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Original Article

GREEN SYNTHESIS AND EVALUATION OF BIOLOGICAL IMPACT OF Zn(II), Cd(II) AND Hg(II) COMPLEXES WITH PHENYLACETYLUREA AND BUTANOATE ION LIGANDS

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ABSTRACT

Objective: To prepare Zn(II), Cd(II) and Hg(II) complexes containing Phenylacetyl urea (PAU), and butanoate (but) ligands and to characterize physic-chemical, spectral and biological properties.

Methods: All the three metal complexes were prepared by the addition of required mole ratios of phenylacetylurea in methanol and sodium butanoate in ethanol to the methanolic metal nitrate solutions followed by microwave irradiation for ~10 seconds after each addition of ligands. The precipitated complexes were filtered, washed with ethanol and dried. The molecular formulae and probable geometry of the complexes were arrived at from the estimation of metals, electrical conductivities and electronic spectral data. The antimicrobial and anti fungal screening of the complexes against the bacteria, viz., *Staphylococcus sp, Escherichia coli, Shigella flexneri, Raoultella planticola, Pseudomonas aeruginosa* and fungi, viz., *Candida albicans, Aspergillus oryzae, Aspergillus niger, Aspergillus flavus and Aspergillus sojae* were carried out by well diffusion method. Antioxidantal activities were carried out by diphenylpicrylhydrazyl (DPPH) free radical scavenging method.

Results: The molecular formulae of the three complexes were arrived as $[Zn(PAU)(but)_2]$, $[Cd(PAU)(but)_2]$ and $[Hg(PAU)(but)_2]$. The antibacterial and anti fungal activities (diameter inhibition) of the complexes were in the range of 25-36 mm at 100 µg/ml concentration and 11-30 mm at 400 µg/ml concentration respectively. The percentage inhibition of DPPH free radical scavenging of Zn(II), Cd(II) and Hg(II) complexes were 44.13, 36.25 and 24.68 respectively.

Conclusion: The results indicate that all the three complexes are biologically active and the biological activities are higher than pure PAU and but ligands.

Keywords: Zn(II), Cd(II) and Hg(II) complexes, Phenylacetyl urea, Antibacterial, Anti fungal.

INTRODUCTION

Metal ions play an important role in biological processes [1-2] and today it is known that metal ions are essential ingredients of life. Especially, transition metal ions and their complexes are essential components in all living organisms [3]. Though first groups metals, namely, sodium and potassium and second group metals namely, magnesium and calcium in the periodic table are present in the biological systems in fairly large quantities, transition metals [4] are present in relatively small quantities. These metals play vital role and are called trace metals in biochemistry. Among the transition metals, chromium, manganese, iron, cobalt, nickel, copper, zinc and silver are widely found in the biological systems. They play the role of enhancing [5] biochemical activities by stabilizing various bio-molecules and active sites and serve as cofactor in many enzymes. Metal ions and their complexes find their use in drugs [6-7] for many diseases including microbial infections, cancer and neurodegenerative disorders. These metal ions are not usually present as free metal ions but in the form of complexes. The ligands involved in such metal complexes have the donor atoms like oxygen, nitrogen, sulphur and occasionally carbon.

Phenylacetylurea [8], commonly known as phenacemide in pharmaceutical science is an urea based drug used for controlling certain seizures in the treatment of epilepsy. This drug acts on the central nervous system to reduce the number and severity of seizures. It is used in the administration of controlling of severe epilepsy, particularly mixed forms of complex, but partial (psychomotor or temporal lobe) seizures, which are refractory to other anticonvulsants.

Phenylacetylurea based drugs accelerate the threshold for minimal electroshock convulsions and prevent the tonic phase of maximal electroshock seizures. It also prevents or modifies seizures induced by pentylenetetrazol and other convulsants.

Microwave assisted [9] preparation, an eco-friendly technique, has become popular and gained much attention in carrying out chemical transformations and in the preparation of compounds. The microwave irradiation technique used for carrying out chemical transformations is almost pollution free, low cost, less time consuming, effluent free and offer high yield [10-11]. The present work aims at the preparation of Zn(II), Cd(II) and Hg(II) complexes with phenylacetylurea and butanoate ion as ligands by microwave irradiation and characterization by physico-chemical, spectral and biological methods.

MATERIALS AND METHODS

Preparation of complexes

All the chemicals, viz. zinc (II) nitrate, cadmium(II) nitrate, mercury(II) nitrate, phenylacetylurea and sodium butanoate were of Analytical Reagent grade. The solvents acetonitrile, dimethylsulphoxide (DMSO), dimethyl formamide (DMF), ethanol and methanol were also of AnalaR grade and used as such. All the three metal complexes were prepared by the addition of required mole ratios of phenylacetylurea (0.60 g, 3.53 mmol; 0.58 g, 3.41 mmol and 0.65 g, 3.82 mmol) in methanol and sodium butanoate (0.75 g, 6.82 mmol; 0.72 g, 6.55 mmol and 0.80 g, 7.27 mmol) in ethanol to the methanolic solutions of Zn(II) nitrate, Cd(II) nitrate and Hg(II) nitrate (1.00g, 3.36 mmol; 1.0g, 3.24 mmol and 1.00g, 3.08 mmol) respectively, followed by microwave irradiation for ~10 seconds after each addition of ligands. Microwave oven model IFB 25PG1S was used for the preparation of complexes. The precipitated complexes were filtered, washed with ethanol and dried.

Characterization

The estimation of zinc, cadmium and mercury in the complexes was carried out by volumetric method by titration with EDTA.

Systronic 304 Conductivity meter was used for the conductance measurements of the 10^{-3} M complex solutions in acetonitrile solvent at 30°C.

The IR spectra of complexes and ligands were recorded on Schimadzu, FT-IR 8400 Spectrometer in 4000-400 cm^{-1} range by KBr pellet technique. Solid state UV-visible absorbance spectra of all the complexes were carried out by using Varian Make, CARY-5000 Model UV-vis Spectrophotometer.

The antibacterial activities of the ligands and their complexes were studied by well diffusion technique[12-13]. Twenty milliliters of sterilized nutrient agar (NA) media was poured into each of the pettri-dish. After solidification 0.1ml of test bacteria were spread over the medium using a spreader. The test complexes in measured quantities were dissolved in DMF to get concentrations of 25, 50, 75 and 100 µg/ml. Using sterile cork borer (6 mm in diameter), four holes were made in each dish, and then test compounds dissolved in DMF were poured into these holes. Finally, the dishes were incubated at 37 °C for 24 hours. At the end of 24 hours, the diameter of the inhibition zone detected around each hole was measured. DMF was used as the control and streptomycin was used as the standard drug under the same conditions for each organism. By subtracting the diameter of the inhibition zone of DMF from the complexes the antibacterial activities were calculated as a mean of three replicates.

The antifungal activities of the ligands and their complexes were studied by Agar plate technique. The complexes were directly mixed to the DMF in 100, 200, 400 µg/ml concentrations. The discs measuring 5mm in diameter were prepared from Whatman No.1 filter paper sterilized by dry heat at 140 °C for 1 hour. The sterilized discs were soaked with the fungus and were placed on the medium with the help of the inoculum needle. The plates were inverted and kept in an incubator at 27 °C for 72 hours. The inhibition zones thus formed were measured after 72 hours. Ketoconazole was used as the standard and the DMF served as a control. The growth of fungus was measured by recording the diameter of fungal colony as a mean of three replicates. The following equation was used for the calculation of the fungal growth inhibition on the presence of the complexes.

Percentage fungal growth inhibition = 100 * (C- T)/ C

Where, C is the diameter of fungal colony in control plate and T is the diameter of fungal colony in test plate.

Antioxidantal activities of the complexes and ligands were carried out by DPPH free radical scavenging method[14]. The complexes were dissolved in ethanol and 5% DMSO in various concentrations (10, 100, 250, 500 and 1000 µg/ml).Vitamin-C was used as a positive control. Assay mixture contained 500µl of the complex, 125µl DPPH (100µM) and 375µl solvent (5% DMSO). This mixture was incubated for 30 min at 25°C in dark condition. The absorbance was measured at 517 nm spectrophotometrically. All determinations were carried out in triplicate. The free radical scavenging activity was calculated from the equation,

Inhibition percentage = 100 * ($Abs_{control}$ - Abs_{sample})/$Abs_{control}$

RESULTS AND DISCUSSION

Physico-chemical properties

The complexes of the three metals were obtained as colourless precipitate. The yield of Zn(II), Cd(II) and Hg(II) complexes were 74.5%, 87.7% and 85.1% respectively. The zinc, cadmium and mercury metal contents[15] in the respective complexes were 15.04%, 24.03% and 36.13% as against the theoretical values of 15.97%, 24.63% and 36.83% respectively. The electrical conductivities [16] of Zn(II), Cd(II) and Hg(II) complexes were 96.01, 70.93 and 65.25 $ohm^{-1}cm^2mol^{-1}$respectively. The results are given in the table 1.

Table 1: Physico-chemical properties of Zn (II), Cd (II) and Hg (II) complexes

S. No.	Complex	Yield, %	Metal, %		Electrical conductivity, $ohm^{-1}cm^2mol^{-1}$
			Theoretical	Experimental	
1	[Zn(PAU) $(but)_2$]	74.5	15.97	15.04	96.01
2	[Cd(PAU) $(but)_2$]	87.7	24.63	24.03	70.93
3	[Hg(PAU)$(but)_2$]	85.1	36.83	36.13	65.25

From the metal percentage, the molecular formulae of the three complexes are arrived as [Zn(PAU)$(but)_2$], [Cd(PAU)$(but)_2$] and [Hg(PAU)$(but)_2$]. The electrical conductance measured was low, which indicate that the complexes are non electrolyte in nature and of 1:0 type.

IR Spectra

In IR spectrum[17]of the ligand PAU, the peaks obtained at the frequencies of 1180 cm^{-1}, 1672 $cm^{-1}$and 3389 cm^{-1} are assignable to the **ν**(C-N) stretching, **ν**(N-H) (secondary amine) bending and **ν**(N-H) (primary amine) stretching vibrations respectively. These vibrations were found almost at the same frequencies in all the three complexes.

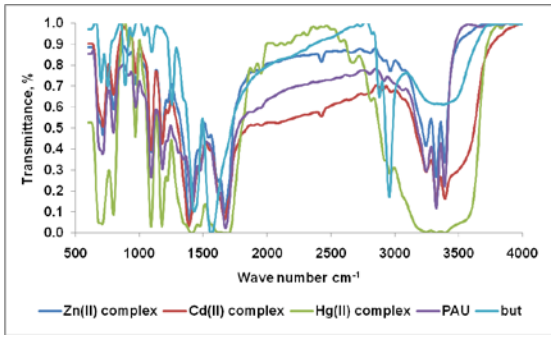

Fig. 1: IR spectra of Zn(II), Cd(II) and Hg(II) complexes, PAU and but ligands

In the IR spectrum of butanoate ligand, the peak appeared at the frequency of 1254 cm^{-1} is assignable to **ν**(C-O) stretching vibrations. This vibration was found shifted at the frequencies of 1386 cm^{-1}, 1386 $cm^{-1}$and 1410 cm^{-1} in Zn(II) complex, Cd(II) complex and Hg(II) complexes respectively.

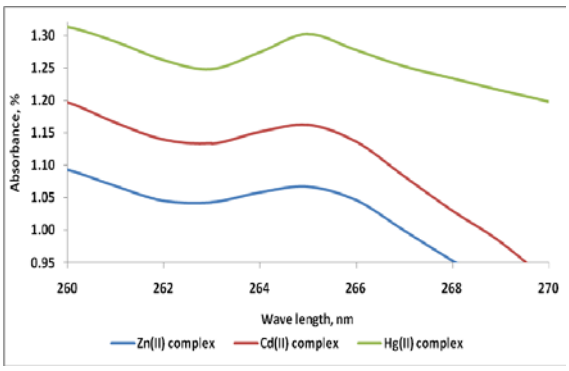

Fig. 2: UV spectra of Zn(II), Cd(II) and Hg(II) complexes

The above IR spectra study ensures the entry of both PAU and but ligands into the coordination sphere. The IR spectra of the complexes and the ligands are given in fig.1.

UV spectra

UV absorption frequencies obtained ~265 nm for all the three Zn(II), Cd(II) and Hg(II) complexes indicate that charge transfer transition. The UV spectra of the complexes are given in fig.2.

Antibacterial activity

All the three complexes exhibit enhanced antibacterial activity[18] than the pure ligands. Streptomycin was used as standard reference. $[Zn(PAU)but)_2]$ exhibits higher activity than $[Cd(PAU)but)_2]$ and $[Hg(PAU)but)_2]$.

Zn(II) complex shows higher activity against *Escherichia coli and Pseudomonas aeruginosa* than Cd(II) and Hg(II) complexes. Hg(II) complex exhibits higher activity against *Staphylococcus sp.* than the other two complexes.

The higher antibacterial activities of the three complexes may be attributed to the toxic nature of the metal itself. However the antibacterial activities of all the three complexes are low when compared to those of the standard, streptomycin. The mean diameter inhibition in mm at 100µg/ml is presented in table 2. and in fig.3.

Table 2: Antibacterial activities of Zn(II), Cd(II) and Hg(II) complexes

S. No.	Complex/Ligand	*Staphylococcus sp*	*Escherichia coli*	*Shigella flexneri*	*Raoultella planticola*	*Pseudomonas aeruginosa*
1	$[Zn(PAU)(but)_2]$	30±0.29	34±0.23	32±0.24	30±0.23	36±0.26
2	$[Cd(PAU)(but)_2]$	25±0.18	33±0.21	29±0.17	32±0.26	28±0.23
3	$[Hg(PAU)(but)_2]$	34±0.17	28±0.17	26±0.15	30±0.17	32±0.22
4	PAU	8±0.06	8±0.12	13±0.10	6±0.06	12±0.12
5	but	9±0.08	11±0.14	16±0.12	13±0.12	19±0.17
6	Streptomycin (standard)	46±0.35	34±0.29	47±0.33	48±0.30	38±0.29

Results are in mean±SD

Table 3: Anti fungal activities of Zn(II), Cd(II) and Hg(II) complexes, PAU and but ligands and ketokonazole

S. No.	Complex/Ligand	*Candida albicans*	*Aspergillus oryzae*	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus sojae*
1	$[Zn(PAU)(but)_2]$	14±0.23	12±0.12	17±0.13	11±0.12	17±0.23
2	$[Cd(PAU)(but)_2]$	20±0.21	13±0.15	18±0.17	22±0.19	13±0.17
3	$[Hg(PAU)(but)_2]$	28±0.22	30±0.23	20±0.22	13±0.15	18±0.17
4	PAU	4±0.08	7±0.09	11±0.15	12±0.13	10±0.12
5	but	17±0.12	19±0.21	15±0.17	19±0.18	16±0.12
6	Ketoconazole (standard)	86±0.36	91±0.32	94±0.30	89±0.40	92±0.35

Results are in mean±SD

Table 4: DPPH free radical scavenging activities of Zn(II), Cd(II) and Hg(II) complexes, PAU and but ligands and vitamin-C

S. No.	Concentration µg/ml	Zn(II) complex	Cd(II) complex	Hg(II) complex	PAU	But	Vitamin-C (standard)
1	1000	44.13±0.09	36.25±0.00	34.68±0.55	12.09±0.65	6.89±0.73	-
2	500	26.92±0.46	27.31±0.09	21.58±0.65	10.01±0.73	5.13±1.02	90.23±0.92
3	250	12.91±0.92	14.47±0.55	9.45±0.09	7.14±1.75	4.06±0.36	92.03±0.21
4	125	10.43±2.21	12.32±0.28	7.63±1.20	6.01±0.44	3.45±0.22	93.22±0.37
5	62.5	9.06±0.83	9.84±0.28	7.17±0.55	4.96±0.51	3.06±0.15	93.09±0.55
6	31.3	8.80±1.01	9.52±0.00	7.50±1.75	4.47±0.22	2.65±0.58	88.92±0.90
7	15.6	8.40±0.46	6.65±0.00	6.85±0.28	4.11±0.58	2.24±0.29	75.23±1.84
8	7.81	6.98±0.27	5.48±0.18	6.91±0.18	4.06±0.36	2.21±0.22	38.72±1.84
9	3.90	6.0±0.92	5.15±0.28	6.71±0.09	4.06±0.36	2.16±0.58	25.68±1.84
10	1.95	4.11±0.09	5.35±0.18	6.65±0.18	3.91±0.58	1.85±0.15	11.99±0.92
11	0.98	3.65±0.00	5.41±0.65	6.32±0.08	-	-	-

Results are in mean±SD

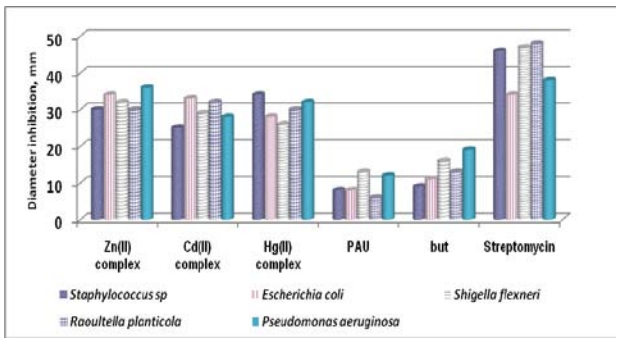

Fig. 3: Antibacterial activities of Zn(II), Cd(II) and Hg(II) complexes, PAU and but ligands and streptomycin

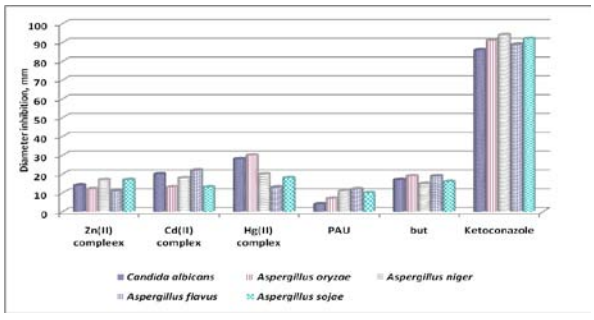

Fig. 4: Comparison of anti fungal activities of Zn(II), Cd(II) and Hg(II) complexes, PAU and but ligands and ketokonazole (standard)

Anti fungal activity

All complexes show lower anti fungal activity[19] than the standard, ketoconazole. [Hg(PAU)(but)$_2$] exhibits higher anti fungal activity than [Zn(PAU)(but)$_2$] and [Cd(PAU)(but)$_2$]. The anti fungal activity of PAU is less than sodium butanoate. The anti fungal activities of Hg(II) complex against *Staphylococcus sp. and Escherichia coli* are higher than those of Zn(II) and Cd(II) complexes. However, the anti fungal activity of the standard, ketokonazole is much higher than the three complexes. The mean diameter inhibition in mm at 400µg/ml is represented in table 3 and in fig.4.

Antioxidantal activity

All the three complexes show lower inhibition of DPPH free radical scavenging activity [20]

at a concentration of 1000 µg/ml than the standard, Vitamin-C. The inhibition percentage of Zn(II), Cd(II) and Hg(II) complexes are 44.13, 36.25 and 34.68 respectively. The inhibition percentage at various concentrations of complexes and ligands are given in Table 4 and in fig.5.

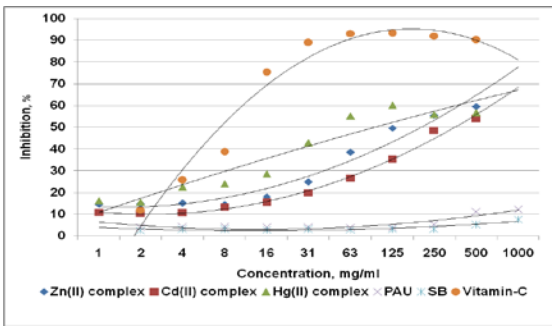

Fig. 5: DPPH free radical scavenging activities of Zn(II), Cd(II) and Hg(II) complexes, PAU, but and Vitamin-C

CONCLUSION

The molecular formulae were arrived at for the complexes as [Zn(PAU)(but)$_2$], [Cd(PAU)(but)$_2$] and [Hg(PAU)(but)$_2$] from the analytical data, UV-visible and IR spectral studies.

All the three complexes were having enhanced antibacterial activities than the pure ligands. [Zn(PAU)(but)$_2$] exhibited higher antibacterial activity than [Cd(PAU)(but)$_2$] and [Hg(PAU)(but)$_2$]. Zn(II) showed higher activity against *Escherichia coli and Pseudomonas aeruginosa* than Cd(II) and Hg(II) complexes. Hg(II) complex exhibited higher activity against *Staphylococcus sp.* than the other two complexes. The toxic nature of the metal ion may be the reason for higher antibacterial activities of the three complexes. However the antibacterial activities of all the three complexes were low when compared to those of the standard streptomycin. All complexes showed lower antimicrobial activities[16] than the standard ketoconazole. [Hg(PAU)(but)$_2$] exhibited higher anti fungal activity than [Zn(PAU)(but)$_2$] and [Cd(PAU)(but)$_2$]. The anti fungal activities of Hg(II) complex against *Staphylococcus sp and Escherichia coli* were higher than those of Zn(II) and Cd(II) complexes. However, the anti fungal activity of the standard, ketokonazole was much higher than the three complexes. All the three complexes showed low inhibition of DPPH free radical scavenging activity at a concentration of 1000 µg/ml when compared to Vitamin-C.

CONFLICT OF INTERESTS

Declared None

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REFERENCS

1. Shazia Rafique, Muhammad Idrees, Anwar Nasim, Haji Akbar and Amin Athar. Transition metal complexes as potential therapeutic agents. J Biotechnology and Molecular Biology Reviews 2010;5(2):38-45.
2. Jenny P Glusker, Amy K Katz and Charles W Bock. Metal ions in Biological Systems. The Rigaku J 1999;16(2):8-17.
3. Rosette M, Roat-Malone. Bioinorganic Chemistry-A short Course. 2nd ed. John Wiley & Sons Inc. New Jersey;2007. p.1-6.
4. Yogesh P Patil, Sachin H Pawar, Sharu Jadhav, and Jitendra S Kadu. Biochemistry of metal absorption in Human Body: Reference to check Impact of Nano Particles on Human Being. Int J of Scientific and Res Publications 2013;3(4):1-5.
5. Walaa H Mahmoud, Gegad G Mahamed, Maher M I El-dessouky. Synthesis, Characterization and in vitro Biological Activity of Mixed Transition Metal Complexes of Lornoxicam with 1,10-phenanthroline. Int J Electrochem Sci 2014;9:1415-38.
6. Warra A A. Transition metal complexes and their application in drugs and cosmetics – A Review. J Chem Pharm Res 2011;3(4):951-8.
7. Joshua A Obaleye, Adedibu C Tella and Mercy O Bamigboye. Metal Complexes as Prospective Antibacterial Agents. A Search for Antibacterial Agents. Varaprasad Bobbarala editor. InTech Janeza Trdine;2012. p.197-218.
8. Drug Bank: Phenacemide (DB01121). www. drugbank. ca/ drugs/DB01121.
9. Ravichandran S, Karthikeyan E.Microwave Synthesis-A Potential Tool for Green Chemistry. Int J of Chem Tech Res 2011;3:466-70.
10. Neha Yadav, Bhojak N. Microwave Assisted Synthesis Spectral and Antibacterial Investigations on Co (II) Complexes with Amide Ligands. Int J of Engineering and Sci 2013;2(2):166-8.
11. Oxana V Kharissova, Boris I Kharisov and Ubaldo Ortiz Méndez. Advances in Induction and Microwave Heating of Mineral and Organic Materials. InTech Europe;2011. p.346-7.
12. Gunasekaran P. Laboratory manual in Microbiology. New Age International Pub;2000. p.75-77.
13. Omar B. Ibrahim, Mahmoud A. Mohamed and Moamen S. Refat. Nano Sized Schiff Base Complexes with Cd(II), Co(II), Cu(II), Ni(II) and Zn(II) Metals. Synthesis, Spectroscopic and Medicinal Studies. J Canadian Chemical Transactions 2014;2(2):108-21.
14. Kaan Kucukoglu, Hayrunnisa Nadaroglu. Assessment of the antioxidant and antiradicalic capacities *in vitro* of different phenolic derivatives. J Free Radicals and Antioxidants 2014;4(1):24-31.
15. Mendham J, Denney R.C, Dbarners J, Thomas M and Shivsankar B. Vogels' Text Book of Quantitative Chemical Analysis. 6th ed. J Longman Scientific & Technical;2009. p.329.
16. Akrawi BA and Ali AMJ. The Electrical Conductivity of Some Transition Metal Complexes with 2,2`-Bipyridyl in Different Solvents at 298.16K. National J of Chemistry 2008;(31):491-500.
17. John Coates. In:R.A. Meyers editor. Interpretation of Infrared Spectra, A Practical Approach in Encyclopedia of Analytical Chemistry. J Ó John Wiley and Sons Ltd Chichester;2000. p.10815-37.
18. Paulina L Páez, Claudia M Bazán, María E Bongiovanni, Judith Toneatto, Inés Albesa, María C Becerra, and Gerardo A. Argüello. Oxidative Stress and Antimicrobial Activity of Chromium(III) and Ruthenium(II) Complexes on Staphylococcus aureus and Escherichia coli. J Bio Med Res Int 2013;1-7.
19. Dominika Hagel, Agata Paneth, Tomasz Pleach:Synthesis, Characterization and Antimicrobial Activity of 4-Benzoyl-1-(pyrazin-2-ylcarbonyl)-thiosemicarbazide. J of Single Molecule Res 2014;2(10):6-13.
20. Butu M, Rodino S, Butu A, Butnariu M:Screening of Bioflavonoid and Antioxidantal Activity of Lens Culinaris Medikus. Digest J of Nanomaterials and Biostructures 2014;9(2):519-29.