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Antibacterial, antifungal and enzymatic activities of azithromycin-heavy metal complexes: newly synthesized and characterized

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Abstract: As part of our continuous research to understand the interaction mechanism of drug and metallo-elements, heavy metal complexes of azithromycin (AZI) were synthesized with arsenic oxide, lead carbonate and silver chloride salts in molar ratio of 2: 1 (L: M). Synthesized heavy metal complexes have shown good percent yield and characterized through spectroscopic parameters including UV-Visible, TLC, FT-IR, NMR and elemental analysis (CHN). Spectroscopic characterization reveals the binding of ligand AZI with heavy metals in bi-dentate manner involving the hydroxide and 9a-NCH_3 group of the aglycone ring of AZI. These newly synthesized heavy metal complexes were evaluated for their antimicrobial response against selected gram positive and gram negative organisms and antifungal species. It was noted that all newly synthesized complexes exhibits increased activity against *B.subtilus* whereas, AZI itself didn't show any activity, while synthesized complexes have low to moderate response against all the studied organisms. Complex A-M12 possess greater enzymatic response against both urease and alpha chymotrypsin among all the studied complexes. Results obtained were then statistically analyzed through one way ANOVA and Dunnett's test by using SPSS version 20.0 suggesting the significant response of complexes against selected organisms.

Keywords: Azithromycin, heavy metals, elemental analysis, antimicrobial activity, antifungal activity.

INTRODUCTION

The group of antibiotics relating to the macrolides class has a profound impact against a variety of infections causing organisms as evident from various researches (Horcajada *et al*., 2012, Orvig and Abrams, 1999). They also plays a pivotal role in determining the extent and level of interactions involving the relevancy of biological targets like DNA, enzymes and protein receptors acting as ligand in coordinating/chelating with the metal ions present in the body, working as co-factor/enzyme (Chandraleka *et al*., 2014, Kirst, 1990, Patil *et al*., 2011). This mode of antibiotics has contributed well in designing metallodrugs/chelates exhibiting potential to have altered drug response. Therefore, the newer drug development advances have focused mainly on the synthesis of these metal complexes. As evident from recent researches, metals like platinum, gold, silver, molybdenum drugs (Yam and Lo, 1999) either alone or in chelation with antibiotics, exhibits potent anticancer, anti-leshmaniasis, antifungal and anti-urease activities (Tevyashova *et al*., 2015, Vladimirova *et al*., 2011).

In light of these evidences, use of coordination compounds in the field of medicinal chemistry having high toxicity profiling has restricted their common use. Therefore, the aim is to synthesize and characterize the metal-based drug complexes, who might have less toxicity profile (Alisir *et al*., 2017). The selection of a suitable ligand plays an important role in the field of coordination chemistry for achieving the modified complex having desired physicochemical, structural and spectroscopic characteristics (Tevyashova *et al*., 2019).

Azithromycin (fig. 1) an important member of the macrolide family, usually used in treating the infections of the upper respiratory tract (RTI's). Chemically, a macrolide having 15 membered aglycone ring, nitrogen substituted with CH_3 relating to its high stability against the acidic environment of the GI, resulting in good absorption than other members of the class. The lactone ring of azithromycin, substituted OH and NH_2 groups are involved in interacting with metal ions, affecting the bioavailability of the drug when co-administered with medications having multivalent cations and antacids (Al^{+2} and Mg^{+2}) (Alisir *et al*., 2017, Mahama and Songuigama, 2020). Previously work reported by Sher *et al*., 1996 and El-Rjoob *et al*., 2008 evident the interaction of AZI with Cu (II), Fe (II) and Fe (III) ion (Hamdan, 2003). As, our research group has reported the *in vitro* interaction of

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different classes of drugs with metal ions including cephalosporins, fluoroquinolones (Gul *et al*., 2013, Arayne *et al*., 2014, Sultana *et al*., 2010a, Sultana *et al*., 2010b) and macrolides (clarithromycin, erythromycin and roxithromycin) (Arayne *et al*., 2014, Sultana *et al*., 2002).

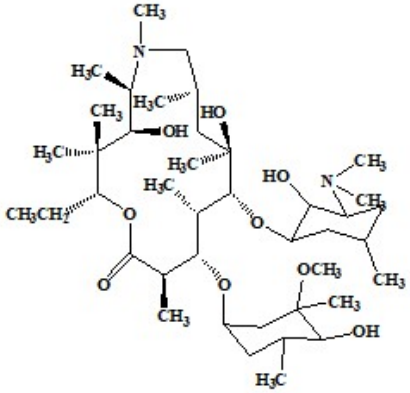

Fig. 1: Structure of Azithromycin

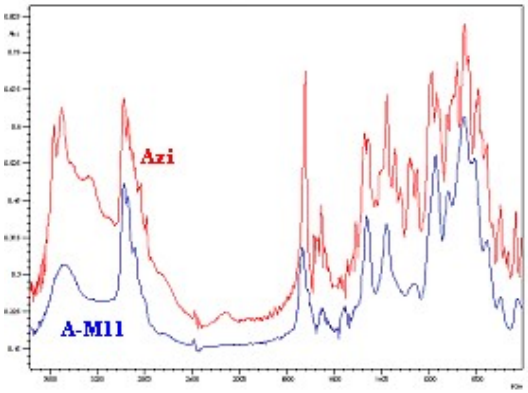

Fig. 2(a): Demonstrates FT-IR spectra of azithromycin-arsenic metal complexes (A-M11)

Our research group has already published the synthesis of AZI-metal complexes with essential and trace elements, having compromising biological activities (Arayne *et al.*, 2014). Here we are reporting the biological activities of newly synthesized heavy metal complexes of azithromycin with As_2O_3, AgCl and $PbCO_3$, which has not been reported previously. For synthesis, same method has been followed as reported for the Azithromycin-essential metal complexes. The complexes were then characterized by conductometric titration, FT-IR, NMR and CHN. Statistical studies were done by *one way* ANOVA and Post hoc Dunnett's test to determine the significant differences with the standard drugs.

MATERIALS AND METHODS

Materials, reagent and instruments: Azithromycin (AZI) was kind gift from Platinum Pharmaceuticals (Pvt.) Ltd. for this work, all other solvents and metallic salts (As_2O_3, AgCl and $PbCO_3$) used were of analytical grade. The spectroscopic characterization was done on FT-IR (Prestige-21 Shimadzu FTIR instrument) and ^{1}H-NMR spectra were recorded on Bruker AMX 400mHz using tetramethylsilane (TMS) as an internal standard. Elemental analysis was done on Carlo Erba 1106. For conductometric titrations Vernier Lab Pro™ was used having Logger pro 3.2 software.

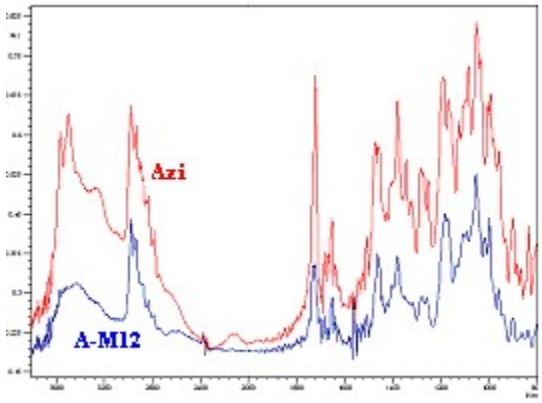

Fig. 2(b): Demonstrates FT-IR spectra of azithromycin-silver metal complexes (A-M12)

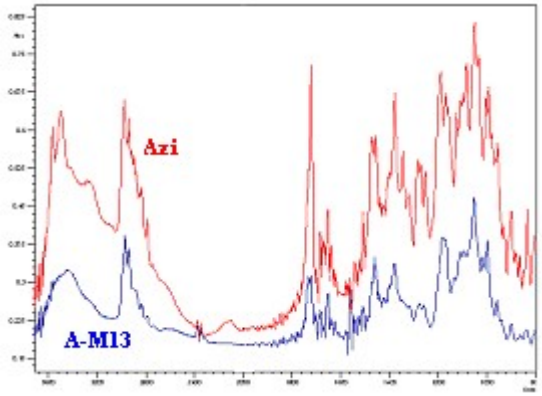

Fig. 2(c): Demonstrates FT-IR spectra of azithromycin-lead metal complexes (A-M13)

Molar ratio determination and synthesis of azithromycin heavy metal complexes

Molar ratio for the synthesis of AZI-heavy metal complexes determined was in the ratio of 2:1 by following the previously published conductometric method. Synthesis of the AZI with selected heavy metal salts was achieved by refluxing the drug and metal salt solution in methanol at 60°C for 3 hrs. The reaction mixture was then filtered and left for slow evaporation, the powder obtained were then characterized both physico-chemically and spectroscopically (Arayne *et al*., 2014).

Antimicrobial activity

Antibacterial activity against selected Gram-positive organisms such as Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus and Streptococcus features and Gram-negative organisms includes *Salmonella typhi, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas*

aeruginosa, Escherichia coli, Citrobacter and *Shigella flexneri* were screened for AZI-heavy metal complexes using a disc diffusion method (Printsevskaya *et al*., 2013) at 5, 10 and 20 mcg/ml. Disc soaked with either AZI / AZI-complexes were placed over agar plates streaked with microbial organisms, following incubation at 37°C for 24 hrs. Inhibitory effects on microbial growth were determinant as zone of inhibition (ZI), measured by using a digital Vernier caliper. All the results were obtained in triads of each concentration.

Antifungal activity

The process followed for the antifungal activity is similar as that of the antimicrobial against selected fungal species like *C. albicans*. The agar used is Sabraoud dextrose having changed incubation period of 48 hrs at 37°C in comparison to antibacterial activity (Gul *et al.*, 2013, Mahama and Songuigama, 2020, Saeed Arayne *et al.*, 2014).

Enzymatic profiling

Urease Assay

Standard method for urease activity by indophenols method has been followed to study the anti-urease activity of the complexes. At 630 nm, absorbance was determined by using Microplater reader (Molecular Device, USA) after 50 min having pH 6.8 and final volume of 200µL (n=3) in entire assay. Rates of change in absorbance were calculated by softMax Pro software (molecular Device, USA).

Formula for % inhibitions:

% inhibition=100-(OD test well/ OD control) ×100 (1)

Thiourea was used as the standard urease inhibitor (Akhtar *et al.*, 2019a, Akhtar *et al.*, 2019b, Sultana *et al.*, 2010a).

Alpha chymotrypsin assay

Inhibitory effects of these heavy metal complexes have been studied at 410 nm absorbance by Cannel's method using chymostatin as standard (Akhtar *et al.*, 2019a, Akhtar *et al.*, 2019b).

STATISTICAL ANALYSIS

Variance in microbial activities (*one way* ANOVA) of the complexes and its standards were studied on SPSS version 20.00 software along with Post hoc dunnett's test at $p \leq 0.05$ level of significance.

RESULTS

Physicochemical properties of AZI-heavy metal complexes are given in table 1, their spectroscopic characterization is explained in experimental and discussion section. Their antimicrobial and antifungal activities are given in table 2 (a & b) and 3 (a-c). Enzymatic profiling of these newly synthesized complexes reveals that complex A-M12 have highest response against the urease and chymotrypsin enzyme illustrated in fig. 4.

DISCUSSION

Conductometric studies

Conductometric titration was performed for the determination of stoichiometric ratio with heavy metals (Patel, 2017). From the conductance measurement data, it was achieved that complexation may occur in the ratio of 2:1 (L: M). Therefore, AZI-heavy metal complexes were synthesized in this experimental ratio.

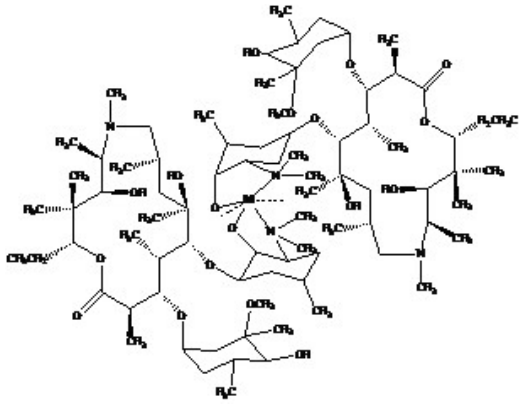

Fig. 3: Proposed structure of azithromycin-metal complexes.

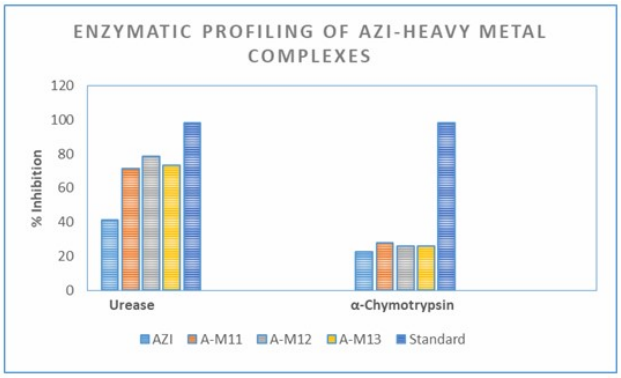

Fig. 4: Graphical illustration showing enzymatic profiling of AZI-heavy metal complexes against urease and alpha chymotripsin.

Synthesis of azithromycin-heavy metal complexes

Synthesis was achieved by adding dimolar alcoholic solution of azithromycin to a unimolar, alcoholic solution of heavy metal salts, following 3-4 hrs refluxing at 60±5°C (water bath), filtered and left for drying by slow evaporation on silica gel at room temperature. Later, dried depositions were taken, washed with methanol and re-dried. Their purity was determined by TLC. Percent yields, color, M.P and solubility of synthesized heavy metal complexes were noted (table 1). Examination of solubility of these complexes shows that they are insoluble in water but soluble in methanol and DMSO.

Table 1: Physical parameters and elemental analysis of azithromycin and its complexes

Compounds	Code	M. P^0C	Color	Yield (%)	C %	H %	N %	Metal %
Azithromycin		198	white	-	61.39 (61.41)	8.65 (8.70)	3.61 (3.65)	-
$[As(Azi)_2(H2O)_2]$ Cl_2	A-M11	139	white	79	55.92 (56.01)	9.39 (9.34)	3.37 (3.39)	1.43 (1.45)
$[Ag(Azi)_2(H_2O)_2]$ Cl_2	A-M12	158	white	55	54.99 (55.06)	9.04 (9.05)	3.30 (3.35)	2.29 (2.30)
$[Pb(AziI)_2(H2O)_2]$ CO_3	A-M12	170	white	63	54.48 (54.50)	9.40 (9.30)	3.22 (3.26)	4.01 (4.07)

Table 2(a): Antimicrobial activity of AZI-heavy metal complexes against selected gram positive organisms

Organism	*M. luteus*			*B. subtilis*		
Conc.	5 μgmL^{-1}	10 μgmL^{-1}	20 μgmL^{-1}	5 μgmL^{-1}	10 μgmL^{-1}	20 μgmL^{-1}
AZI	28.2±0.03	30.09±0.05	32.34±0.02	0±0	0±0	0±0
A-M11	8.21±0.07* 70.89	10.26±0.07* 65.9	12.02±0.01* 62.83	6.28±0.25*0	7.31±0.1*0	9.21±0.06*0
A-M12	1.21±0.18* 95.17	12.24±0.11* 59.32	14.27±0.19* 55.88	9.24±0.07*0	11.28±0.22*0	14.43±0.07*0
A-M13	10.35±0.13* 63.3	12.19±0.11* 59.49	14.06±0.04* 56.52	10.21±0.22*0	12.22±0.21*0	14.27±0.06*0
ANOVA (P<0.001), df = 3, 8.						
F- value	18989.47	31541.59	27631.25	2280.75	3630.62	45852.58

Table 2(b): Antimicrobial activity of AZI-heavy metal complexes against selected gram positive organisms

Organism	*S. features*			*S. aureus*		
Conc.	5 μgmL^{-1}	10 μgmL^{-1}	20 μgmL^{-1}	5 μgmL^{-1}	10 μgmL^{-1}	20 μgmL^{-1}
AZI	16.31±0.13	18.19±0.23	20.28±0.15	26.18±0.15	28.21±0.14	30.38±0.02
A-M11	11.31±0.11* 30.66	13.32±0.22* 26.77	15.25±0.15* 24.8	1.24±0.08* 95.26	12.19±0.12* 56.79	14.39±0.01* 52.63
A-M12	12.18±0.16* 25.32	15.2±0.19* 16.44	17.17±0.19* 15.34	13.18±0.18* 49.66	15.08±0.06* 46.54	17.23±0.03* 43.29
A-M13	14.13±0.09* 13.37	15.25±0.23* 16.16	16.35±0.15* 19.38	12.23±0.08* 53.28	14.31±0.1* 49.27	16.22±0.17* 46.61
ANOVA (P<0.001), df = 3, 8.						
F- value	928.39	251.81	534.754	9497.56	14283.03	21133.66

Mean ± S.D, % ZI * indicates significance and -ve sign shows increase in activity.

Elemental analysis of these complexes was also carried out and characterized by spectroscopic analysis as UV, IR and ^{1}H-NMR. These newly formed complexes were evaluated for their altered antimicrobial spectrum against series of Gram-negative, Gram-positives and antifungal species by the disc diffusion method.

Spectral studies

Infrared absorption studies

Initially, to identify the important bands of AZI involved in interaction with heavy metals, the spectra of the azithromycin was acquired. Several characteristics bands were observed in the spectra, among which few were observed to be indicative of metal-drug interactions. The spectra of AZI and its synthesized new complexes were then compared to have the complete characterization of involved spectral changes.

In AZI-heavy metal complexes, some important peak changes has been observed in terms of peak shift and intensities, determinant of azithromycin chelation with heavy metals. The main spectral bands involved are (i) diminished aliphatic amine stretch in complexes which is present at 1100cm^{-1} in AZI; (ii) peak shifting of 1200 cm^{-1} (AZI) to 1140-1160cm^{-1} (Complexes), (iii) reduced intensity and shifted maxima of OH band from 3550cm^{-1} (AZI) to 3500-3434cm^{-1} (Complexes) concluding the bidentate involvement of desosamine N $(CH_3)_2$ group and OH group o AZI in the complex formation (Robaina *et al.*, 2013, Tevyashova *et al.*, 2019) as given in fig. 2 (a-c).

^{1}H NMR studies

NMR structural analysis technique was acquired for having more accurate and conclusive picture of synthesized complexes. ^{1}HNMR spectra of AZI and its heavy metal complexes were obtained using DMSO as solvent. The spectra of the AZI found to be well agreed with the previously reported spectra, which were then compared with complexes. Changes in resonance were determinant of the absorption at (i) 3"-OCH_3 of cladinose sugar at 3.352 (3 H, s), 9a-NCH_3 (15-membered aglycone ring) at 2.316 (3 H, s) and the 3'-N $(CH_3)_2$ of desosamine sugar at 2.288 (6 H, s), respectively (Brennan and Barber, 1992, Zervou *et al.*, 2014).

^{1}H NMR spectra of metal complexes have the similar chemical shifts as that of AZI at 3.51, 2.43 and 2.29 except reduced intensities in signals of 9a-NCH_3 group of the 15-membered aglycone ring. Absence of intense signal at 2.327 ppm in AZI-heavy metal complexes was

Table 3(a): Antimicrobial activity of AZI-heavy metal complexes against selected gram negative organisms

Organism	*P. mirabilis*			*S. typhi*			*E. coli*		
Conc.	5 μgmL^{-1}	10 μgmL^{-1}	20 μgmL^{-1}	5 μgmL^{-1}	10 μgmL^{-1}	20 μgmL^{-1}	5 μgmL^{-1}	10 μgmL^{-1}	20 μgmL^{-1}
Azi	22.34±0.09	26.3±0.18	30.12±0.08	18.21±0.16	20.18±0.19	22.14±0.19	18.13±0.11	20.24±0.17	22.2±0.16
A-M11	10.17±0.0* 54.48	13.36±0.0* 49.2	16.22±0.1* 46.15	8.33±0.1* 54.26	11.25±0.05* 44.25	14.23±0.1* 35.73	9.41±0.09* 48.1	10.2±0.22* 49.6	13.35±0.11* 39.86
A-M12	14.27±0.0* 36.12	16.24±0.0* 38.25	18.22±0.1* 39.51	12.38±0.01* 2.02	14.3±0.11* 29.14	16.25±0.1* 26.6	13.38±0.05* 26.2	15.21±0.1* 24.85	17.29±0.14* 22.12
A-M13	13.1±0.11* 41.36	15.16±0.0* 42.36	18.25±0.1* 39.41	12.2±0.1* 33	11.24±0.11* 44.3	16.34±0.1* 26.2	9.17±0.12* 49.42	12.39±0.15* 38.78	16.18±0.11* 27.12
ANOVA (P<0.001), df = 3, 8.									
F- value	12690.43	9243.21	8499.83	2817.009	3310.955	1284.534	5978.19	2065.28	2307.74

Table 3(b): Antimicrobial activity of AZI-heavy metal complexes against selected gram negative organisms

Organism	*K. pneumonia*			*P. aeruginosa*		
Conc.	5 μgmL^{-1}	10 μgmL^{-1}	20 μgmL^{-1}	5 μgmL^{-1}	10 μgmL^{-1}	20 μgmL^{-1}
Azi	18.27±0.09	20.23±0.21	22.34±0.23	16.26±0.15	18.38±0.13	20.3±0.21
A-M11	0.27±0.09* 98.52	7.27±0.22* 64.06	12.17±0.08* 45.52	10.4±0.15* 36.04	13.27±0.07* 27.8	16.43±0.05* 19.06
A-M12	5.32±0.08* 70.88	7.22±0.17* 64.31	9.33±0.11* 58.24	14.25±0.2* 12.36	16.29±0.14* 11.37	18.25±0.17* 10.1
A-M13	8.29±0.17* 54.36	10.28±0.0* 49.18	12.17±0.1* 45.52	10.25±0.19* 36.96	14.19±0.13* 22.8	17.29±0.17* 14.38
ANOVA (P<0.001), df = 3, 8.						
F- value	13595.86	3655.93	4100.56	883.6	1037.67	312.9

Table 3 (c): Antimicrobial activity of AZI-heavy metal complexes against selected gram negative organisms

Organism	*S. flexneri*			*Citrobacter species*		
Conc.	5 μgmL^{-1}	10 μgmL^{-1}	20 μgmL^{-1}	5 μgmL^{-1}	10 μgmL^{-1}	20 μgmL^{-1}
Azi	20.37±0.09	22.23±0.05	24.35±0.12	8.18±0.2	1.36±0.07	14.18±0.13
A-M11	8.19±0.12* 59.76	10.21±0.1* 54.07	12.19±0.2* 49.94	10.07±0.02* -22.06	12.16±0.05* -19.1	14.37±0.08* -1.13
A-M12	10.34±0.1* 49.24	13.24±0.1* 40.44	15.15±0.0* 37.78	14.23±0.18* -72.84	16.38±0.12* -60.43	18.37±0.07* -29.28
A-M13	10.16±0.6* 50.12	12.28±0.1* 44.76	14.19±0.0* 41.72	12.33±0.07* -49.45	14.07±0.08* -37.81	16.2±0.24* -14
ANOVA (P<0.001), df = 3, 8.						
F- value	7234.14	5284.62	4169.4	5921.91	8683.2	12223.79

Mean ± S.D, % ZI * indicates significance and -ve sign shows increase in activity.

Table 4: Antifungal activity of AZI-heavy metal complexes

Organism	*C. albicans*		
Conc.	5 µgmL-1	10 µgmL-1	20 µgmL-1
Azi	9.31±0.15	12.35±0.07	15.25±0.14
A-M11	7.29±0.11* 21.53	8.36±0.12* 31.53	9.38±0.14* 38.41
A-M12	8.42±0.06* 9.36	12.32±0.12* -0.9	16.23±0.09* -6.57
A-M13	9.34±0.2* 10.54	11.11±0.08* 9.01	13.14±0.08* 13.72
ANOVA (P<0.001), df = 3, 8.			
F- value	3829.31	3570.92	4520.3

Mean ± S.D, % ZI * indicates significance and -ve sign shows increase in activity.

Table 5: Enzymatic profiling of AZI and AZI-heavy metal complexes

Enzymes	Urease		α-chymotripsin	
Complexes	Inhibition %	IC50±SEM (µm)	Inhibition %	IC50±SEM (µm)
AZI	41.3	0	22.56	0
A-M11	71.2	155±0.52	27.7	0
A-M12	78.8	169.23±0.25	26.05	0
A-M13	73.3	145.56±0.069	25.9	0
Standard[1,2]	98.3	21.00±0.2	98.1	5.7±0.13

[1] Thiourea, [2] Chymostatin

observed. While the shifting of signals from 3.12--3.30 (triplet) from quadruplet was found in AZI-heavy metal complexes as compare to AZI, confirming the bidendate chelation with heavy metals due to the involvement of OH and 9a-NCH_3 group of 15-membered aglycone ring (table 1 and fig. 3) (Al-Dmour *et al.*, 2019, Tevyashova *et al.*, 2016).

Microbiological screening for heavy metals-azithromycin complexes

Formed complexes were then evaluated for their antimicrobial spectrum against a series of Gram-positive, Gram-negative and antifungal species at three different concentration levels of complexes (5, 10 and 20µgmL^{-1}). One way ANOVA study was carried out to check any differences at ($p<0.001$)

Antibacterial activity

Against selected gram positive organisms

Variance study reveals significant differences ($p<0.001$) among synthesized complexes with AZI against *M. luteus* with order of inhibition at 5 µgmL^{-1} was *A-M13 >A-M11 >A-M12*, 10 and 20 µgmL^{-1} it is *A-M12 >A-M13 >A-M11*. Against *B. subtilis*, inhibition order at all concentrations was *A-M13 >A-M12 >A-M11* in comparison to AZI as it didn't show any activity against *B. subtilis.* Analysis against *S. features* reveals that all complexes showed significant increase ($p<0.001$) having inhibitory order of *A-M13 >A-M12 >A-M11* at 5 and 10 µgmL^{-1} while at 20 µgmL^{-1}, it was *A-M12 >A-M13 >A-M11*. At 5 and 20 µgmL^{-1} concentration levels, the order of inhibition was *A-M12 >A-M13 >A-M11*, whereas for 10µgmL^{-1} it was *A-M13 >A-M12 >A-M11* against *S. aureus* (table 2a & b).

Against selected gram negative organisms

Results against *P. mirabilis* have inhibition order of *A-M12 >A-M13 >A-M11* at 5 and 10 µgmL^{-1}, which was *A-M13 > A-M12 > A-M11* at 20 µgmL^{-1}. While against *S. typhi,* it was found to be *A-M12 >A-M13 >A-M11* at 5 µgmL^{-1}, A-M12 >A-M11 >A-M13 for10 µgmL^{-1} and *A-M13 >A-M12 >A-M11* for 20µgmL^{-1}. Inhibition order against both *E. coli* and *P. aeruginosa* was *A-M12 >A-M13 >A-M11*, respectively. Significant differences against *K. pneumonia* were determined to be *A-M13 >A-M12 >A-M11* for 5 and 10 µgmL^{-1} and *A-M11 >A-M13 > A-M12* at 20µgmL^{-1}. Dunnett's test and ANOVA were applied in combination and showed significant differences between all synthesized complexes with azithromycin against *S. flexneri* and *Citrobacter species* at 5, 10 and 20 µgmL^{-1} having order of inhibition as *A-M12 > A-M13 > A-M11* table 3(a-c).

Antifungal activity

ANOVA showed significant differences between all synthesized complexes with azithromycin against *C. albicans*, only as they were found to have no activity against other fungal species. Dunnett's test reveals that activity of complexes against *C. albicans* was found to be significantly decreased ($p<0.001$), at 5µgmL^{-1} it was *A-M13 > A-M12 >A-M11*. At 10 and 20µgmL^{-1} the order was *A-M12 > A-M13 > A-M11* (table 4).

Enzymatic profiling of Azi-heavy metal complexes

Newly synthesized AZI-heavy metal complexes/chelates has been determined against urease and α-Chymotrypsin (table 5, fig. 4) for their enzymatic profiling. Results obtained revealed that synthesized complexes exhibits

good-moderate activities against both studied enzymes (urease and α-Chymotrypsin,) in comparison to AZI and standards[1, 2] (thiourea and chymostatin).

CONCLUSION

The synthesized heavy metal complexes of azithromycin were obtained in good percent yield, which were then further characterized by spectroscopic techniques. Through characterization it has been confirmed that the hydroxide and 9a-NCH_3 group of the aglycone ring of AZI (15-membered) binds to metal ions, in bidentate manner. Microbiological evaluation of all the synthesized heavy complexes revealed that against Gram negatives *P. mirabilis, S. typhi, E. coli*, *P. aureogenosa, K. pneumonea, S. flexneri, Citrobacter* and *C.albicans* synthesized complexes exhibits reduced activity with respect to azithromycin. While against Gram positives *M.luteus*, *S. features* and *S. aureus* all complexes showed activity less than azithromycin. Except against *B. subtilus,* where all complexes have increased activity whereas azithromycin didn't show any activity. These newly synthesized AZI-heavy metal complexes exhibits good activity against both urease and α-Chymotrypsin enzymes. Complex A-M12 (silver complex) possesses the better activity among the complexes. Future emphasis and research on these complexes may found their place as specific inhibitors of urease and alpha chymotripsin.

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