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Synthesis and Characterization of Metal Complexes Containing Curcumin ($C_{21}H_{20}O_6$) and Study of their Anti-microbial Activities and DNA Binding Properties

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Abstract

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], was extracted form turmeric and also synthesized by the condensation of vanillin substituted by acetyl acetone. Its metal complexes (M = Eu, Ce, La, Y, Cr, Pd) were prepared and characterized by IR and UV-vis spectroscopy. The result shows that curcumin coordinates with metal ions in bidentate mode in deprotonated form. Anti-bacterial study of the synthesized complexes indicated that this complexes has anti-bacterial activity against Klebsiella pneumonia and Escherichia coli. The result of agarose gel electrophoresis suggested that the complexes had good interaction with genomic DNA.

Keywords: Curcumin-metal; DNA binding; Anti-bacterial; Electrophoresis; DNA damage.

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1. Introduction

Metal bound organic compounds are known to possess potential activities in the areas of biological, clinical, analytical, catalytic, microbial, insecticidal, antibiotic, growth factors, food additive, tumor inhibitor, cell division etc. This is due to either the unused coordination sites present on the metal and ligand systems, or due to the selective oxidation state of the complexed metal ions in the coordination sphere [1]. The usefulness of metal chelates in various branches of theoretical and applied chemistry is now generally recognized. These reagents which form metal chelates are used extensively in both qualitative and quantitative analyses. According to scientific research, curcumin (Scheme 1) the main yellow bioactive component of turmeric has been known to have the medicinal activity since ancient times and this molecule has been the object of several investigations in the field of biology, medicine and pharmacology over the last decades, such as antioxygenation, antibiosis and antitumor activities and also shown to have a wide

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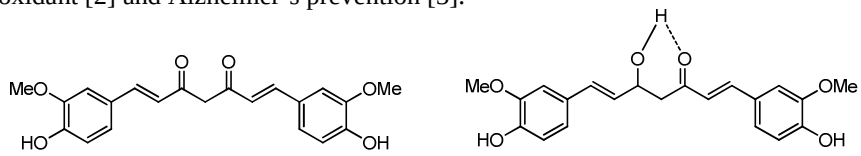
spectrum of pharmacological effects including anti-carcinogenic, anti-inflammatory, antioxidant [2] and Alzheimer's prevention [3].



Curcumin in the ketone form Curcumin in the enol form

Scheme 1. Structure of curcumin in the ketone and enol forms.

From the chemical structure of curcumin {1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione}, it can be said that curcumin has a highly conjugated ß-diketone moiety and can be a powerful natural chelating agent with its safety evaluation even administered at high dose in human [4-5]. Complexation of curcumin with metals has attracted much interest over the past years as one of the useful requirements for the treatment of Alzheimer's disease and *in vitro* antioxidant activity. Moreover, several metal complexes of curcumin have been synthesized, characterized and evaluated for various biological activities [6].

The strong spectrochemical and electrochemical characteristics of these complexes provide sensitive utensils to study their interaction with DNA molecules. The changes in the intensities of the spectral and electrochemical behavior can be used to explore the nature and potency of the interaction between the chromophore and DNA base pairs. Complexation of metal with a known antioxidant, curcumin, has the potential to improve synergistically the potency of a metal-based hypoglycemic agent. These *ß*-diketoenolate ions form very stable chelate complexes with a great range of metal ions. Among the commonest types of diketo complexes are those with the stoichiometries $M(dike)_3$ and $M(dike)_2$. Tetradiketo complexes $M(ß\text{-}dike)_4$ are usually nonrigid. Substance of composition $M(dike)_2$ are almost invariably oligomeric, unless the replacement groups are very bulky ones such as $(CH_3)_3C$-. Moreover, the methylene moiety in the middle of acetylacetone molecule can also bonded with the metal anion, notably for rhodium, iridium, palladium and platinum. The present investigation is aimed at extraction of curcumin from turmeric along with the chemical synthesis. Furthermore, synthesis and characterization of new rare earth metal complexes of crucumin and study of their biological activity such as investigation of the antibacterial activity and the cleaving double stranded DNA (interaction of complex with DNA) in order to gain a better understanding of the biological activity.

2. Experimental

2.1. *Materials and Measurement*

All the chemicals used were collected from Merck (Germany), Wako Pure Chemicals Industries Ltd. and JHD (China). These chemicals were analytical, spectroscopic grade and were used without further purification unless otherwise specified.

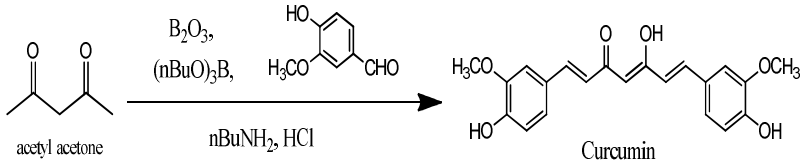
Melting points of the ligand and the complexes were obtained with an electrothermal melting point apparatus GALLENKAMP made in England. UV-Visible spectra of the ligand and complexes were recorded with a UV-Visible spectrophotometer Model No: UV- SHIMADZU UV-1800 series, Japan. Infrared spectra were recorded on KBr pellets with an IR spectrometer of model No- SHIMADZU IP Prestidge-21, FTIR spectrometer, Japan in the 4000-400 cm^{-1} range. DNA binding and antimicrobial studies were done in Molecular Biotechnology Laboratory of National Institute of Biotechnology, Dhaka, Bangladesh

2.2. *Extraction of curcumin from turmeric*

20 g of ground turmeric in 50 mL of dichloromethane was magnetically stirred and heated under reflux for 1 h. The mixture was suction-filtered and the filtrate was concentrated in hot-water bath maintained at 50°C. The reddish yellow oily residue was triturated with 20 mL of hexane and the resulting solid was collected by suction filtration. TLC analysis (3% methanol-97% dichloromethane) showed the presence of three components. The crude material obtained after trituration with hexanes was dissolved in a minimum amount of 99% dichloromethane- 1% methanol (v/v) and loaded into a column packed with 30 g of silica gel. The column was eluted with the same solvent. TLC analyses of the various fractions showed the presence of three components. The fractions containing the least polar color component were combined and the solvent was removed on water bath to give a yellow solid. Yield 76%, melting point 184-187 °C.

2.3. *Synthesis of curcumin*

Curcumin was synthesized according to the following scheme. Acetyl acetone (1.03 mL, 10 mmol) followed by tributyl borate (10.8 mL, 40 mmol) were added to a solution of boric anhydride (0.35 g, 5.0 mmol) in ethyl acetate (30.0 mL) at 50°C for 15 min. Vanillin (3.04 g, 20 mmol) was added to the resulting boron complex and stirred at 50°C for 5 min. Butylamine (0.4 mL, mmol) was then added dropwise over 40 min at 50°C and the reaction mixture was refluxed for 4 h. Then the reaction mixture was cooled, combined with 1N HCl (30 mL) and stirred for 30 min. The organic layers were separated, extracted three times with ethyl acetate, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by recrystallization from methanol to give curcumin as a yellow solid: yield 81%, melting point 183-185°C.



Scheme 2. Synthesis of curcumin.

2.4. *Synthesis of metal complexes*

2.4.1. *Synthesis of $Cr(Curc)_3$ complex*

The $Cr(Curc)_3$ complex was synthesized by mixing curcumin with chromium chloride at a molar ratio 3:1 in ethanolic solution. The reaction was accomplished by adding a stoichiometric quantity of an ethanolic solution of $CrCl_3 \cdot 6H_2O$ (7.994 mg, 0.03 mmol) dropwise with stirring to an ethanolic solution containing the anion of the curcumin (33.144 mg, 0.09 mmol), prepared by neutralization with NaOH (3.6 mg, 0.09 mmol). The clear mixture was heated at 80°C until a clear purple-red solution was formed. The solvent was removed under reduced pressure and the remaining solid was dried under vacuum, yield 84–89%.

2.4.2. *Synthesis of $Y(Curc)_3$ complex*

The synthesis of $Y(Curc)_3$ complex was accomplished by dropwise additon of a stoichiometric quantity of an ethanolic solution of $Y(NO_3)_3 \cdot nH_2O$ (27.49 mg, 0.1 mmol) to an ethanolic solution containing the anion of the curcumin (110.5 mg, 0.3 mmol), prepared by neutralization with NaOH (12.0 mg, 0.3 mmol) and the red powder precipitate was produced immediately. The reaction mixture was refluxed for 4 h at 80°C. The reaction mixture was then cooled to room temperature and the complex precipitated was separated by filtration, washed with a small amount of ethanol and dried under vacuum, yield 93–95%.

2.4.3. *Synthesis of $La(Curc)_3$ complex*

The complex $La(Curc)_3$ was prepared in the following steps. A solution of curcumin (77.357 mg, 0.21 mmol) in ethanol (10 ml) was added dropwise to a solution of $La(NO_3)_3 \cdot 6H2O$ (30.310 mg, 0.07 mmol) in ethanol (5 ml) under stirring. The pH value of the mixture was adjusted to 6 by adding an aqueous solution of Na_2CO_3 (11.13 mg 0.105 mmol) and refluxed at 80°C for 4 h. The reddish precipitate obtained was separated by filtration, washed three times with ethanol and dried in vacuum for 24 h. The yield was about 83% based on the amount of $La(NO_3)_3 \cdot 6H_2O$ used.

2.4.4. *Synthesis of $Ce(Curc)_3$ complex*

For the preparation of $Ce(Curc)_3$ complex, to a solution of curcumin (110.5 mg, 0.3 mmol) and Na_2CO_3 (15.9 mg, 0.15 mmol) in ethanol (10 mL), a solution of $Ce(NO_3)_3 \cdot 6H_2O$ (43.43 mg, 0.1 mmol) in ethanol (5 mL) was dropwise added and the red precipitate was produced immediately. The reaction mixture was refluxed at 80°C for 4 h. The red solid product was filtered and washed by cold ethanol and water to remove the residue reactants, and then the product was dried in vacuum overnight, yield 89%.

2.4.5. *Synthesis of Eu(Curc)$_3$ complex*

For the preparation of Eu(Curc)$_3$ complex, to a solution of curcumin (110.5 mg, 0.3 mmol) and Na_2CO_3 (15.9 mg, 0.15 mmol) in ethanol (10 mL), a solution of $Eu(CH_3CO_2)_3 \cdot nH_2O$ (43.43 mg, 0.1 mmol) in ethanol (5 mL) was dropwise added and the reddish precipitate was produced immediately. The reaction mixture was refluxed at 80^{o}C for 4 h. The red solid product was filtered and washed by cold ethanol and water to remove the residue reactants, and then the product was dried in vacuum overnight, yield 85%.

2.4.6. *Synthesis of Pd(Curc)$_2$ complex*

For the preparation of Pd(Curc)$_2$ complex, to a solution of curcumin (29.46 mg, 0.08 mmol) and Na_2CO_3 (4.24 mg, 0.04 mmol) in methanol (5 mL), a solution of $PdCl_2$ (7.09 mg, 0.04 mmol) in methanol (3 mL) was added. The cloudy mixture was heated at 60^{o}C until a clear blood-red solution was formed. The solvent was reduced under reduced pressure and allowed to stand for crystallization. A reddish-black crystals were obtained, with 38% yield.

2.5. *Antibacterial activity assay*

In vitro antibacterial activities of curcumin and its complexes were studied by using the disc diffusion method. The chosen strains included E. coli belong to G(-) strains, Klebsiella pneumonia belong to G(-) strains, Proteus spp. belong to G(-) strains and Pseudomonas sp. belong to narrow strains. Small circular scraps of filter paper of diameter 5 mm were prepared for the purpose of bacteriostatic slices. Drug (5 mg) (curcumin and its complexes) was dissolved in 10 mL DMSO (1%) to make concentrations of 0.5 mg/mL. The solution (0.1 mL) was poured into a small bottle containing ten paper slices and it was ensured that the solution was completly blotted up. The bottle was covered by gauze, sterilized for 20 min and then kept in an oven at 80°C for subsequent testing.

Bacterial strains were inoculated onto the medium plates of corn meal agar with absorbent cotton, and two previously prepared bacteriostatic slices containing the same drug were put on a medium plate. Finally, all plates were incubated at 35°C for 24 h and then examined.

2.6. *Procedure for the DNA binding studies*

Prior to binding studies DNA was extracted from goat blood manually by utilizing minicale preparation method and also by using EZ-10 Spin Genomic DNA Minipreps extraction Kit from human Blood. Then binding study was conducted by agarose gel electrophoresis method as follows:

5 µL of the metal complex solution was taken to a 0.5 mL of eppendorf centrifuge tube. Then 12 µL of DNA solution dissolved in Tris-EDTA buffer was added in the tubes containing the metal complexes and for DNA control just the DNA was taken into the tubes. The tubes were incubated at 37°C for 30 min to 2 h for short time interaction studies and 16 h for long time interaction studies. After incubation, the solutions containing tubes were kept in a refrigerator at O°C for few min. 3 µL gel loading buffer was taken into the tubes and used for electrophoresis.

3. Result and Discussion

3.1. *Spectral data of curcumin*

3.1.1. *UV-visible spectra*

The electronic spectra of the curcumin was recorded in methanol and DMSO as shown in Fig. 1 and the data is given in Table 1. Curcumin in methanolic solution showed a broad characteristic UV-visible absorption at around 300-500 nm with maximum absorption band at wavelength 424 nm and shoulder near 360 and 460 nm, and a weak absorption band at 262 nm while in DMSO the maximum absorption band appeared at 435 nm and a weak absorption band at 268 nm. The maximum absorption is due to the electronic dipole allowed π-π* type excitation of its extended conjugation system. Since there is electrostatic interaction between polar solvent (methanol) and polar chromophores in curcumin molecule, this solvent tends to stabilize both the bonding electronic ground states and the π* excited states. This interaction causes the n-π* transition which occurs at lower energy than the π-π* transitions to move to higher energy and π-π* transition to move to lower energy. Thus, the π-π* and n-π* absorptions of curcumin move close to each other [7].

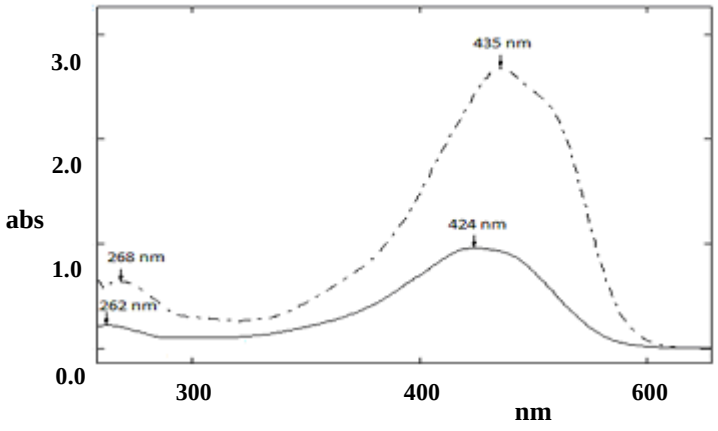


Fig. 1. UV-Vis spectra of curcumin in methanol (—) and in DMSO (- -).

3.1.2. *FT-IR spectrum of curcumin*

IR spectrum of curcumin (Fig. 2 and Table 2) showed a sharp peak at 3510 cm^{-1} indicating the phenolic O-H stretching with a broad band at a range from 3200-3500 cm^{-1}, which is due to the ν(OH) group (in enol form). The low intensity bands observed in the IR spectrum at 3079–3000 cm^{-1} are assigned to the aromatic ν(C-H), while the lower frequency bands are attributed to the methyl group motions. The important absorption band at 1629 and 1603 cm^{-1} correspond to the mixtures of stretching vibrations of ν(C=C) and ν(C=O) in curcumin.

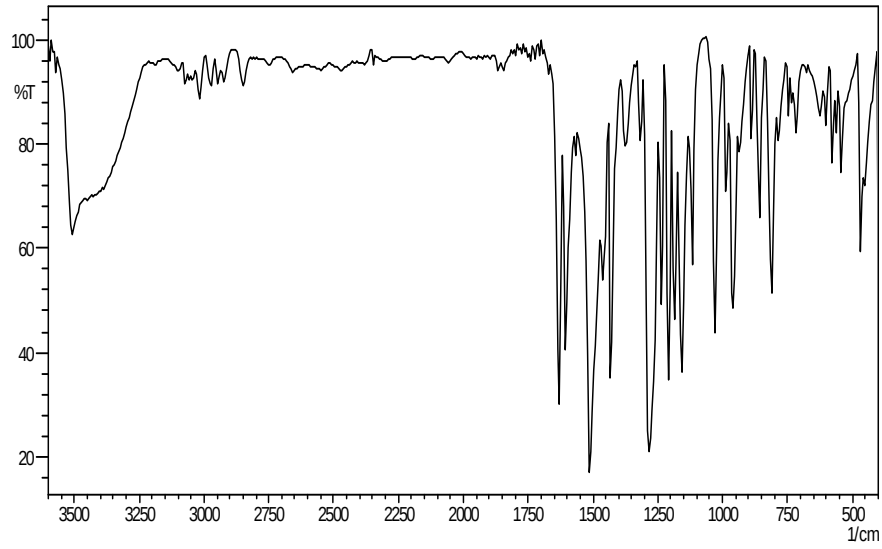


Fig. 2. FTIR spectrum of curcumin.

The most prominent band in the IR spectrum is at 1510 cm^{-1} attributed to highly mixed vibrations of ν(C=O), δ(CCC), δ(CC=O) and aromatic ν(CC), ν(CCH). Deformation vibrations of the two methyl groups are pure. These are observed at 1460–1430 cm^{-1}. Most bands in frequency region 1450–1300 cm^{-1} are highly mixed. The bands at 1282 cm^{-1} belong to the pure in-plane C-H vibrations of aromatic rings. The bands in 1235–1117 cm^{-1} are attributed to the in-plane deformation vibration of phenyl rings and skeletal in-plane deformations. The band at 886 cm^{-1}, belongs to the C-H out-of-plane vibration of aromatic rings, could be described as pure vibrations. The IR bands at 856 cm^{-1} are assigned to the highly mixed γ(C-H) and aromatic γ(CCH). The out-of-plane vibrations of both –OH groups are found at 438 cm^{-1} [6, 8].

3.2. *Spectral data of metal complexes*

3.2.1. *UV-Vis spectra*

The comparative electronic absorption spectra of curcumin and its metal complexes in DMSO is shown in Fig. 3**.** The curcumin ligand showed a main absorption band of π-π* transition at 415–430 nm and compared to curcumin, the complexes showed maximum absorption shifted by (1–8 nm) which indicated the involvement of the carbonyl group of curcumin in metal complexation. The shoulders at (410–413 nm) and (448–454 nm) are attributed to a curcumin (L) → metal (M^{n+}) charge transfer specific complex formed [9]. We believe that the variation of the absorption peak of curcumin and shoulders in diferent complexes depend on the nature of metal (M^{n+}) ion. The spectral detail has been summarized in Table 1. The UV spectrum of $Eu(Curc)_3$ complex in acetone (Fig. 3) showed that the absorption maxima (419 nm) corresponds to π-π* transition also shifted to shorter wave length. The important feature of the spectra is the three weak absorption band at 586 nm, 615 nm and 634 nm which are due to the f-f transition of Eu and assigned as $^7F_1 \rightarrow {}^5D_1$, $^7F_1 \rightarrow {}^5D_0$ and $^7F_2 \rightarrow {}^5D_0$, further supported the complexation [10] (Fig. 4).

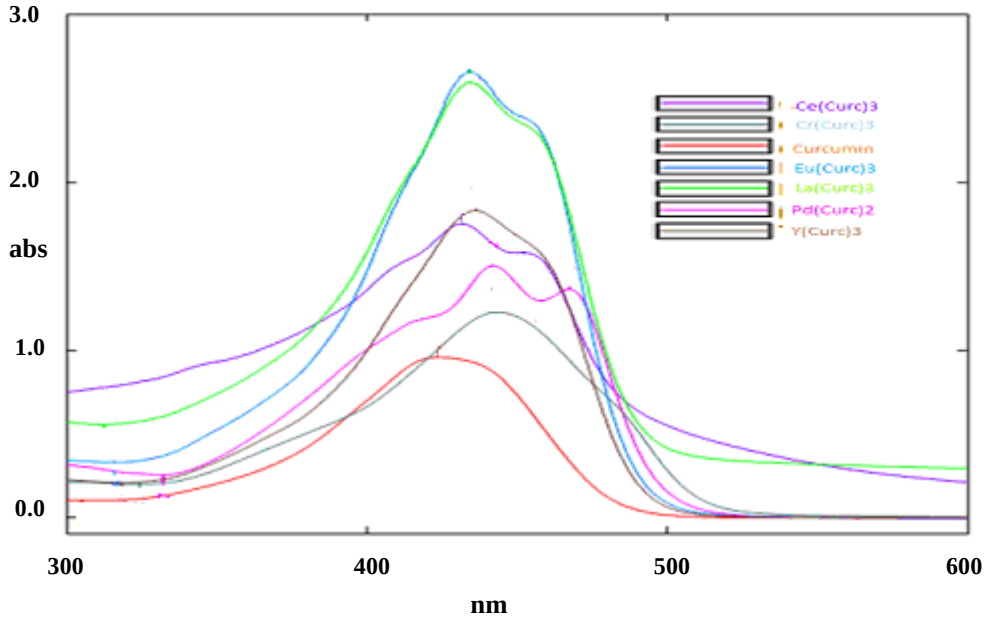


Fig. 3. Comparative UV-visible spectra of curcumin and its metal complexes.

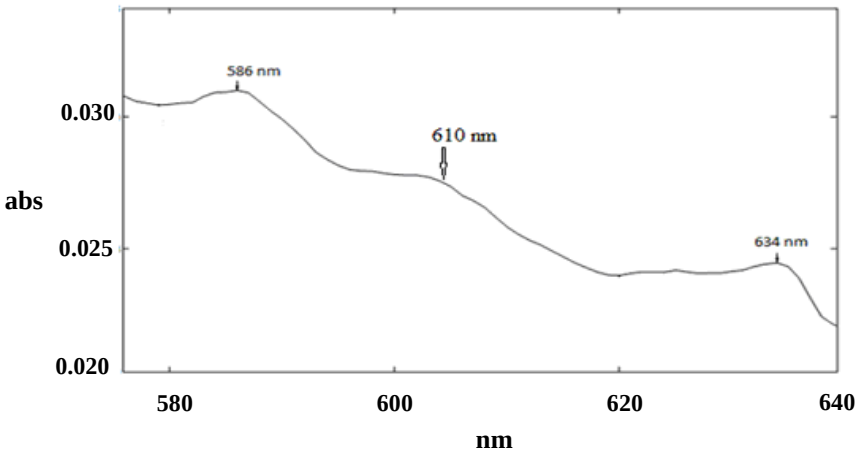


Fig. 4. UV-Vis spectra of $Eu(Curc)_3$ in acetone.

Table 1. UV-Vis Spectra data of curcumin and its complexes in DMSO.

Compound	UV-Vis (nm) peaks		
Curcumin	435 (λ_{max})	-	268
$Cr(Curc)_3$	444 (λ_{max})	480	375
$Y(Curc)_3$	437 (λ_{max})	460	271
$La(Curc)_3$	436 (λ_{max})	456	271
$Ce(Curc)_3$	432 (λ_{max})	452	264
$Eu(Curc)_3$	434 (λ_{max})	455	268
Pd(Curc)2	442 (λ_{max})	468	410

3.2.2. *FT-IR Spectrum*

The IR spectra of all complexes are similar, but different from that of the free ligand. A representative IR spectra of complex $Eu(curcumin)_3$ has been depicted in Fig 5. The spectral details are summarized in Table 2. The salient feature of solid state IR spectra of the compounds exhibited by the presence of O-H stretching, C-H stretching, C=O stretching, C=C stertching, C-O stretching in OCH_3 and phenol, and C-H stretching in aromatic. In complexes the bands due to C=O stretching should be shifted to lower frequencies during complexation. IR spectraum of ligand curcumin showed ν(C=O) at 1629 cm^{-1} and ν(C=C) at 1603 cm^{-1}, shifted to lower energy in the metal complexes of the

same ligand. And the broad band at 3200-3600 cm^{-1} may be due to the presence of water molecule in the compound. This suggest that the ligand coordinates with the central metal ions through the C=O group. The new bands occurred at 519-523 cm^{-1} furthur supported the formation of M-O band [6, 11, 12].

Table 2. IR spectrum data of curcumin and its complexes.

Compound	IR (cm^{-1})			
	ν(O-H)	ν(C=O)	ν(C=C)	ν(M-O)
Curcumin	3510, 3200-3500	1629	1603, 1511	-
$Cr(Curc)_3$	3200-3600	1624	1593, 1512	648
$Y(Curc)_3$	3200-3600	1622	1591, 1504	-
$La(Curc)_3$	3200-3600	1616	1587, 1495	520
$Ce(Curc)_3$	3200-3600	1618	1585, 1499	519
$Eu(Curc)_3$	3200-3600	1622	1589, 1501	522
$Pd(Curc)_2$	3200-3600	1624	1601, 1510	496

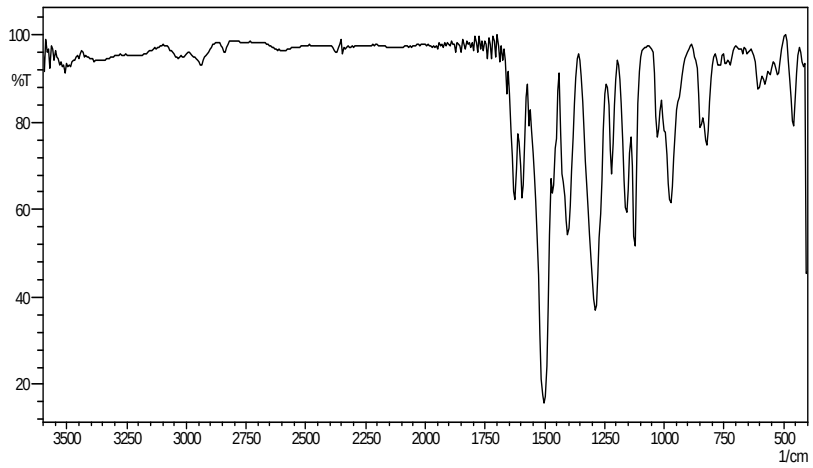


Fig. 5. FTIR spectra of $Eu(curcumin)_3$ complex.

3.3. *Antibacterial activity of the ligand and its complexes*

The antibacterial activity of the complexes are expressed as the diameter of growth inhibition area in mm. The metal complexes of curcumin exhibited some anti-bacterial property. Among the complexes, $Cr(Curc)_3$ had the most antibacterial activity against E. Coli (4 mm), Klebsiella pneumonia (3 mm) and Pseudomonas sp. (3 mm). The $Pd(Curc)_2$

also showed small inhibitory zone of 2 mm and 1 mm for E. Coli and Klebsiella respectively. $Y(Curc)_3$ complex showed a inhibitory zone of 1 mm and 1.5 mm against E. Coli and Pseudomonas sp., respectively. Thus $Cr(Curc)_3$ exhibited good antimicrobial activities against E. coli, Klebsiella pneumonia and Pseudomonas sp. than other metal-curcumin complexes. The other metal-curcumin complexes had little or no antibacterial activity. The antibacterial mechanism is presumably because the compounds affected the functions associated with cell division such as cell wall, protein, and/or DNA biosyntheses or killed the exponentially growing cells through binding to the protein synthesizing enzyme thus inhibited the protein synthesis for bacterial growth [13].

3.4. *DNA binding properties*

We have reported binding studies and effect of light on the conductance of intercalated curcumin into DNA [14]. In our recent studies on DNA-metal complexes (with phendione) interactions, DNA binding and damaging properties of complexes have been discussed [13]. The electrophoresis data of curcumin and its complexes after 1 h of incubation has been shown in Fig. 6. In the lane 1-4 which correspond to $Eu(Curc)_3$ (lane 1); $La(Curc)_3$ (lane 2); $Ce(Curc)_3$ (lane 3) and DNA control (lane 4), the intensity of the DNA-metal bands for the complexes are less than the DNA control and the intensity decreased in order of control DNA<La(III)<Ce(III)<Eu(III). The mobility of Eu(Curc)3 (lane 1) and $La(Curc)_3$ (lane 2) decreased compared to $Ce(Curc)_3$ (lane 3) and DNA control (lane 4). For the lane 5-8 which corresponds to $Pd(Curc)_2$ (lane 5); $Y(Curc)_3$ (lane 6); curcumin (lane 7) and DNA control (lane 8), it is also observed that the intensity of the DNA-metal bands for the Y(III) and Pd(II) complexes decreased compared to curcumin and DNA control. The mobility of $Pd(Curc)_2$, (lane 5) and $Y(Curc)_3$, (lane 6) are less than curcumin, (lane 7) and DNA control (lane 8). This decrease in intensity of the DNA bands can be explained in terms of intercalation of the complexes into DNA. Because of the intercalation of metal complex into DNA, ethidium bromide could not intercalate effectively to DNA and thus intensity due to the fluorescence of ethidium bromide became less. Furthermore, the decrease in mobility of the bands implies that because of the interacalation of complexes, the conformation of the DAN strands have been changed.

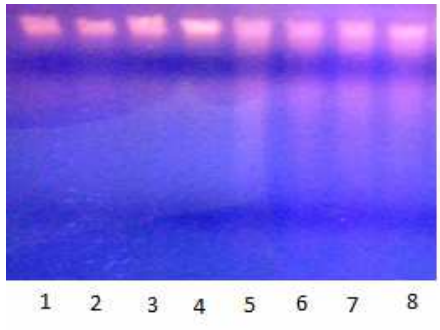


Fig. 6. Agarose gel electrophoresis pattern for the DNA binding studies of curcumin and its complexes after 1 h of incubation (1: $Eu(Curc)_3$; 2: $La(Curc)_3$; 3: $Ce(Curc)_3$; 4: DNA control; 5: $Pd(Curc)_2$; 6: $Y(Curc)_3$; 7: curcumin and 8: DNA control).

The electrophoresis diagram of curcumin and its complexes after 16 h of incubation has been shown in Fig. 7, where lanes 1-6 corresponds to DNA control (lane 1); $Eu(Curc)_3$ (lane 2); $Ce(Curc)_3$ (lane 3); $La(Curc)_3$ (lane 4); $Pd(Curc)_2$ (lane 5) and $Y(Curc)_3$ (lane 6). From the diagram, it is observed that the mobility of the DNA bands for the metal complexes increased with time. The mobility of metal-DNA band for Eu(III) (lane 2) is more than other and for the complexes of Pd (lane 5) and La (lane 4) had very similar mobilities. The least mobile band of the metal complexes has been found for the Ce complex (lane 3). It is also observed that the intensity decreased for the metal-DNA bands compared to DNA control. The intensity of the DNA-metal bands for La (lane 4) and Eu (lane 2) complexes are less than other metal complexes. These result for the increased mobility of the DNA bands of complexes can be rationalized by the DNA degradation effect exerted by the metal complexes.

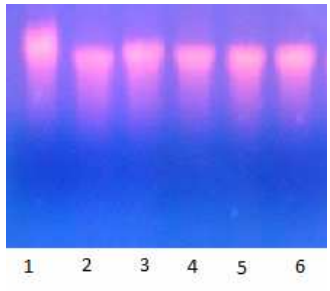


Fig. 7. Agarose gel electrophoresis pattern for the DNA binding studies of curcumin and its complexes after 16 h. of incubation (1: DNA control; 2: $Eu(Curc)_3$; 3: $Ce(Curc)_3$; 4: $La(Curc)_3$; 5: $Pd(Curc)_2$; 6: $Y(Curc)_3$).

From the above two experiments it is observed that when the icubation time is short, the metal complexes showed mainly the intercalate mood binding with DNA and when the incubation period is extended to 16 h, the metal complexes showed the more DNA cleaving activity. These results suggest that with time the degradative activity of the metal complexes increases. Among the complexes the Eu(III) complex showed better DNA cleaving activity while the Ce(III) has less effect.

4. Conclusion

Curcumin was extracted from turmeric and also synthesized by the condensation of vanillin and acetyl acetone. A comparative study of UV-Visible and FTIR spectra confirmed the structure of synthetic curcumin. The Cr(III), Y(III), La(III), Ce(III), Eu(III) and Pd(II) complexes of this ligand were synthesized. UV-Visible and FTIR spectroscopic methods were adopted for the confirmation of the synthesized complexes. The DNA

binding study of curcumin and its complexes through agarose gel electrophoresis suggested that the complexes had DNA cleaving ability and with time the degradative activity of the metal complexes was increasesd. Among the complexes the $Eu(Curc)_3$ showed excellent DNA cleaving activity. The antibacterial testing showed that the Cr(III) complex exhibited better antibacterial activity against E. Coli, Psydomonus and Klebsiella than other metal-curcumin complexes. The Y(III) and Pd(II) also showed a little activity against the testing bacterium.

Acknowledgement

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