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X-ray diffraction, spectral and antimicrobial actvity of bivalent metal (Zn, Cd, Hg, Pb and Sn) chelates of 2-hydroxy 1-4 naphthoquinone

A. B. Pawar, A. A. Killedar[1], K. D. Jadhav and R. G. Sarawadekar*

**Bharati Vidyapeeth Deemed University, Pune ,
Yashwantrao Mohite College, Pune 411 038,India.
[1]A Vishay Components India Pvt. Ltd. Loni Kalbhor Pune 412 201,India.**

***Corres.author: rgsarawadekar@yahoo.co.in
Phone: 020- 25433383, Fax: 020- 25440201***

Abstract: Five metal chelates of the type M [Lw] $_2$ where M = Zn, Cd, Hg, Sn & Pb and Lw = 2- hydroxy-1,4- naphthoquinone have been synthesized. All chelates have been characterized by modern methods such as elemental analysis, FTIR, Electronic spectra, Thermogravimetry and Differential thermal analysis and electron microscopy with EDAX analysis. All chelates are found to be coloured. These chelates are thermally stable up to 500^{0}C and all are crystallite in nature. Their particle sizes are in the range of 38 -157 nm. The ligand and the metal chelates have been screened for antimicrobial activity on gram positive, gram negative and antifungal activity.
Keywords: Lawsone, Metal chelates, IR, NMR, SEM, Antimicrobial activity, Electronic spectra.

Introduction

The anti – helicobactor pylori activity of 2-hydroxy 1-4 naphthoquinone (Lawsone) was evaluated using the paper disc assay which had shown very strong activity at 0.1 and 0.5 mg / disc but weak activity at doses of 0.01 mg / disc or lower (1). Lawsone exhibited inhibitory effects upon comman nosocomial urinary tract pathogenes such as E. Coli, P. Mirabilis, K. pneumonia, P. aeroginosa and S. aureus at certain concertrations. (2). J. Prasirst et.al. reported that lawsone have shown to be effective against oral Candida albicans isolated from patiants with HIV / AIDS (3). W.B. Wendel (4) studied the influence of naphthoquinones upon respiratory and carbohydrate metabolism of malaria parasites. It is reported that in vitro leishmanicidal activity of monomeric and dimeric naphthoquinones (5). Antimicrobial activities of lawsinia inermis a review have been published by P. Dinesh Babu and S. R. Subhasree (6). Naphthoquinones have been studied for their effects on prokaryotic and eukaryotic cella. (7, 8). Lawsone have shown antibacterial effects on several species of both aerobic and anaerobic organisms (9). Bactericidal and bacteristatic activity of lawsone on Staphylococcus epidermidis have been reported by S.T. Karpe et.al. (10). The probable mechanism of antibacterial activity of lawsone is proposed by Marco A. et. Al (11). Cobalt chelates of lawsone have been studied for preliminary antimicrobial activity by Kelkar et.al. (12). The 2-hydroxy-1,4-naphthoquinone (Lawsone) from Lawsonia inermis were found to exhibit strong fungal toxicity (13). From literature survey it is seen that the work on metal chelate of lawsone is sparse and incomplete. Hence the studies have been undertaken to get more

insight of the metal chelates of lawsone. This paper describes physicochemical properties of metal chelates of, under studies, such as X-ray diffraction, MID, IR and FAR IR, thermal analysis of electronic spectra in solids and in methanol. We studied the antimicrobial activity of ligands and metal chelates for *Escherichia coli, Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus and Candia albicans.*

Computational details

The calculations of the title compound were carried out with Gaussian 09 mechanics program. Geometry optimization was calculated using RHF / 6-31 G* level of theory. The wave number value computed by HF method and 6-31 G* level contain known systematic errors due to negligence of electron correlation (14). We therefore have used the scaling factor as 0.90 for HF/6-31G* (d p) set.

2. Materials and Methods

The ligand 2-hydroxy 1-4 naphthoquinone was used as it is, supplied by AR grade Thomas Baker chemicals. A stock solution of chlorides of Zn (II), Cd (II), Hg (II) Pb (II) and Sn (II) were prepared by using AR grade chemicals. Deionised water was used during synthesis.

2.1 Preparation of metal chelates

The chelates were prepared by mixing metal salt solution and ligand in 1:2 for all metals. The mixture was constantly stirred for one hour with magnetic stirrer. The pH of the mixture was maintained between pH 5.0-6.0 by adding ammonia solution to it. Mixture was warmed on water bath for about 15 minutes. On cooling it was filtered and found to be colored.

2.2 Instrumental Analysis

The IR spectra were recorded on a JASCO FTIR in the region of 4000 to 350 cm^{-1} model in a KBr matrix. Electronic spectra were recorded on JASCO 530 UV-VIS spectrometer, in solid state in KBr matrix and in methanol solution. DTA/TGA curves were recorded on Shimatzu 60H model using 10 C^o / min. heating rate, 800 C^o maximum temperature in air. X-ray diffraction patterns were obtained by using Brucker D8 advanced diffractometer. Elemental analysis was carried out with a Perkin Elmer 2400 series for C, H, O, & N. Scanning electron microscopy was carried out on Vega 2SB model and EDAX on OXFORD INCA PENTA with TECAN VEGA 2SB.

2.3 Bioassay

Test organisms: The antimicrobial activity ligands, metal salt and synthesized metal chelates were examined against bacteria and fungi [*Escherichia coli* (NCIM 2065), *Bacillus subtilis* (NICN 2063), *Klebsiella pneumonia* (NCIM 5082), *Staphylococcus aureus*(NCIM 2079) *and Candia albicans* (NICM 3471)] strains collected from NCL Pune India.

2.4 Maintenance and Culture

The culture of bacteria and fungi were maintained on Nutrient agar (Himedia Laboratories Pvt. Ltd. Ref. M 002-500G 99% Purity and sub cultured accordingly. These plates were incubated at 35^{o}C for 24 hour in incubator.

2.5 Inoculums preparation

One loopful growth of bacteria and fungi were transferred in to the 100µL of the organism suspension. Finally 100µL of ligand, metal salt and metal chelates were placed in to each well.

3. Result and discussion

The chelates of lawsone are stable at room temperature, insoluble in water and protic solvents while soluble in aprotic solvents such as DMF and DMSO.

3.1 Infra red spectra

The IR frequencies of ligand and its metal chelates are given in table-1.

Table-1: Characteristics IR (cm^{-1}) Bands of Lw and its metal chelates

Sr.No.	Compound	OH	C=O	C=O	C-O
1	Lw	3172	1662	1678	1224
2	ZnLw	3168	1644	1677	1285
3	CdLw	3153	1644	1675	1277
4	HgLw	3265	--	--	1274
5	SnLw	3175	1643	1676	1283
6	PbLw	3323	1642	---	1253

In comparison with Lw spectrum (15), the spectra of the metal chelates displayed bands ranging between v(C-O) group (16-17) and confirming the coordination through the phenolic oxygen. The other frequencies of C=O are shown common in all chelates. The main peak for OH from ligand is not observed in chelates. In The region 1700-1200 cm^{-1}, all the chelate showed a number of intensive bands, which are due to normal modes of vibrations of ligand affected by its coordination. The v(C=O) stretching frequency of coordinated carbonyl group is similar that of ligand. All the chelates exhibits v(C=O) band at lower energy. It can be explained on the basis of mono anionic nature of the ligand which acts as a ring with bidendate ligand. In results form a ring with metal ion. A considerable delocalization of the π electron density exhibits such a strong ring formation. This can be explained on the basis of absorption bands by the chelate. The most important region is v 600-300 cm^{-1} exhibits several absorptions are sensitive to the central metal ion and the structure of the chelate and can be attributed to the normal mode of vibrations of the MO_4 moiety. Generally the ligand to metal ratio is 1:2 and they have D_{2h} symmetry and band can be assigned in the region of v 590-420 cm^{-1} to the v_{sym} (MO) stretching vibration species B_{lu} and the other band in the v390-320 cm^{-1} to the v_{asym} (MO) stretching to B_{2h}. These two vibrations are sensitive to the nature to the central metal ion.

3.2 Electronic spectra

The UV-VIS spectra of the ligand and its metal chelates were recorded in a solid state in the KBr matrix, also in methanol solution, data is given in Table-2. Ligand Lw exhibited electronic transition bands at 240, 322 and 402 nm in solid state and assigned to Benzenoid electronic transition (π-π*) and n to π* ($^1B_{1u} - {}^1A_{1g}$) respectively. These bands are compared to theoretically calculated by using Gaussian 09 and using RHF / 6-1 G* (d,p) level which gave results as 331.80, 289.31, 266.93, 251.20, 20.41 and 189.67 nm which shows good arrangement with experimental results. In methanol solution, the calculated transition state values are at 268, 255 and 190 nm, while the experimental values are 248 and 330 nm. These transitioned to π to π*. In the case of ZnLw, it shows transition state at 261, 345 and 491 nm as against calculated values at 350, 365 and 357 nm respectively. The remaining metal chelates show similar results.

3.3 X-ray diffraction

X-ray diffraction patterns of Lw, ZnLw and PbLw are shown in fig-1. Lw crystallizes in the triclinic group which is calculated by using McMaille Version-4 computer code (18) and it has crystallographic parameters are a = 7.6180, b = 7.1189 and c = 11.3240 α = 99.460, β = 70.494, γ = 93.344, its volume is 570.996 $(A^o)^3$ and space group H-M symbol P1.

Table-2: Electronic Spectra (UV-VIS) of Lawsone and its Metal Chelates λ1 nm in solid state and λ2 nm in Methanol

Sr. No.	compound	Colour	λ1 nm	λ2 nm	λ 1 nm	λ2 nm	λ1 nm	λ2 nm
1	Lw	Yellow	278	248	322	330	402	--
2	ZnLw	Brown Red	261	234	335	350	491	--
3	CdLw	Yellowish orange	261	236	325	--	425	406
4	HgLw	Reddish orange	249	244	300	330	488	--
5	SnLw	Yellowish Green	261	238	320	342	460	--
6	PbLw	Dark Red	288	232	344	--	--	446

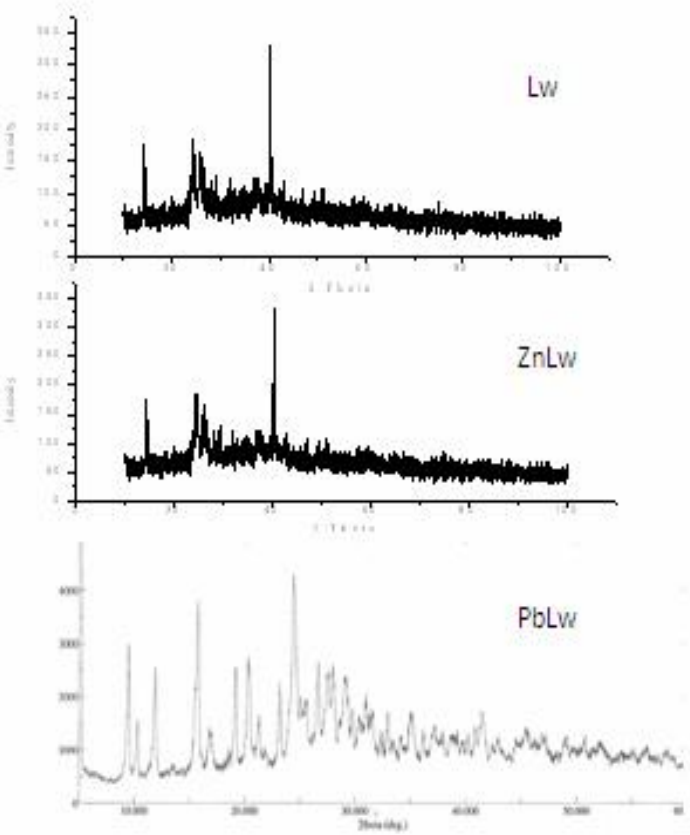

Fig -1: XRD Patterns of 1) Lawsone (Lw), 2) Zinc Lawsonate (ZnLw) and 3) Lead Lawsonate (PbLw)

The values for miller indices and observed 2 θ for Lw are given in Table-3.

Table-3 Miller indices and observed 2 θ for Lw

Sr.No.	H	k	l	Observed2θ
1	1	0	0	18.365
2	0	1	1	18.785
3	1	-1	-1	32.970
4	0	1	2	33.850
5	1	0	-2	35.985
6	0	0	3	38.015
7	2	-1	1	39.010
8	0	1	-3	39.895
9	2	-1	0	41.490
10	2	1	2	44.145
11	0	1	3	45.410
12	1	0	4	48.455
13	0	2	-4	60.180
14	1	3	0	62.0005
15	1	-1	-4	67.410
16	2	-2	5	70.545

Zinc Lawsone (ZnLw) crystallizes in the triclinic group and it has crystallographic parameters are a = 5.0555, b = 10.4411 and c = 10.3535 α = 64.141, β = 89.935, γ = 101.984, its volume is 478.453 $(A^o)^3$ and space group H-M symbol P1.The values for miller indices and observed 2 θ are given in Table-4 .

Table-4: data for h k l and 2 θ values

Sr. No.	h	k	L	TH(OBS)	TH-ZERO	TH(CALC)	DIFF.
1	0	0	1	14.185	14.204	14.194	0.010
2	0	1	-1	24.415	24.434	24.428	0.006
3	0	1	2	25.570	25.589	25.613	-0.024
4	0	2	1	25.935	25.954	25.946	0.008
5	1	0	**1**	29.165	29.184	29.180	0.004
6	1	0	-1	31.875	31.894	31.883	0.011
7	1	-2	0	34.875	34.894	34.906	-0.012
8	1	0	2	37.465	37.484	37.488	-0.004
9	1	1	-1	40.225	40.244	40.256	-0.012
10	1	0	3	49.235	49.254	49.246	0.008
11	1	-3	-3	51.085	51.104	51.098	0.006

Lead Lawsonate (PbLw) crystallizes in the triclinic group and it has crystallographic parameters are

A	B	C	ALPHA	BETA	GAMMA
9.0770	7.6246	9.7726	82.784	72.981	78.913

VOLUME (A**3) :632.920 and space group H-M symbol-1**.** Dmin = 3.100570 g/cm^3

The values for miller indices and observed 2 θ are given in Table-5 .

Table-5: data for h k l and 2 θ values

Sr. No.	h	k	L	TH(OBS)	TH-ZERO	TH(CALC)	DIFF.
1	0	0	1	9.460	9.497	9.482	0.015
2	1	0	0	10.280	10.317	10.323	-0.006
3	0	1	-1	15.700	15.737	15.740	-0.003
4	1	-1	0	16.940	16.977	16.979	-0.002
5	1	1	-1	19.180	19.217	19.230	-0.013
6	2	1	1	21.300	21.337	21.347	- 0.011
7	0	1	-2	23.180	23.217	23.221	-0.004
8	1	0	-2	24.080	24.117	24.111	0.006
9	1	2	1	24.420	24.457	24.449	0.008
10	2	1	2	24.560	24.597	24.587	0.010
11	2	0	-1	25.100	25.137	25.152	-0.015
12	2	-1	0	25.580	25.617	25.609	0.008
13	2	1	-1	26.660	26.697	26.679	0.018
14	1	-2	0	27.540	27.577	27.577	0.000
15	1	2	2	28.000	28.037	28.043	-0.007
16	1	1	3	28.580	28.617	28.622	-0.005

3.4 Metal-Organic Frameworks

Crystalline porous coordination polymers (PCPs), also called Metal-Organic Frameworks (MOFs), are a fascinating class of solid-state inorganic-organic hybrid materials. Research in these compounds is expanding very rapidly owing to their exciting combination of properties for advanced functional materials in gas storage and gas separation, catalysis, chemical sensing, as well as medical applications. Fig 2 shows SEM of a) Lw b) ZnLw c) PbLw d) CdLw e) HgLw f) SnLw

Its synthesis involves following steps -

Metal ion + Organic linker------Nucleation------ Crystal growth

The growth orientation [100] on the layer by layer growth of the same MOF requires coordination through Lw-metal interaction, which forms the two dimensional layer of the MOF, was impeded by the presence of oxime and hydroxyl group. Therefore, selective coordination-modulation method enhanced the relative crystal growth in the [001] direction. The material grown under coordination modulation was significantly enhanced compared to that of the usually obtained microcrystalline power of M-(Lw) 2. The growth of metal chelates is observed as bulk crystals. The crystallite size of chelates is found to be nano range as 69.9, 157.74 and 38.35 nm for Lw, ZnLw and PbLw respectively which is calculated using XRD data and Scherrer formula. This difference was attributed to the higher structural defects of the usual material as compared with the coordination modulation nano-MOF. Nevertheless, direct evidence of coordinated oxime groups during crystallization in the framework as a powder has not been reported. Even more generally, the fabrication and characterization of well-defined, stable self-assembled mono layers (SAMs) of organic ligands at a crystal face of a MOF has not been documented to date.

SEM image of Lw shows plates made of various crystals which is nothing but polymerization of crystals through hydrogen bonding. ZnLw image clearly shows a needle shaped formation of bulk crystals which are belonging to nano size materials. The EDAX data is obtained for elemental analysis and the calculated values are presented in Table-6.

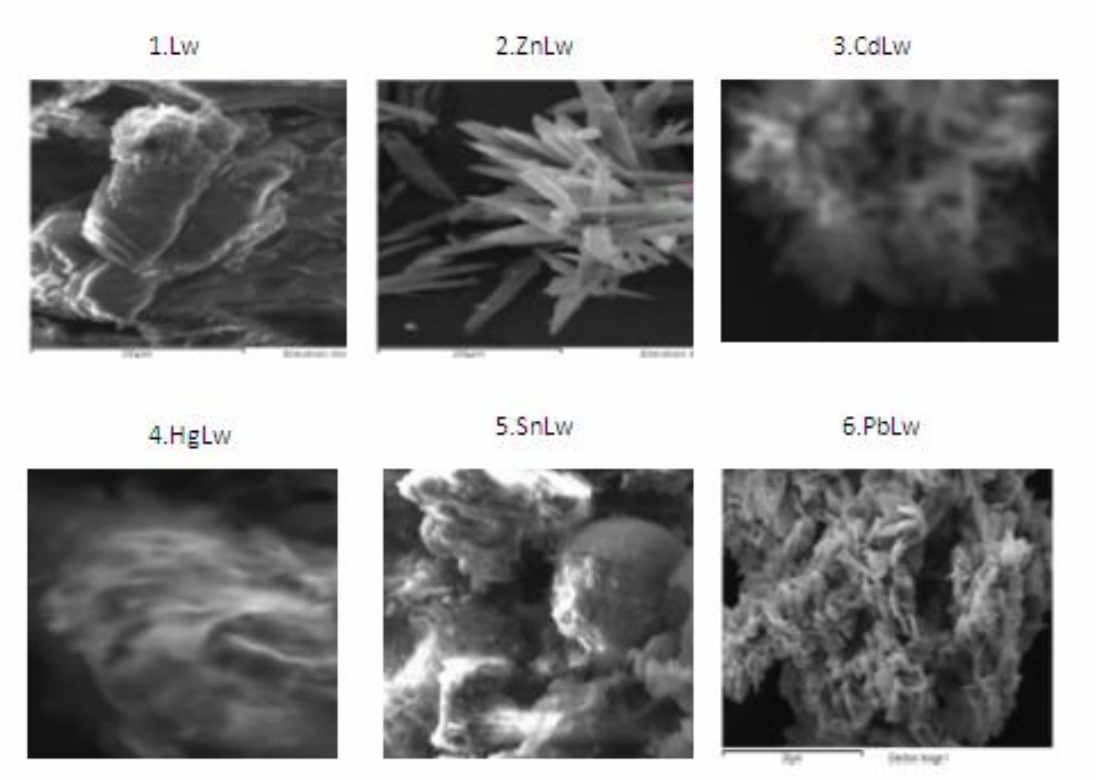

Fig: SEM Photographs of Ligand and Metal Chelates

Table-6 Data for elemental analysis by EDAX and calculated

Sr.No.	Compound	Method	% C	% O	% H	% Metal
1	Lw	Obsd.	64.59	35.41	--	--
		Cald.	68.96	27.58	3.64	--
2	ZnLw	Obsd.	61.76	17.64	--	20.60
		Cald.	58.29	23.31	2.62	15.95
3	CdLw	Obsd.	55.65	22.71	--	21.64
		Cald.	52.37	20.93	2.18	25.40
4	HgLw	Obsd.	33.93	20.91	--	45.16
		Cald.	43.93	17.55	1.82	36.68
5	SnLw	Obsd.	48.22	33.32	--	18.47
		Cald.	51.67	20.64	2.15	25.23
6	PbLw	Obsd.	34.73	13.73	--	51.54
		Cald.	31.34	17.34	1.84	37.39

It is observed that this can be a qualitative analysis of the metal chelates.

3.6 Thermal analysis (TGA/TG)

DTA/TG of lawsone (Lw) was carried out in air using 10^0C/min. heating rate and maximum temperature up to 800^0C. DTA of Lw showed one endotherm and one exotherm.

Endotherm -132.52 to 250.68^{0}C

Exotherm- 500.15 to 652.81^{0}C

The endotherm is due to decomposition of lawsone and the weight loss is 52.374% which is due to loss of $C_6O_1H_1$ moiety .The second step is exothermic decomposition of remaining mass which corresponds to 20.69% loss and it loses C_{20} O_1 H_5 moiety. On further heating, it finally shows only carboneous matter.

DTA / TG of ZnLw shows one endotherm in the temperature range 145 to 180 ^{o} C which is due to loss of water molecules .The TG data shows 8.267% weight loss which corresponds to two molecules of crystalline water. Later it shows, second step in the temperature range of 315 to 509^0C and the weight loss is 40.4%. It is observed that it loses $C_{12}H_{11}O_3$ moiety and the remaining mass is ZnC_8HO.

3.7 Antimicrobial activity studies

Antimicrobial Scanning Results

The Lw ligand and its metal chelates are screened for their antimicrobial activities against *Escherichia coli, Bacillus subtilis, Candida albicans, Staphylococcus aureus* and *Klebsiella pneumonia*. The testing against growth of micro-organisms was carried out by using well diffusion method employing Mueller Hinton Agar (MAH) and culture in nutrient broth in each case of micro-organisms. The concentration of ligand Lw and its metal chelates were chosen as 10^{-4}M. the plates were incubated at 35^{o}C for 24 hours in incubator. The clear zone of inhibition of growth for the organism was measured in mm and the data is given in Table-7. Dimethyl sulphoxide i.e. solvent used shows no inhibition for all organisms under studies.

Table: 7 Antimicrobial activities of 2-hydroxy -1, 4- naphthoquinone (Lw) and its metal chelates (inhibition zone diameter in mm)

Sr. No.	Compound	Escherichia coli	Bacillus subtilis	Staphylococcus aureus	Klebsiella pneumoniae	Candida albicans
1	DMSO	Nil	Nil	Nil	Nil	Nil
2	ZnLw	11	--	27	17	18
3	CdLw	--	--	25	19	12
4	HgLw	13	19	30	20	22
5	SnLw	29	42	12	17	26
6	PbLw	11	13	18	14	12

All the metal chelates showed microbial activity against all organisms studied in this work.

SnLw showed good activity against all organisms and showed highest activity for Bacillus subtilis.

The inhibition of the micro-organisms growth for metal chelate was found to be in the following order for Candida albicans

SnLw > HgLw > ZnLw > PbLw

The studies demonstrate that metal chelation can increase the antimicrobial activity than metal free ligand. It is responding that metal chelation reduce the polarity of the metal ion mainly due to partial sharing of its positive change with the donor group and possibly the δ electron delocalization occurring within the whole chelate ring system formed during co-ordination and results in increase of the lipophilic nature of the central metal atom (20). It favors for its penetration through the lipoid layer of the membrane.

The transition metal chelates possess high degree of inhibition which can be due to the greater number of δ electrons which increase the electrostatic field around the metal ion.

4. Conclusions

The metal chelates are thermally stable up to 500^{o}C which is a unique characteristic property. All these chelates are crystalline in nature and generally belong to triclinic. The coordination ability of ligand Lw towards M(II) chelates were examined by different spectroscopic methods that unequivocally determine the coordination sites of ligands Lw. It is observed that the ratio of the metal chelates is 1:2 for chelates of Zn, Cd, Hg, Sn and Pb. This data is supported by LC-MS work. Biological activity screening proved the good antimicrobial activity of ligand Lw and its metal chelates. The antimicrobial activity explored on the basis of overtone concept of cell permeability.

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References

1. Park B.S., Lee H.K., Lee S.E., Piao X.L., Takoka G.R. , Wong R. Y., Ahn Y.J. and Kim J.H., Antibacterial activity of Tabebuia impetiginisa Martius ex DC (Taheebo) against Helicobactor pylori, *J. Ethanopharmacol*, 105 (1-2) 255-62 (2006).
2. Bhuvaneshwari K.S. Gnana Poongthai, A. Kuruvilla and A. Appala Raju, Inhibitory concentrations of Lawsonia inermis dry powder for urinary pathogens, *Indian J. Pharmacol.* 34, 260-163 (2002).
3. Prasirst J.T. Leewatthanakom, U. Piamsawad, A. Derjudee, P. Panichayupakaranent, R. Teanpaisan and W. Nittyananta, Antifungal activity of potassium Lawsone methyl ether mouthwash in comparison with Chlorhexidine mouthwash on oral Candida isolated from HIV/AIDS subjects, Abstract, 5th World Workshop on Oral Health and disease in AIDS, Phaket, Thailand, pp 6-9 (2004).

4. Wendel W.B. The influence of naphtha - quinones upon the reapiratory and carbohydrate metabolism of malaria parasites, Fed. Proc., 5, 406-7 (1946).
5. Kayser O., A. F. Kiderlen, H. Laatsch and S. L.Croft., In vitro leishmanividal activity of monomer and dimer naphthoquinones, *Acta. Tropica*,77 (3) 307-14 (2000).
6. Dinesh Babu P. and R. S. Subhasree., Antimicrobial Activities of *Lawsonia inermis* - A Review ,*Academic Journal of Plant Science*, 2(4) 231-232 (2009).
7. Perry N. B., Blunt J. W. and Munro M. H. G., A cytotoxic and antifungal 1,4 naphtho - quinone and related compounds from a New Zealand brown alga, Landshurgia quercifolia, *J. Natural Product*,54,978-985 (1991).
8. Thomson R. H., Natural, Occurring Quinones, Academic Press, London, England.
9. Didry N., Pinkas M. and Dubreil L.,Activate antibacterinne de naphthoquinone of 0rigine vegetable, Annales, *Pharmaceutiques Francaises*,44,77-78 (1986).
10. Karpe S. T., S .R. Kulkarni, S .A. Shaikh and M. Manikrao, Bactericidal and bacteristatic activity of isolated naphthoqionone fraction of lawsonia inermis and synthetic lawsone on Staphylococcus epidermidis, *Pharmaco - logyonline*, 2,256-163 (2011).
11. Marco A., Biffi G. Zaavag L., Bacteristatic action of quinines on normal and resistant strains of S. aureus Sperimentale, *Sperimentele*,100,481-91(1951).
12. Kelkar V.D., Gokhale R.R .and Gholap H.R. Synthesis, characterization and antimicrobial studies of cobalt(III) chelates of some lawsone derivatives, *Synthesis and Reactivity in Inorganic and Metal-organic chemistry*.,28 (8) 1253-64 (1998).
13. Wang, H., Ng, T.B. Isolation of a novel deoxyribonucleic with antifungal activity from Asparagus officinal is seeds *Biochem. Biophys. Res. Commun*., 288, 765(2001).
14. J. R. Holden and C.W.Dickinson, Crystal structures of three solid solution phases of ammonium nitrate and potassium nitrate. *Journal of Physical Chemistry* , 79(3): 249-256 (1975).
15. A.B. Pawar, K. D. Jadhav, M. P. Wadeker, and R.G. Sarawadekar, Spectral and theoretical investigation on 2- hydroxy- 1,4-naphthoquinone (Lawsone), *Journal of Pharmacy Research*, 4(6), 1745-1747 (2011).
16. Gupta K.C. H.K. Abdulkadir, S. Chand, Metallation of tetra dentate N_2O_2 Schiff base with Mn (II), Co(II), Cu(II) and Zn(II): synthesis, characterization and formation constants measurement, *J. Molecular catalysis A: Chemical*. 202, 253 (2003).
17. El-Bindary A.A. Spectral studies on metal-ligand bonding in complexes of 1-acetyl-2-(coumariniminecarboxamide-3-yl)hydrazine , *Spectrochimica Acta., Part A*, 57 49 (2001).
18. Le Bail, A. (2002), http:// www.cristal.org /mcmaille.
19. R.G. Sarawadekar and I.B. Gade, Software for Kinetic Analysis of non Isothermal Thermogravimetry (TG) Data, HEMRL REPORT , PYRO, TR 253 (2006).
20. Z.H. Chohan, C. T. Supuran and A. Scozzafava, Unsymmetrical 1,1′-disubstituted Ferrocenes: Synthesis of Co(ii), Cu(ii), Ni(ii) and Zn(ii) Chelates of Ferrocenyl -1-thiadiazolo-1′-tetrazole, -1-thiadiazolo-1′-triazole and -1-tetrazolo-1′-triazole with Antimicrobial Properties, *J. Enzyme Inhihitian and Medicinal chemistry*, 20, 303 (2005).

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