Synthesis, Chracterization and Antimicrobial Studies of Chromium(II), Manganese(II) and Iron(III) Urea Complexes from Cow Urine

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Absract: Carbamide (urea) was extracted from cow urine and complexed with chromium (II), manganese (II) and iron (III) in a 1:3 metal-ligand ratio. The physicochemical parameters such as colour, melting point, pH, and solubility of the isolated urea crystals was determined at 37°C and compared with standard urea crystals. The characterization of the complexes was carried out using UV-VIS, FT-IR and XRD methods. There were shifts in the UV-VIS spectra of the metal salts from 350nm, 380nm and 360nm to 389nm, 383nm, and 389nm of the corresponding complexes respectively. These confirm the formation of complexes at those regions. The FT-IR spectra showed stretching symmetry and asymmetry frequency peaks V_s (NH_2) and V_{as} (NH_2), C=O and V (C-N) all stretched. The prepared complexes of Cr (II), Mn (II) and Fe (III) have a tentative coordinate points at O and N atoms. The X-ray diffraction pattern of the complexes showed sharp peaks at 2θ=30.1, 23.2 and 23.1which is a strong indication that they are crystalline in nature. The anti-bacterial study of the synthesized complexes indicated that they are potentially active against *Staphylococcus aureus* and *Escherichia coli*. Cr (II), Mn (II) and Fe (III) complexes were active at 50mg/ml concentration while all the complexes are active at 200mg/ml concentration.

***Keywords**: Carbamide, Physicochemical, Characterization, Symmetry, Asymmetry.*

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I. INTRODUCTION

Chemical synthesis (combination) is the artificial execution of useful chemical reactions to obtain one or several products (Vogel et al., 2013). This occurs by physical and chemical manipulations usually involving one or more reactions. In modern laboratory uses, the process is reproducible and reliable (Rafigue et'al., 2010). The synthesis of organic and inorganic compounds embraces an immense range of techniques and approaches. New organometallic molecules for example might require multi-step organic reactions in the successful production of ligands followed by precision handing and manipulation to form the desired complexes under anaerobic conditions (Suresh et al., 2020). Even though urine is nontoxic, it contains compounds eliminated by the body considered as undesirable, and irritating to the skin and eyes. The irritating odour of ammonia gas that diffuses out of urine is also a major threat to the environment and human health, especially the obstruction of pure – free atmospheric oxygen during respiration (Omar, 2012).

Cow urine is very much popular from the time of human civilization and is placed among high medicinal liquid because of its antibacterial, antifungal, antiviral, antioxidant and many other effects (Gurpreet and Rajiv, 2015). Besides its biological effects, it is popular as a fertilizer, source of urea, insecticidal, as a pesticide and other diverse uses. Cow urine contains 95% water, 2.5% urea, minerals, 24 types of salts, hormones and 2.5% enzymes. It also contains iron, calcium, phosphorus, carbonic acid, potash, nitrogen, ammonia, manganese, iron sulfur, potassium, uric acid amino acids enzymes, cytokine and lactose (Bhadauria, 2002). Carbamides, carbonyl diamides or the most famous name urea (CH_4N_2O) was first prepared by wohler in 1828 by evaporating a solution containing a mixture of potassium isocynate and ammonium sulphate. Ammonium isocynate which is formed first, undergoes molecular rearrangement to give urea as:

$$Pb(OCN)_2 + 2NH_3 + 2H_2O \longrightarrow Pb(OH)_2 + 2NH_4(OCN)$$

$$NH_4(OCN) \longrightarrow NH_3 + HOCN \longrightarrow (NH_2)_2CO$$

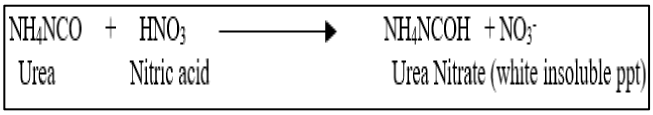
A coordination complex is the product of a lewis acid – base reaction in which neutral molecules or anions (called ligands) bond to a central metal atom (or ion) by coordinate covalent bonds. Coordination metal complexes are popular because of their multipurpose applications that extend to biomolecules chelating agents as therapeutics, coloring agents and analytical reagents (Strohfeldt, 2015). The metal complexes are formed as a result of interaction of the positively charged metal center with ligands that surround it, which in turn determines the properties of these complexes. Millions of coordination compounds have been synthesized from chemicals and reagents and studied over the years. However, with the rising costs of chemicals and calls for recycling of waste, it is necessary to explore other means of sourcing alternatives for chemicals and reagents.

II. MATERIALS AND METHOD

A. Sample Collection and Isolation Procedure

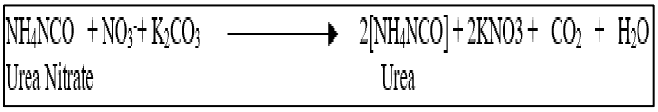
Early morning urine sample was collected from the dairy farm for four days into a Twenty-litre Yellow, transparent, clean and sterile sample bottle. The raw Cow urine (twenty liters) was poured into a clean stainless pot of about five liters capacity in a batch procedure to eliminate excess water. It was then concentrated by heating gently to a volume of 250 ml. The resulting 250ml concentrated urine was added to the first portion inside the beaker each containing 500ml of the concentrate for the first and second batch respectively. The Concentrated “Urine syrup” was allowed to cool at room temperature and was-vacuum filtered (Gurpreet, et al., 2015). The filtrate from the preceding step, which was suspected to contain urea was poured into a clean 1000 ml beaker and concentrated nitric acid was added slowly, with constant mixing, (the reaction is exothermic). Urea was recovered or regenerated from the urea nitrate by reacting the urea nitrate with concentrate potassium carbonate (K_2CO_3) in a clean 1000ml beaker. The urea- potassium carbonate solution was heated gently (not to boil) on a water bath to evaporate all of the water. Urea is separated from potassium nitrate based on their solubility difference in hot ethanol. The resultant mixture was filtered, and washed with iced-cold ethanol to further decolorize. The ethanolic solution of the urea was evaporated to a thick syrup. It was then recrystallized with a mixture of water and ethanol (1:3). This was heated again to evaporate the water and ethanol and allowed to cool at room temperature. The growing urea crystal was placed inside the freezer overnight. The urea crystal was scraped out and allowed to cool to room temperature. This was poured inside a clean transparent glass bottle for further use. The isolation follows these processes.

- *Urea Nitrate Formation*



$$\underset{\text{Urea}}{NH_4NCO} + \underset{\text{Nitric acid}}{HNO_3} \longrightarrow \underset{\text{Urea Nitrate (white insoluble ppt)}}{NH_4NCOH + NO_3^-}$$

- *Regeneration of Urea from Urea Nitrate*



$$\underset{\text{Urea Nitrate}}{NH_4NCO + NO_3^- + K_2CO_3} \longrightarrow \underset{\text{Urea}}{2[NH_4NCO]} + 2KNO3 + CO_2 + H_2O$$

B. Complexation of Cr, Mn and Fe-Urea Complex with Isolated Urea Crystals

The complexation of all the Urea-metal complexes was carried out following a modification of the synthesis described by Shatha *et al.* (2020). The dry chemical-free synthesis method was applied to synthesize isolated urea crystals. The complexes were prepared in 1:3 (Metal-ligand) ratio for $CrCl_3 \cdot 6H_2O$ (0.4529 g, 2mmol), $MnCl_2 \cdot 4H_2O$ (0.394 g, 2 mmol) and $FeCl_3 \cdot 6H_2O$ (0.476 g, 2 mmol). 3g of isolated urea crystals and 1g of each of Cr, Mn, and Fe were first weighed accurately using the AR2130, Ohaus Cooperation, Japan Weighing Balance, into a clean-dry 250ml beaker. It was then covered with porcelain to prevent any gas from escaping (Prablu et’al., 2014) The mixtures were heated in an oven between 60ºC to 70ºC with a drop wise addition of ammonia and stirred for about 2 hours simultaneously. The heating process continues at 60°C until colour change. Each mixture was refluxed for 3hours for each of the five complexes. On cooling at room temperature, colored compounds were extracted in every case. These complexes have been filtrated, washed with acetone as well as dried over anhydrous calcium chloride.

C. Characterization of Cr, Mn and Fe-Urea Complex

Instrumental methods were applied to characterize the UC and IUC formed. First, the UC crystal was characterized by FT-IR and UV. The metal-urea complexes obtained was further characterized spectrally using UV, FT-IR and XRD using the modified methods of Gongden et’ al(2020). These were all carried out at the Multi usher Laboratory A B U Zaria, Kaduna State, Nigeria.

III. RESULTS AND DISCUSSION

The physical properties of the synthesized urea crystals and pure urea crystals are shown in Table 1.

Table 1: Physical Properties of Pure Urea Crystals and Isolated Urea Crystals

Parameter	**Pure Urea Crystals**	**Synthesized Urea Crystals**
Water (27oC)	Very soluble	Very soluble
Ethanol (27oC)	Very soluble	Very soluble
Methanol (27oC)	Slightly soluble	Slightly soluble
pH at (27oC)	5.70	7.90
Refractive Index (n)	1.491	1.433
Melting Point (oC)	132- 36	135-141

Electrical Conductivity (27oC and atm pressure)	1.5 x 10-4	1.4 x 103
Colour and Particulate Nature	White Powder	Yellowish White needle like

The UV–vis spectrum of the pure urea crystals and synthesized urea crystals shows a distinct peak at 308.06 nm and 308.00 nm respectively.

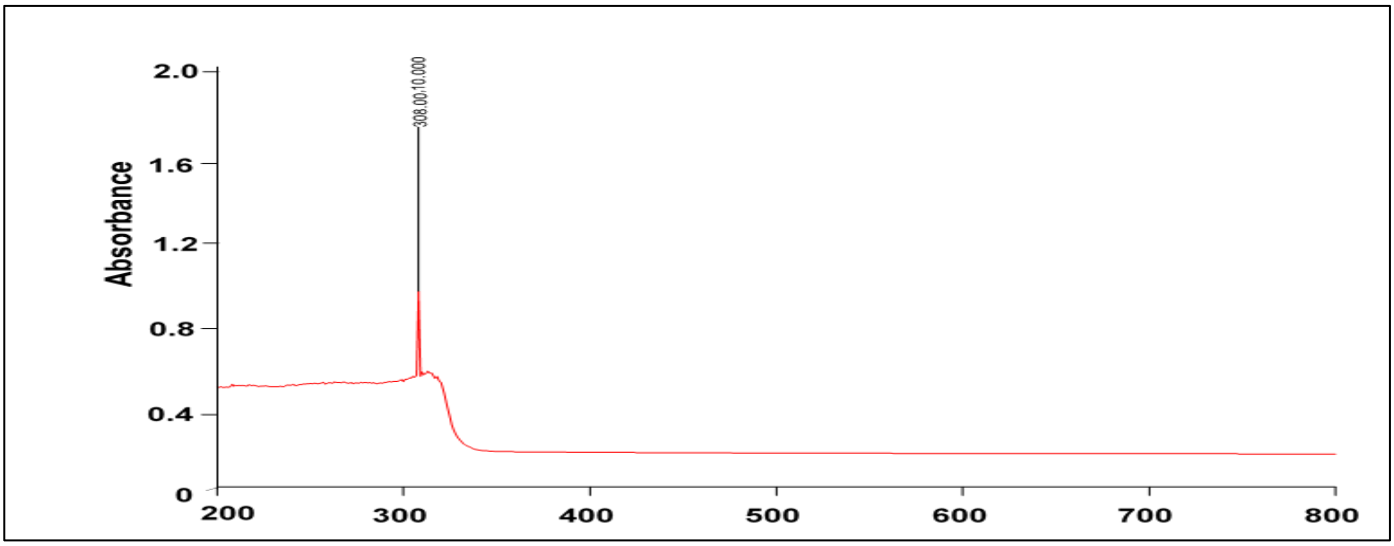


Fig 1: UV-Vis of Pure Urea Crystals

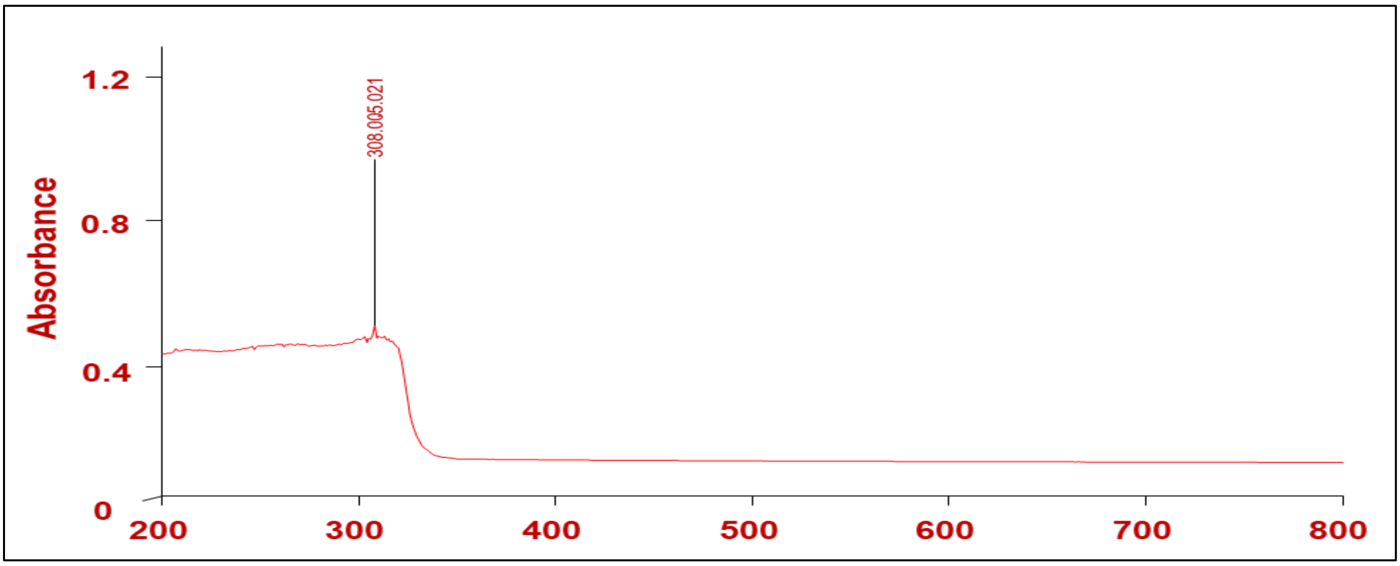


Fig 2: UV-Vis of Synthesized Urea Crystals

A. *UV-VIS spectra of Cr, Mn and Fe urea complexes*

- *Chromium*

The scanned UV-vis spectrum of the chromium (II) shows a peak absorption at 0.465 and a wavelength of 350 nm. The crystal is found to be violet in the region between 350-400 nm. This result is slightly different from the result of the scanned UV- vis spectrum of chromium complex (Figure 3) which shows absorptions of 0.501 and a wavelength of 389 nm. The crystal complex is found to be green in the region between 490-560 nm.

The slight difference may be attributed to Complexation with the ligand which shifted the wavelength from 350nm to 389nm, chromium salt is a silvery grey crystalline solid, while the chromium complex is a transparent crystalline solid, and this might be as a result of the ligand present in the chromium complex. This result is in agreement with the result of Yasmin et al., (2018) who studied synthesis, characterization and comparative thermal degradation of Co(II), Ni(II) and Cu(II) complexes with Asparagine and Urea as mixed ligands.

- *Manganese*

The scanned UV-vis spectrum of the manganese (II) shows a peak absorption at 0.760 at a wavelength of 380 nm. The crystal is found to be violet in the region between 380-435 nm. This result is slightly different from the result of the scanned UV-vis spectrum of the manganese complex (Figure 4) which shows absorptions at 0.740 at a wavelength of 383 nm. The crystal complex is found to be transparent in the region between 230-300 nm. The slight difference in their wavelength may be attributed to the difference in their appearance and Complexation with the ligand. The manganese salt is a pink crystalline solid while the manganese complex is a colourless crystalline solid.

- *Iron*

The scanned UV-vis spectrum of the pure shows a peak absorption at 0.740 at a wavelength of 360nm. It is found to be green in the region between 435-490 nm. The result is slightly different from the result of scanned UV-vis spectrum of iron complex (Figure 5) which shows absorbance at 0.650 at a wavelength of 389 nm. The crystal complex is found to be transparent in the region between (230-300 nm). The difference in their wavelength may attributed to the difference in their appearance, iron (III) salt is a green amorphous solid, while the iron complex is a purple-red crystalline solid, this might be as a results of the ligand present in the iron complex (Omar, 2012).

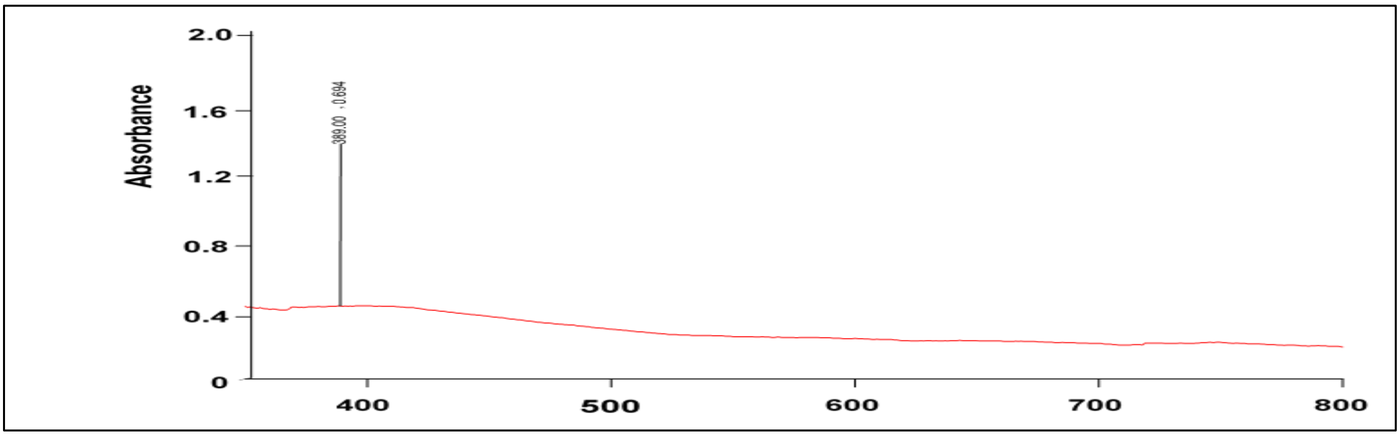


Fig 3: UV-vis Spectrum of Chromium (II) Complex

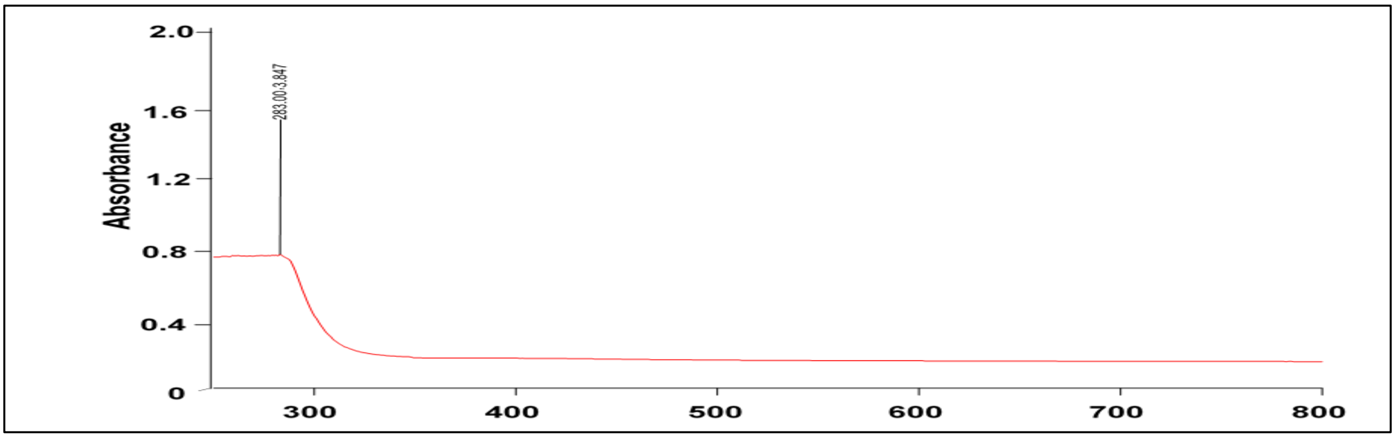


Fig 4: UV-vis Spectrum of Manganese (II) Complex

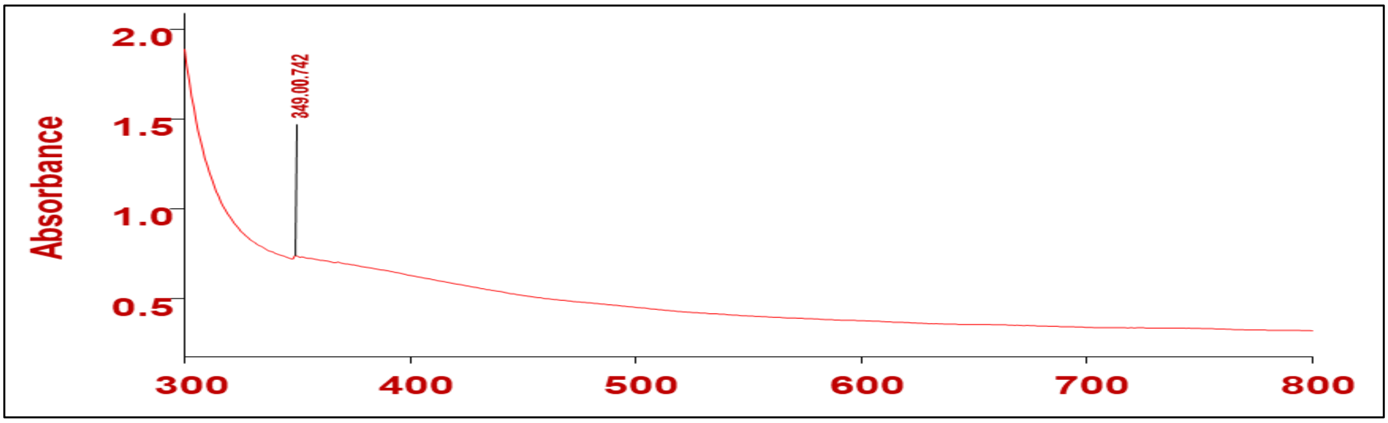


Fig 5: UV-Vis Spectrum of Iron (III) Complex

B. *FT-IR Spectroscopic Studies of Pure and Isolated Urea Crystals*

The FT- IR analysis of pure urea crystals and isolated urea crystals were recorded on FTIR-ATR (Shimadzu 8400S) utilizing KBr disc process and all samples were scanned over a range of 750-4000cm^{-1}. The IR spectra of pure urea crystals and isolated urea crystals are given in Figure 8 and 9 respectively. The FT-IR spectrum of the isolated urea crystals shows a dual peak at 2925.0cm^{-1} and 2903.0 cm^{-1} of N-H resulting to the symmetric stretch which requires slightly more energy (2925.0 cm^{-1}) for a transition. The second peak however is an antisymmetric stretch, which requires slightly less energy (2903.0 cm^{-1}). The symmetric and antisymmetric coupling observed at these energies may be due to the vibration of neighboring bonds in the compound, which correspond to the vibrational mode of N-H group of a secondary amide (Omar, 2012). The band which corresponds to -NH$_2$ stretching of PUC and IUC are 3405.2 cm^{-1}and 3398.5cm^{-1} respectively. These characteristic vibrational frequencies are reported in the literature by Gongden et al., (2020). An intense sharp peak occurred at 1687.8 and 1645.6 cm^{-1} for pure urea crystals and isolated urea crystals respectively which are attributed to the presence of the carbonyl group. The peak corresponding to vC-N is observed at 1221.0cm^{-1} and 1201.7 cm^{-1} for both crystals respectively.

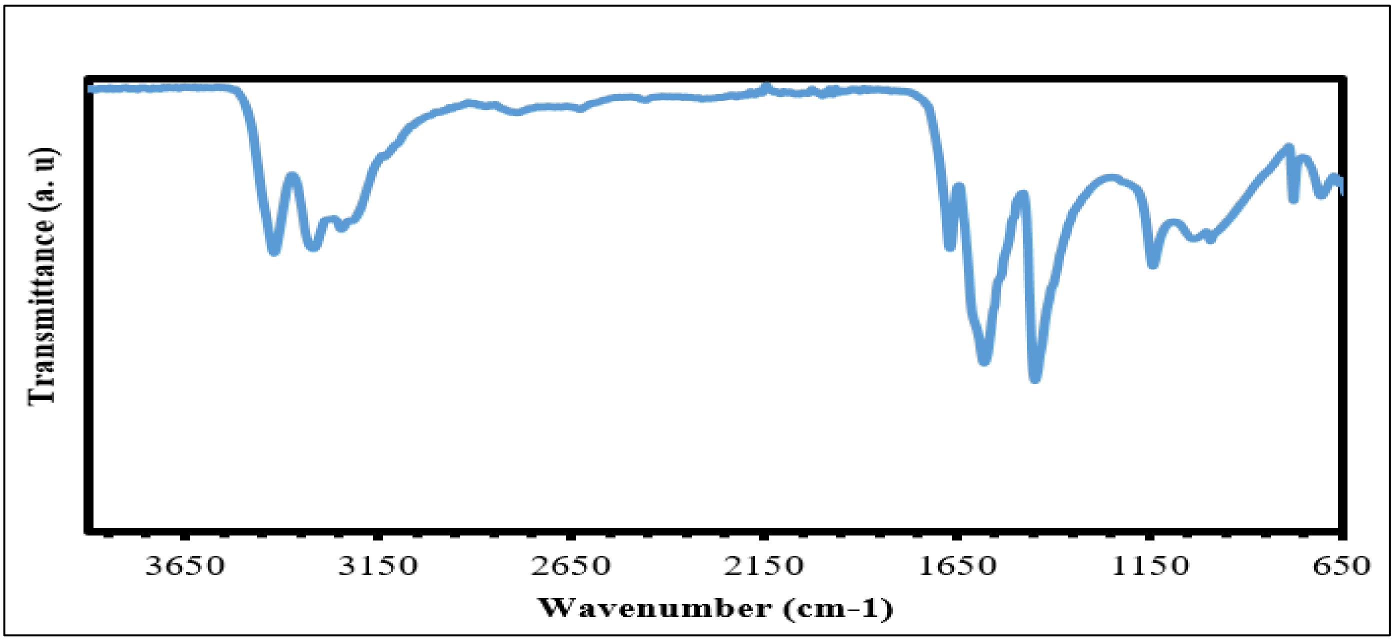


Fig 6: FT-IR Spectrum of Pure Urea

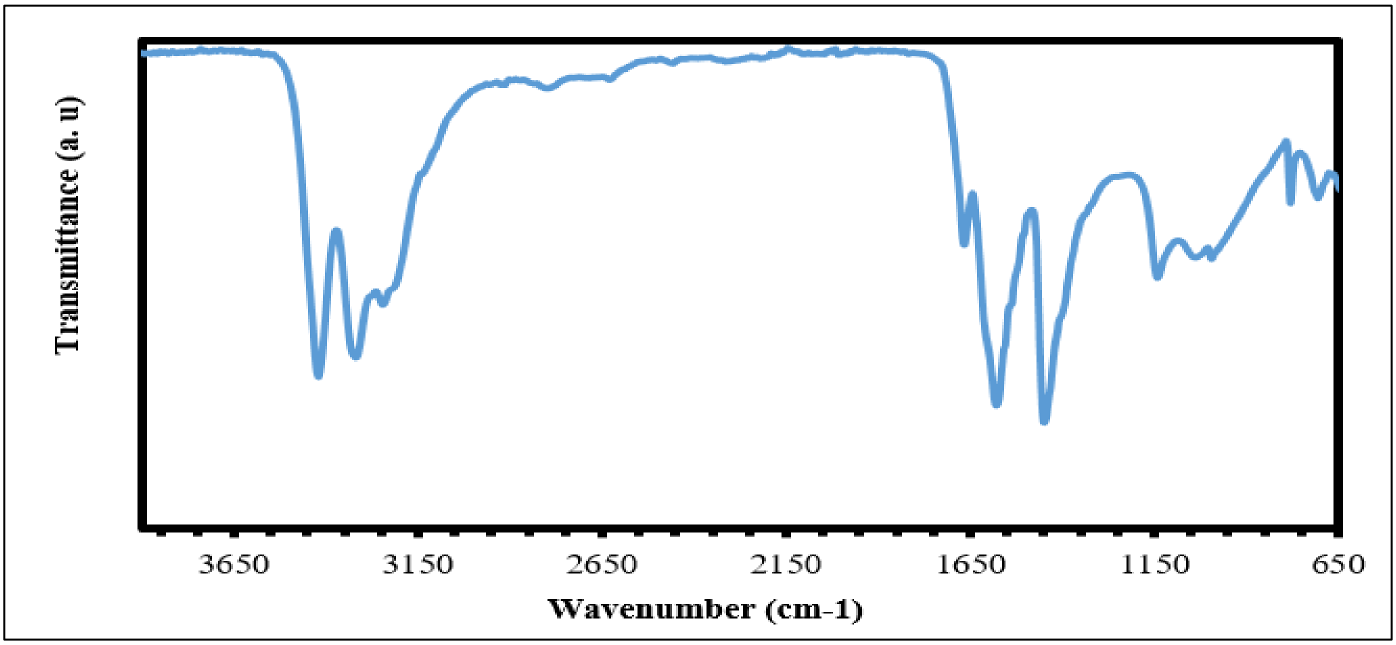


Fig 7: FT-IR Spectrum of Isolated Urea

C. FT-IR Spectroscopic Studies of Cr, Mn and Fe Urea Complexes

The FTIR spectra of the Cr, Mn and Fe-urea complex were recorded on FTIR-ATR (Shimadzu 8400S model) utilizing KBr disc process and all samples were scanned over a range of 650-4000 cm^{-1}. The FT-IR spectra are shown in figure 10-14 respectively. The band assignments have been given in comparison with the data obtained for the isolated urea, that is, uncoordinated. From Table 6, the bands assigning to vC-N of all the complexes shifted to lower wave number in comparison with that of isolated urea crystals (1201.7 cm^{-1}) as a results of coordination. This is in agreement with the literature of Yasmin et al. (2018). The complexes shows bands at 1640.0, 1606.5 and 1595.3 cm^{-1} respectively due to vC-O significantly higher than the carbonyl functional group of the free ligand, this is indicating that coordination occurs through nitrogen and oxygen atoms respectively (Omar, 2012).. From literature, the mode of coordination of urea with metal ions seems to be dependent upon the type and nature of metal, if a nitrogen-metal bond is present, the vibrational spectrum of the complex will be significantly different from the free urea molecule (Omar, 2012). The peak corresponding to N-H is observed at 3387.0, 3392.7 and 3300.7cm^{-1} respectively also occurred at lower wave number as compared with 2925.0 cm^{-1} of the free ligand which confirmed coordination of the metal-ligand complex, since the vibrational spectrum of nickel complex differs significantly from that of the isolated urea complex, there is possibility of the present of nickel-nitrogen bond this is in agreement with the literature of (Omar, 2012). The spectra of the complexes also shows higher wave numbers shifts of the bands assigned to NH_2 which indicates that the NH_2 group is imminently involved in the coordination since urea acts both as monodented and bidented ligand (pramod et'al., 2011).

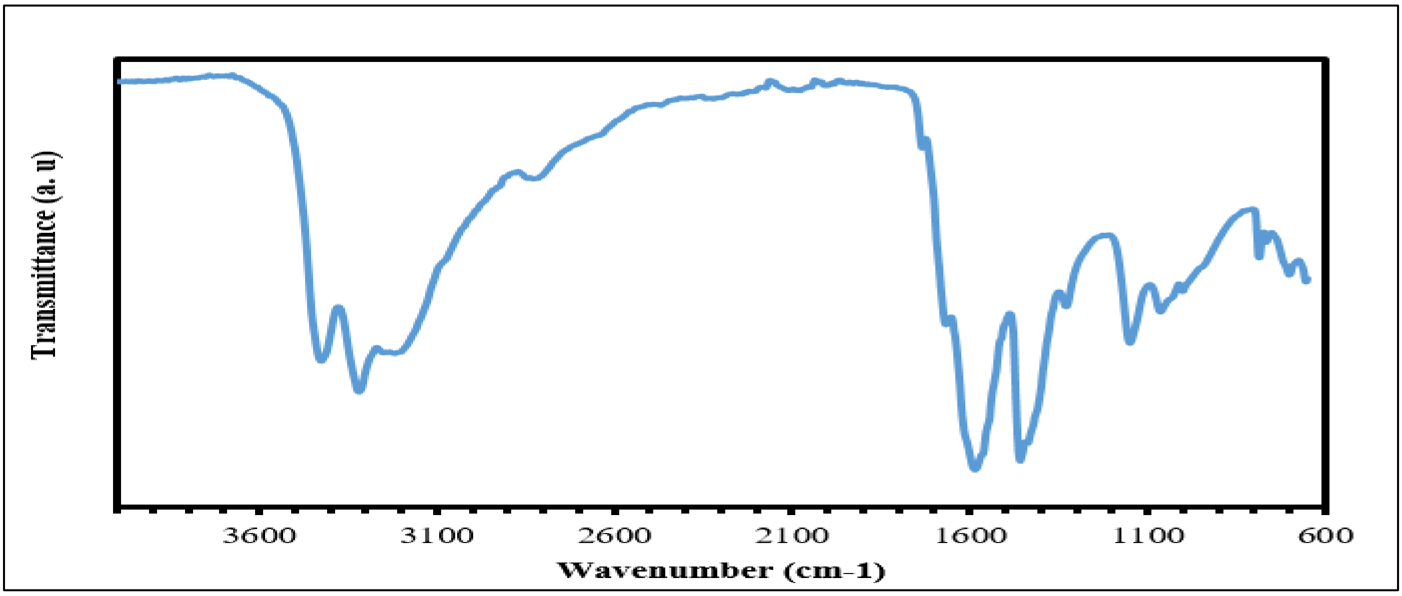


Fig 8: FT-IR Spectrum of Urea-Cr Complex

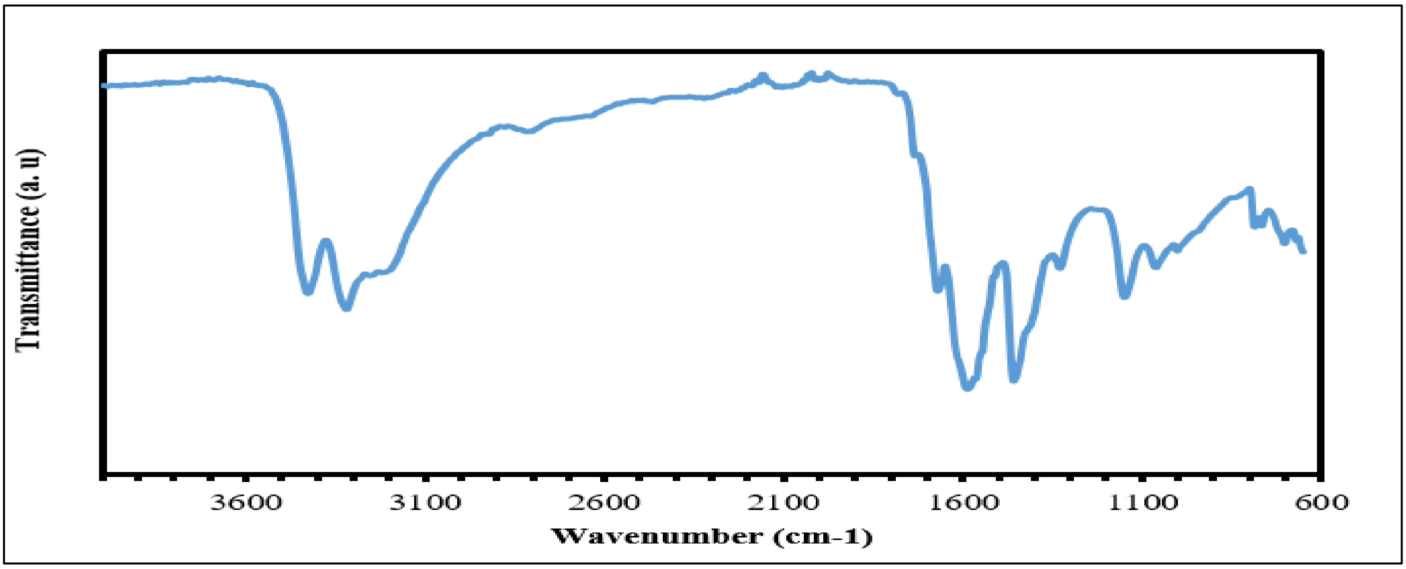


Fig 9: FT-IR Spectrum of Urea-Mn Complex

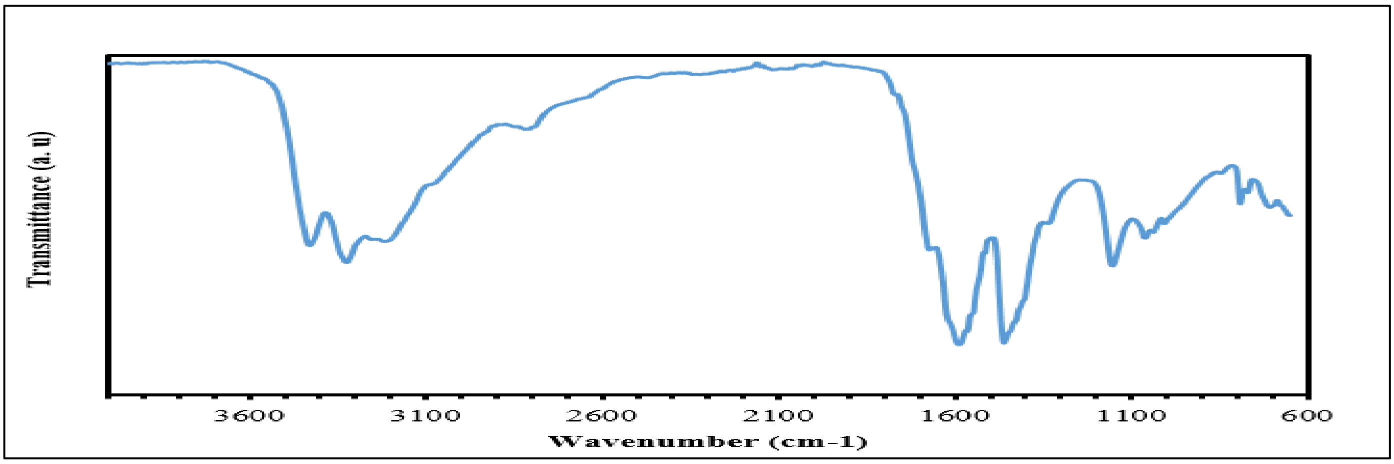


Fig 10: FT-IR Spectrum of Urea-Fe Complex

D. XRD Spectroscophic Analysis of Cr, Mn and Fe Urea Complexes

➢ *Chromium*

The XRD pattern of urea-chromium (II) chloride complex in Figure 15 showed the diffraction peak of the complex at about 30.10, which indicate that urea-chromium (II) chloride complex has been synthesized successfully. The size of the crystal was evaluated by using the Debye-Scherrer equation $D_s = \frac{K\lambda}{\beta cos\theta}$where θ is the Bragg's peak; κ is a constant that is dependent on crystal morphology, usually assumed as unity; β is the half-width of the diffraction peak; and lastly λ is the X-ray wavelength equal to 0.14507 nm. The average crystal size of urea-chromium (II) chloride complex has been calculated to be 18 nm using the Scherrer equation and Bragg's law, the average lattice space of the complex was obtained to be 0.3 nm. The peak at 2θ = 23.20 corresponds to the (81) reflection, while 2θ = 36.50 and 37.50 mainly from (59) and (51) reflections merged into a single peak. This revealed that the complex is crystalline in nature.

➢ *Manganese*

The XRD patterns of the synthesized urea-manganese (II) complex in Figure 16 showed that the phases present in the urea-manganese (II) complex are identified by comparison of the XRD pattern with the joint committee for powder diffraction standards (JCDPS) data. The XRD pattern of the complex shows an intense peak at 2θ = 23.20, which indicated that the urea-manganese is the main phase present in the complex. The peak that appeared at 2θ = 21.00 and 37.80 arises from the (156) and (203) reflections respectively. Using the Scherrer's equation, the average crystalline size of the complex has been calculated to be 14 nm and by using Bragg's law and 0.6 as the average lattice spacing of the synthesized urea-manganese (II) complex was obtained. The result of the complex was found to be crystalline in nature.

➢ *Iron*

The X-ray diffraction pattern of urea-iron (III) complex in Figure 17 showed that the background of the XRD pattern is small, the diffractive intensity is strong, and the result revealed that the complex has a fine crystalline state. The XRD pattern of the complex shows an intense peak at 2θ = 23.10, which indicated that the urea-iron is the main phase present in the complex. The index calculation of the X-ray diffraction data is based on the computer programs of least squares method. The results obtained revealed that the urea-iron (III) complex is a single multiphase compound and the crystal structure of the complex belongs to the polyclinic system which is in agreement with the literature of (Omar, 2012).

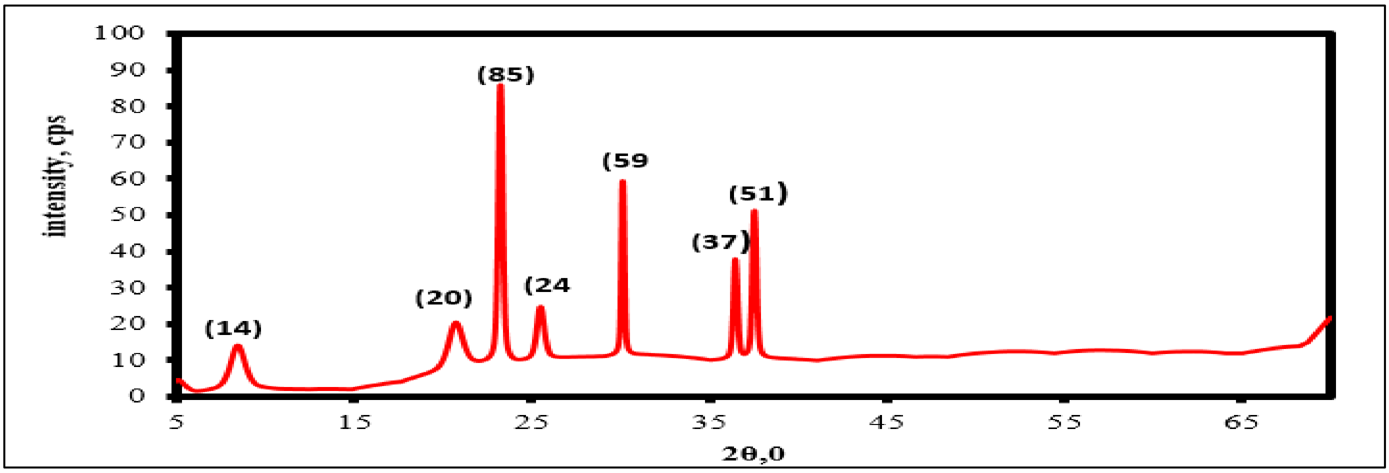


Fig 11: XRD Spectrum of Urea-Cr Complex

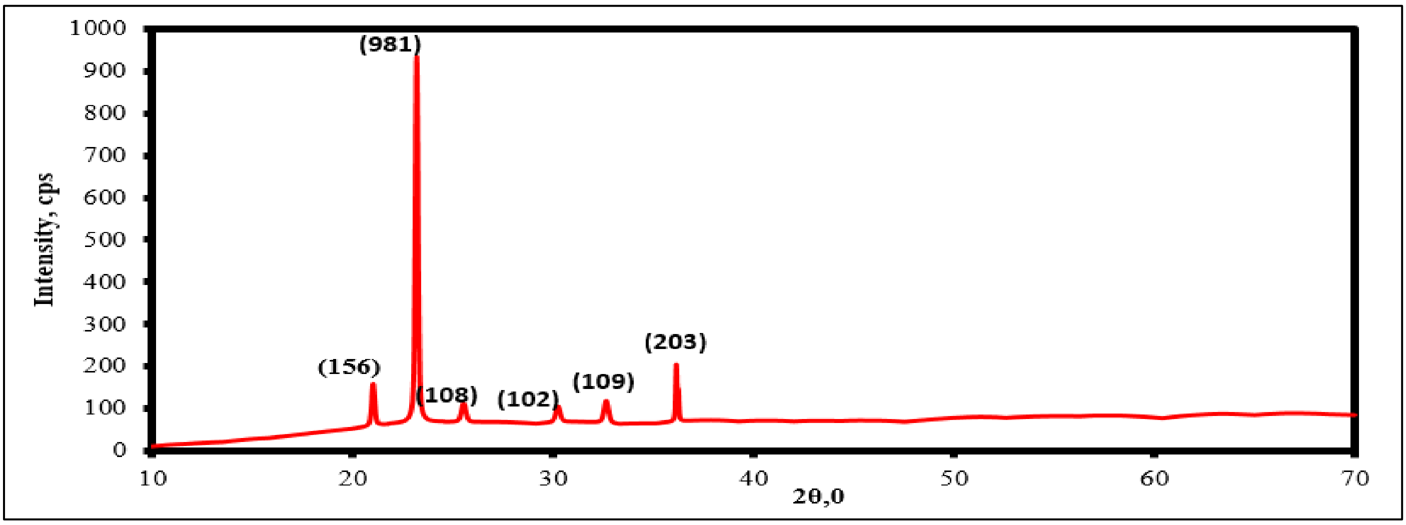


Fig 12: XRD Spectrum of Urea-Mn Complex

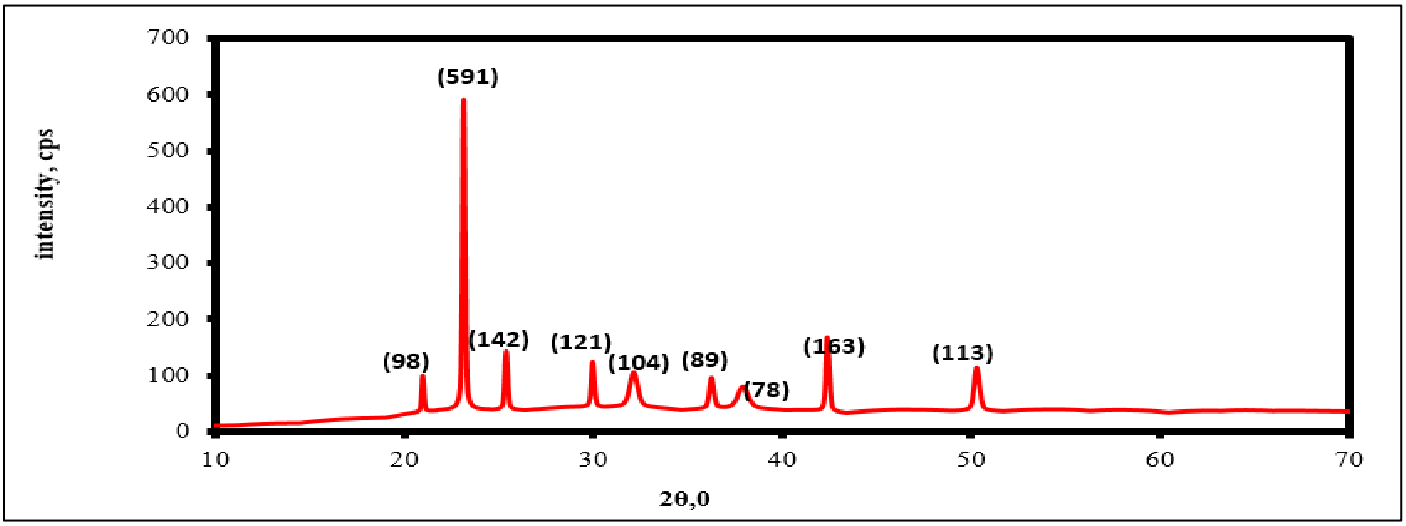


Fig 13: XRD Spectrum of Urea-Fe Complex

E. Antimicrobial Studies of CR, Mn and Fe-Urea Complexes

Metal complexes have been reported with enhanced biological activity (Matar et al., 2003). The antibacterial potency have been enhanced upon complexation as compared to neat ligand, thus opening new avenues in the fight against antibiotic resistance (Matar et al.,2003). Therefore, the antibacterial activities of these complexes were tested against gram positive (Staphylococcus aereus) and gram negative (Escherichia coli) bacteria.

The results shows zone of inhibition of all the metal complexes against Staphylococcus aereus and Escherichia coli. At lower concentration, no inhibition zone was found, whereas at concentrations of 50mg, 100mg and 200mg, most of the synthesized metal complexes show some level of inhibition varying in the ranges of 15.00 – 31.00 mm zone of inhibition. Literature reveal that for metal ions, geometry around metal ions and the counter ions impart great deal of effect on the inhibition of bacterial activities (Omar, 2012). The results for Escherichiacoli show that Urea-Fe complexes is found to be moderate. The reason may be attributed to geometries and presence of counter ions (Omar, 2012). In the case of their activities against Staphylococcus aereus, it has been observed that all the metal complexes show intermediate inhibition zone at higher concentration.

The results also shows that at higher concentrations all the complexes showed intermediate inhibitory zones for Staphylococcus aereus and Escherichia coli. Overall, it may be revealed that from the data obtained Urea-Cr, Urea-Mn, and Urea-Fe complexes are moderately active against the tested bacterial strains. The results for antibacterial activities show that the complexes inhibit bacteria growth significantly different from each other. These difference may be attributed to anion variation (Matar et al., 2003). It was already shown that the decomposition behavior and the formation constants of these complexes vary due to the presence of different anions. Also, the behavior of these complexes for the pyramidal geometry formation is varying due to anions presences (Liebowitz et al.,2013).

It has been suggested earlier that the oxidation state and apparent charge of the metal ion affects the biological activities. The apparent charge of the metal ion is partially shared with the donor ligands resulting in electron delocalization over the whole chelate system including the counter anions, thereby effectively influencing the solubility of the metal complex (Omar, 2012). It has been seen earlier that chelation make the ligands more powerful and potent bactericidal agents in comparison to either the metal ion or the uncoordinated ligand. The factors like solubility, conductivity, dipole moment and cell permeability are also responsible for enhancing the biological activity (Omar, 2012). In conclusion the synthesized complexes (Urea-Cr, Urea-Mn, and Urea-Fe Complexes) are found to be potentially active.

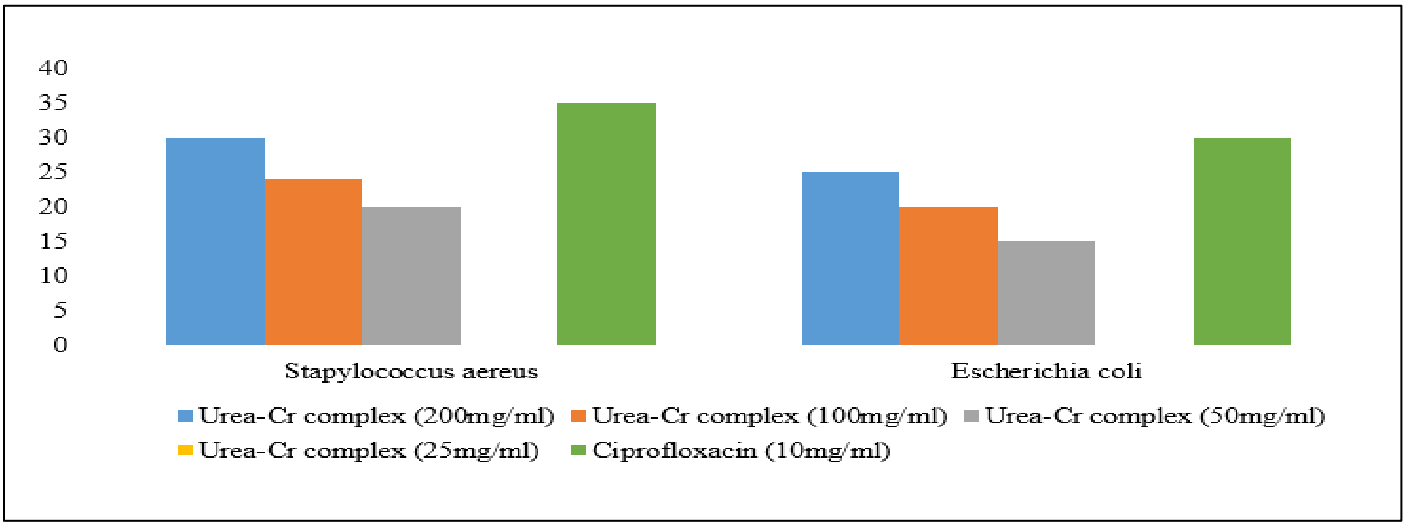


Fig 14: Antimicrobial Activity Urea-Cr Complex against Stapylococus Aereus and Escherichia Coli

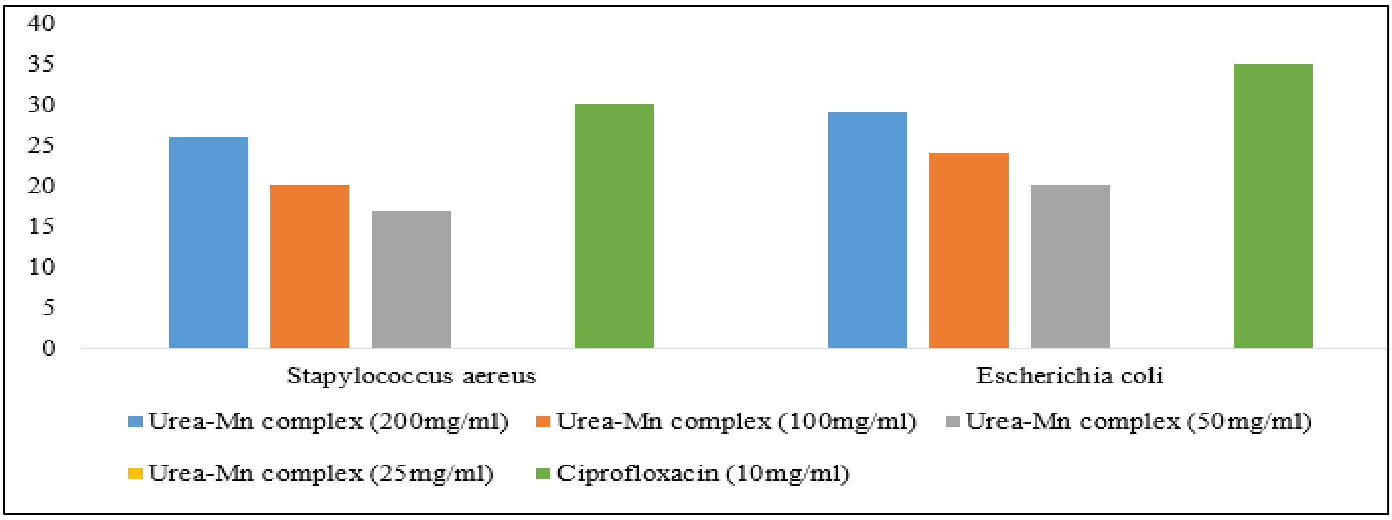


Fig 15: Antimicrobial Activity Urea-Mn Complex against Stapylococus Aereus and Escherichia Coli

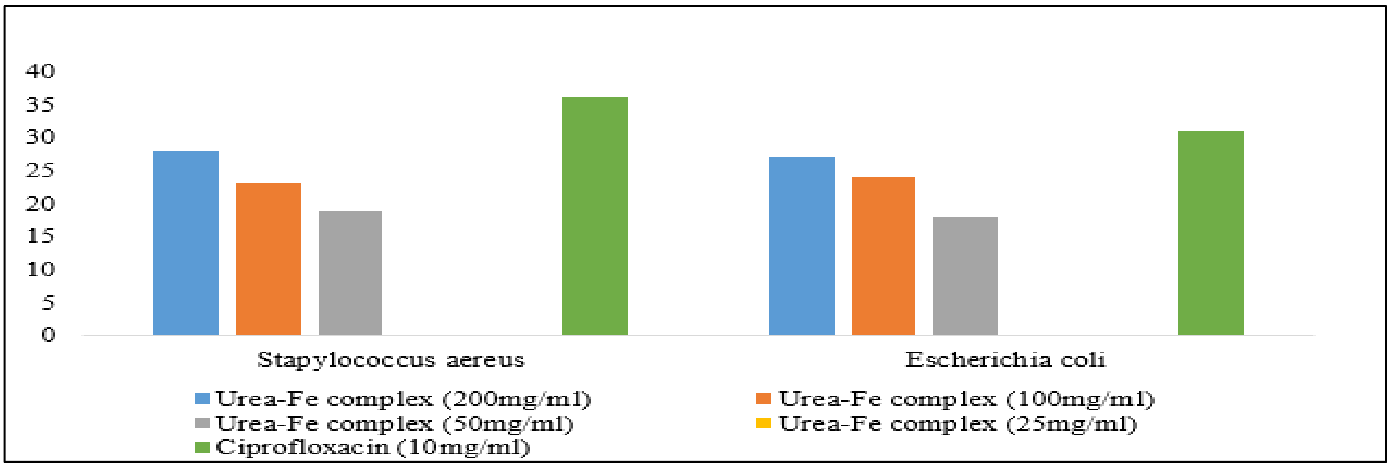


Fig 16: Antimicrobial activity Urea-Fe Complex against Stapylococus Aereus and Escherichia Coli

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