17
7.	PL	C11H8O3	70.20	70.87	4.28	4.74	25.50	25.01	-	-
8.	Nd-PL	C33H25O11	53.42	58.841	3.37	2.749	23.47	22.99	19.45	18.89

From the percentage of carbon, hydrogen and oxygen, (Table-1) chemical composition of the complexes is determined which is $[ML_3.2H_2O].nH_2O$.

V. THERMAL STUDY

Thermal decomposition curves of the chelates are recorded in nitrogen atmosphere and show a two to three step weight loss pattern (Fig-3).

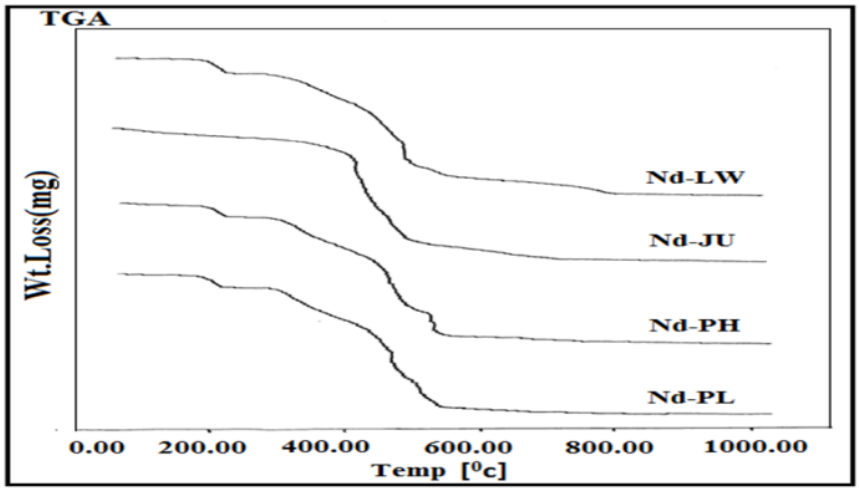

Fig-3 Thermograms of Isomeric Juglone Chelates Nd (III)

From the initial weight loss in the thermograms, the number of water molecules associated with the metal chelates were determined. All the chelates consists of two coordinated water molecules and one or two lattice water molecules[6]. This observation is consistent with the results of microanalysis. In general, the second to fourth stages of decomposition gives partial weight loss of the three ligands to give residue. The decomposition of Nd(III) lawsonate and Nd(III) phthicolate takes place at higher temperatures than their respective isomers Nd(III) juglonate and Nd(III) plumbaginats.

VI. INFRARED SPECTROSCOPY

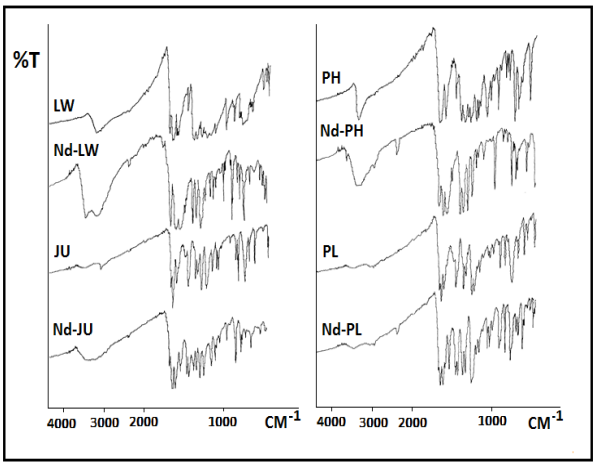

Fig-4 IR Spectra of Ligands and their Chelates

Table-2 Significant IR Frequencies of Isomeric Juglones and its Chelates

Sr.No	Compound	ν (OH)(cm-1)	ν (C=O) (cm-1)		ν (C-O) (cm1)	-CH3(cm-1)
			Chelated	Free		
1)	LW	3170	1592	1678	1214	---
2)	Nd-LW	3446	1567	1656	1218	---
3)	JU	---	1643	1664	1225	---
4)	Nd-JU	3433	1577	1633	1291	---
5)	PH	3325	1590	1656	1208	2942
6)	Nd-PH	3563	1570	1640	1288	2922
7)	PL	---	1644	1663	1230	2965
8)	Nd-PL	3465	1609	1643	1253	2924

The IR spectra are presented in Fig-4 and significant IR frequencies are listed in Table-2. The ligands lawsone and phthiocol exhibit hydroxyl frequencies at 3170cm-1and 3325cm-1 [12]which are not exhibited by juglone and phthiocol as their hydroxyl groups are engaged in intramolecular hydrogen bonding. The two frequencies which are due to C=O and C-O are important frequencies which are involved in coordination to central metal ion ie Nd(III).In all four ligands two C=O groups are present which are not equivalent. The C=O which is present next to OH group is called as chelated C=O because it further gets involved in coordinating the metal.The other carbonyl group is called as free C=O. The other frequency which takes part in coordination is due to C-O bond which is involved in bonding after deprotonation.

After chelation with ligands the chelated C=O frequency is shifted to lower wavenumbers indicating weakening of the bond due to donation of electron density to metal ion. The C-O frequency show shifting toward higer wavenumbers which is again a consequence of chelation and delocalization of electron density in the napthoquinone moity. The free C-O frequency also decreases as it might have involved in the delocalization. Therefore shifting of these frequencies confirms the donation of electron density is through O from –OH and O from chelated C=O [7].

VII. ELECTRONIC SPECTROSCOPY

Sr. No	Compound	Principle band wavelength			
		BET λ nm.	QET λ nm.	n→π*/L→M/ f-f transition λ nm	Shifting of third band
1)	LW	260	343	394	--
2)	Nd-LW	265	343	485	91
3)	JU	247	367	425	--
4)	Nd-JU	247	343	515	90
5)	PH	265	352	418	--
6)	Nd-PH	340	350	522	104
7)	PL	248	363	407	--
8)	Nd-PL	248	342	461	54

Table-3 Significant Electronic Spectral Bands of Isomeric Juglones and its Chelates

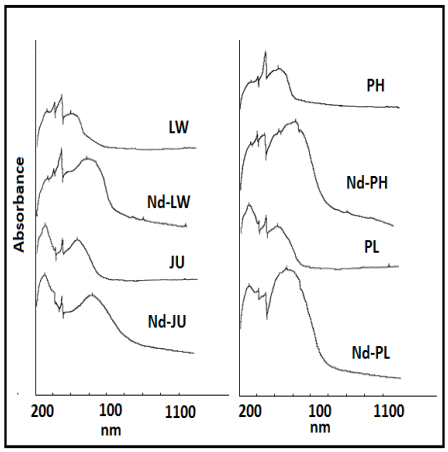

Fig-5 Electronic Spectra of Ligands andtheir Chelates

The electronic spectra of the juglones are normally interpreted in terms of BET(benzenoid electron transfer), QET(Quinonoid electron transfer) and n→π*transitions[13].The spectra and principle bands observed are shown in Fig-5 and Table-3 respectively. When the coordinating molecules are powerful donors, there is a possibility of ligand to metal charge transfer transitions in the metal chelate. Since the metal belongs to f block, f-f transitions may take place along with these two transitions. In the all ligands the last band is attributable to n→π* transitions. A large shifting of the last band is observed after chelation. This shifting is a additive effect of three bands which are n→π*, L→M charge transfer and f-f transitions in the metal chelates.

VIII. SCANNING ELECTRON MICROSCOPY (SEM)

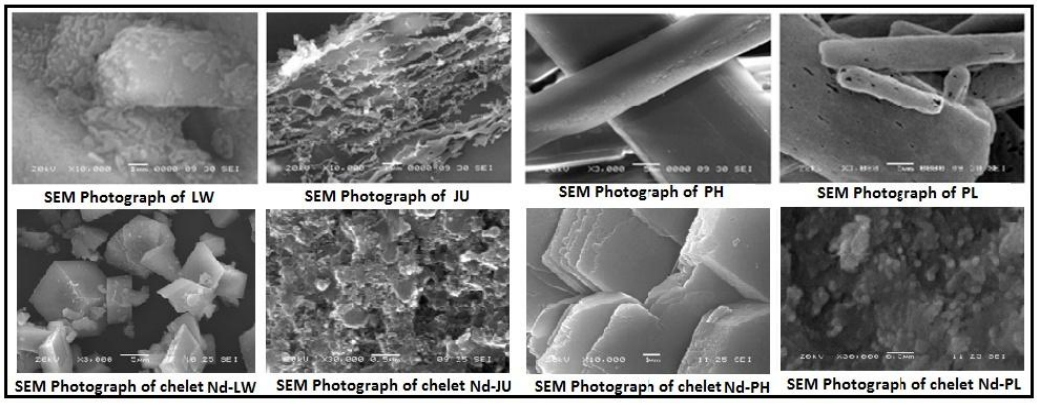

Fig-6 Scanning Electron Microscopy of Ligands and their Nd(III) Chelates

SEM photographs of ligands and Nd(III)juglonates provide information about the shape and size of the particles. Distinct change in the morphology of ligands after chelation with Nd(III) is observed (Fig-6). The photographs are also useful to compare the shape of isomeric metal chelates. Particles of juglone are in form of big lumps which are covered by small particles while particles of juglones are in the form of thin layers developed one over the other. Phthiocol exhibits round rod shaped particles while Plumbagin shows small and porous rod like particles. As a result of chelation shape of Nd(III) lawsonate is observed as big square particles and its isomer Nd(III) juglonate is appeared as dense cluster matrix with merged boundaries. Nd(III) phthiocolate shows big and rectangular plates while its isomer, Nd(III) plumbaginate shows network of fine capillary structure.

IX. ANTIBACTERIAL ACTIVITY OF JUGLONES AND NYODYMIUM JUGLONATES

The ligands and the metal chelates are screened against four bacterial strains which are B. subtilis, S. aureus, E. coli and P. aeruginosa using well assay method. The systematic procedure of the method was followed as described in our previous communication[7].The strains were obtained from National collection of Industrial Microorganisms division of National Chemical Laboratory, Pune. Metal salt (NdCl3.6H2O) purchased by Aldrich was used for comparative purpose of antimicrobial activities. All compounds were screened against selected bacteria using concentration 1.5mg/ml dissolving the compounds in DMSO.

Table-4 Antibacterial activities of Ligands and their Nd (III) Chelates

Compounds	Circular area of inhibition zone (mm2)			
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***
DMSO	---	---	---	---
Nd Cl3.6 H2O	---	---	---	---
LW	-	132.66	-	-
Nd-LW	-	-	-	-
JU	113.04	153.86	153.86	78.5
Nd-JU	113.04	176.62	226.865	200.96
PH	78.5	200.96	-	-
Nd-PH	-	-	-	-
PL	-	94.985	94.985	78.5
Nd-PL	78.5	200.96	314.00	615.44

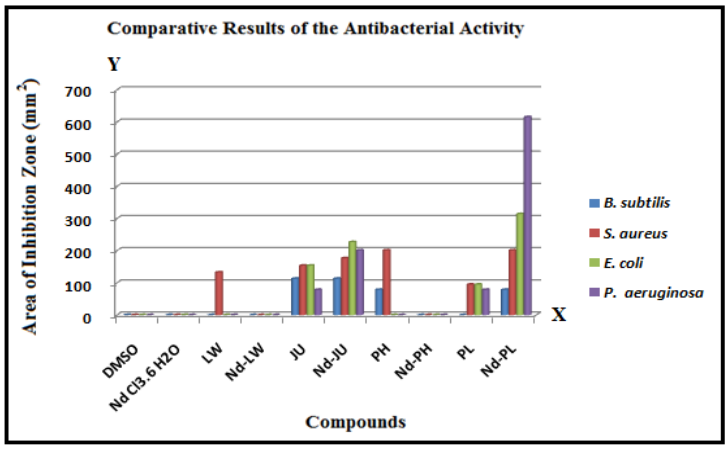

Fig- 7 Bar Diagram of Comparative Result of Antibacterial Activity

The antibacterial activity of the ligands and the Nd(III) juglonates are depicted in Table-4 and comparison of the results is expressed in the bar diagram(Fig-7). The DMSO and the salt NdCl3.6H2O show no activity against selected bacterial strains. The diameters of inhibition zone are converted in 'area of inhibition zones' using formula πr2 where r is the radius of inhibition zone, which are more convenient for comparing the activities of isomeric pairs. The ligand lawsone show moderate activbity only against S.aureus. After chelation with Nd(III), the activity is totally diminished. In general, its isomer, juglone shows significant activity against all four bacterial strains. It is notable that activity Nd(III) juglonate is increased after chelation. The ligand phthiocol shows activity only against B.subtilius and S.aureus and activity is lost after chelation with nyodymium.Plumbagin exhibits small activity against all organisms except that of B. subtilis. After chelation with Nd(III) the activity is greatly enhanced. Nd-plumbaginate shows highest activity against p.aeruginosa (615.44mm2).

It is note worthy that five membered lawsone and phthiocol chelates loose antibacterial activity after chelation but six membered juglone and plumbagin chelate show increased activity as result of chelation with Nd(III). Nd-JU and Nd-PL exhibit greater activity against gram negative bacteria than gram positive bacteria under study.

X. ANTICANCER ACTIVITY

The preliminary anticancer activity of the compounds were tested using SRB assay method following standard procedure[14]. The activity was studied at four different concentrations (10µg/ml, 20µg/ml, 40µg/ml, and 80µg/ml.) by dissolving the ligands and the Nd(III) chelates in DMSO. In the present study ADR (Adriamycin) is used as positive control which is a standard anticancer drug, for comparison. The results are presented in Table-5-6 and Fig 8-9. Amoung the four ligands lawsone, juglone, phthiocol and plumbagin, juglone is found to be the most active ligand which exhibits its maximum activity (% control growth = -60.2) at concentration 10µg/ml. Lawsone and phthiocol exhibit moderate activity while phthiocol is showing less activity at studied concentrations. It is not possible to determine the GI50 values of lawsone, juglone and plumbagin because concentration required to find exact GI50 could be less than 10 µg/ml. The GI50 value of phthiocol is 32.2 µg/ml.

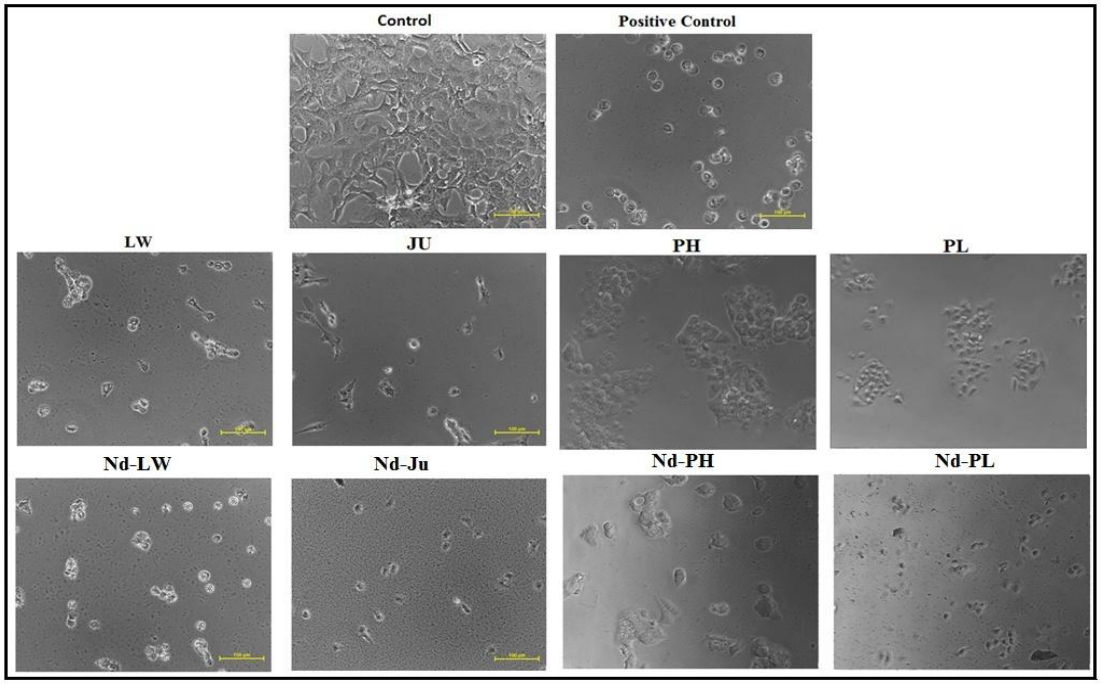

Fig-8 Microscopic images of Control, Positive control(ADR), Juglones and their Nd(III) juglonates

Table-5 Average Values of Anticancer Activity on Human Breast Cancer Cell Line of Ligands and Nd(III)Juglonets

Sample Code	Human Breast Cancer Cell Line MCF-7			
	% Control Growth			
	Drug Concentration (µg/ml)			
	Average Value			
	10	20	40	80
LW	8.3	-30.7	-53.0	-45.6
Nd- LW	96.9	106.1	94.9	-24.0
JU	-60.2	-61.0	-60.0	-28.3
Nd- JU	7.7	2.1	-15.5	-9.4
PH	72.3	52.1	44.7	15.0
Nd- PH	43.1	14.4	2.1	-0.4
PL	13.7	21.0	21.8	19.1
Nd- PL	-42.6	-47.3	-47.8	-36.9
ADR	-42.4	-51.1	-74.4	-65.4

As a result of chelation with Nd(III), the activity of lawsone and juglone is found to be suppressed but activity of another two ligands phthiocol and plumbagin is found be increased. The concentration required for half inhibition of cancer cell by phthiocol is is 32.2 and for complete inhibition it could be greater than 80 µg/ml. It is remarkable that due to Nd(III) phthiocolate the total growth inhibition occurs at concentration 66.8 µg/ml.The activity of Nd(III) Plumbaginate is remarkably increased than ligand plumbagin. Amoung all metal chelates highest activity is exhibited by Nd(III) plumbaginate. A graphical presentation of the activity is shown in Fig-7.The images of the ligands and their Nd(III) chelates (Fig-6) clearly show the morphological changes as a result of chelation. The distraction of cancer cells is notable in case of Nd(III) plumbaginate.

Table-6 LC50, TGI, GI50 Values of juglonates and Nd(III)Juglonets against MCF-7

	Concentration (µg/ml)		
	LC50	**TGI**	**GI50**
LW	NE	NE	<10
Nd- LW	NE	NE	47.5
JU	NE	NE	<10
Nd- JU	NE	NE	<10
PH	>80	>80	32.2

Nd- PH	>80	66.8	<10
PL	NE	NE	<10
Nd- PL	NE	NE	<10
ADR	11.1	<10	<10

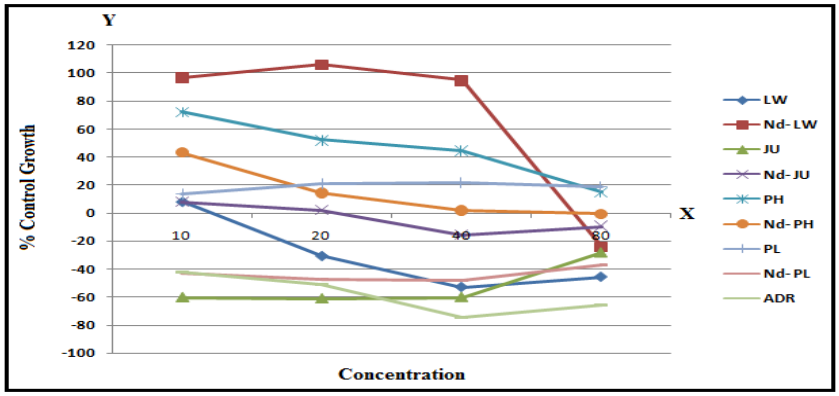

Fig-9 Graphical presentation of anticancer activity of Nd(III) Juglonates

XI. CONCLUSIONS

Four metal chelates of lawson, juglone, phthiocol and plumbagin with Nd(III) were prepared and characterized. The Nd(III) coordinated by eight oxygens, six from ligands and two from water molecules as confirmed from microanalysis, TGA and IR studies.The spectral and biological properties of isomeric chelates are compared. Six membered juglonates and plumbaginates are thermally more stable than five membered lawsonates and phthicolates as seen from TGA. Donor sites of the chelates are confirmed from IR studies which are phenolic and carbonyl oxygens. Nd(III) juglonate and Nd(III) plumbaginate are significantly active against bacterial strains and show enhanced activity as compare to respective ligands. Their activity against gram negative bacteria is greater than gram positive bacteria. These two chelates also show considerable anticancer activity against MCF-7 cancer cell which is again greater than their respective ligands. Nd(III) plumbaginate show highest activity among the four chelates.

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