Synthesis, Characterization and Investigation of the Antibacterial Activity of Potassium Trisoxalatoferrate (III) Trihydrate

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***Abstract*: - Potassium trisoxalatoferrate (III) trihydrate complex, $K_3[Fe(C_2O_4)_3].3H_2O$ was synthesized and characterized using UV-Visible and FT-IR spectrophotometry. Its antibacterial properties was evaluated using three(3)different bacteria strains namely: Escherichia coli, Staphilococus aureus and Bacillus subtillus. A commercial drug, ciprofloxacin was used as control. The results of the analysis showed that escherichia coli was not sensitive to the complex. However, staphilococus aureus and bacillus subtillus were sensitive to the complex at a concentration range of 100 - 400mg/ml and 200 – 400 mg/ml respectively. The results of minimum inhibitory analysis and minimum bactericidal analysis showed that the complex could not inhibit the growth of escherichia coli, but it inhibited and destroyed staphilococus aureus and bacillus subtillus at some range of concentration.This study has revealed that the complex, potassium trisoxalatoferrate(III) trihydrate has some medicinal potentials as likely antibacterial agents**

Key words: **Coordination complexes, potassium trisoxalatoferrate(III) trihydrate, antibacterial activity.**

I. INTRODUCTION

The treatment of infectious diseases still remains an important and challenging problem due to the outbreak of infectious diseases caused by different bacteria, and the development of antibiotic resistance. Therefore, researchers are searching for new antibacterial agents, for the treatment of the resistant diseases. Inorganic elements play a crucial role in biological medical processes and it is evident that many organic compounds used in medicine do not have a purely organic mode of action as some are activated or biotransformed by metal ions (Afanasev, *et al.*, 1989).

Coordination complexes of transition metals have been widely studied for their antibacterial, antifungal and potential chemotherapeutic agents. They have been evaluated against several pathogenic fungi and bacteria with promising results. One of the approaches aimed at increasing the efficacy of the drugs is the modification of their physical and chemical factors. In addition to its ability to combat infections or neoplastic diseases, these new agents must exhibit selective toxicity, chemical stability, and optimum rates of bio-transformation and elimination (Johari, Kumar, & Kumar, 2009). In more recent time, a stable metal coordination complex based on the element platinum, cis $[PtCl_2(NH_3)_2]$ (cisplatin) has become the most well known metal based drugs and hundreds of articles have been published on the synthesis and activity of complexes derived from the parent cisplatin molecule (Merchant, 1998).

In recent times, much attention has been given to the synthesis of new metal complexes and their evaluation for antibacterial, antifungal and anticancer activities. Studies have been done on the structure and chemical behavior of several metal complexes to find out alternative against the drugs.

Potassium trisoxalatoferrate(III) trihydrate complex, $K_3[Fe(C_2O_4)_3].3H_2O$ has been extensively investigated for its wide applications in photochemical studies, actinometry sensors and magnetic materials (Bencini, *et al.*, 2000; Fen, *et al.*, 2014; Teinkink & Whitehouse, 1995). Its antibacterial properties have also been investigated (Atheer, *et al.*, 2015). This paper describes the antibacterial studies of potassium trisoxalatoferrate(III) trihydrate complex.

II. EXPERIMENTAL

Materials

All chemicals used were of reagent grade. They were used without further purification.

Synthesis of potassium trisoxalatoferrate(III) trihydrate complex

The complex was prepared according to a known procedure as reported in the literature (Rendel, *e.t al.*, 1969) by dissolving 5.5g (0.03 mole) of potassium oxalate monohydrate in $100cm^3$ of water with the aid of a steam bath. 2.7g (0.01mole) of iron (III) chloride hexahydrate was then added with stirring. The resulting solution was kept on an ice bath for crystallization and the deposited green crystals were collected by Buchner filtration. The crystals were then purified by recrystallization. This was achieved by dissolving it in hot water and allowing it to cool gradually. The dissolved impurities were removed via filtration, leaving the pure solid. The crystals were washed with $10cm^3$ portion of propanone and then dried in a dark cupboard before weighing.

Characterization of the complex

The complex was characterized using UV-Visible and IR-spectrophotometry as well as molar conductance measurements. UV analysis was carried out using *Pharmacia Biotec Ultra Spec 2000* [Ultraviolet – Visible] Spectrophotometer. The cells of the spectrophotometer [silica cell] were washed with detergent, rinsed with distilled water and acetone and after drying; it was rinsed again with distilled water (solvent used). A very dilute solution of the sample was prepared by weighing 0.02g of the complexes (MM = 494) into 10ml volumetric flask and making it up to the mark with distilled water. 2.5ml aliquots were then withdrawn with the aid of a dropping pipette into the cell of the spectrophotometer. A matched cell containing the pure solvent was also prepared and used as a reference. Both cells were placed in their appropriate holders in the spectrophotometer and scanned between 200-800nm to obtain the λmax. The spectrums were then printed out from the inter-phased computer.

The infrared spectra of the sample were obtained using a *Matson Infrared* Spectrophotometer (FT-IR Genesis II Series). 2mg of the sample was weighed into a small agate mortar and a drop of Nujol added and finely ground. The mull obtained was suspended on the cell of the spectrophotometer (NaCl cells) and scanned at normal laboratory temperatures between 4000 – 500cm^{-1} at 32 runs per minute. The charts were plotted on a computer inter-phased with the spectrophotometer, using a WIN-FIRST plot composer software and then printed. The electrical conductivity of the complex was recorded at room temperature in DMF solution using PW-9527 Philips Digital conductivity meter.

Invitro-antibacterial test using the agar diffusion method

(i) Standardization of Inoculums

Three (3) microorganisms; namely, Escherichia coli, Staphilococus aureus and Bacillus subtillis were used for testing the antibacterial activity of the complex. The organisms were collected from a clinical laboratory. The pathogens were sub-cultured to nutrient agar (NA) slants using a wire wop (done aseptically) and incubated for 24 hour at 37^0C. Growth of micro organism was indicated by turbidity. The turbidity produced was adjusted to match 0.5 Mc farmland (100^8 cfu/ml) which was further adjusted to 10^5 (cfu/ml).

(ii) Inoculation of the Plates and Application of the Extract

`To inoculate the plate, one drop of the adopted sub-cultured broth was applied to the surface of the nutrient agar (Seaboard dextrose agar) and evened to cover the surface of the nutrient agar with microbes. One microbe was inoculated to one plate making a total of three (3) micro organisms. After 30 minutes four wells were punched on the plate using a sterile cork borer of 5mm diameter, two for the water extract, one for negative and one for positive control. A 0.1ml of the solvent (equivalent to 20mg of the extract) was dropped into each of appropriate labeled wells into the remaining two wells, distilled water and the tricycle of the same concentration as the extract was to serve as negative and positive controls respectively for the bacteria. The inoculations were left on the table for 1 hour to allow for proper diffusion. Agar plate were incubated aerobically at 37^0c while the saborand dextrose agar containing microsporunaudoinii and trichophyton mentagrophyte, was incubated for 48 hours at 25^0c. Zone of inhibition produced after incubation was measured by linear measurement of diameter.

(iii) Minimum Inhibitory and Bactericidal Concentration

The minimum, inhibitory concentration was determined using the tube dilution method by preparing different concentration of the metal complex solution (100mg/ml). Cleaned test tubes where taken and the volume of medium made up to 20ml with nutrient broth. The control was prepared with 2ml of nutrient broth without the metal complex. Both were then sterilized at 121^0C temperature at 151bs pressure for 15 minutes in an autoclave.

After sterilization the medium was allowed to cool and 0.2ml of overnight culture of each organism was dispensed into sterile medium and incubated for 48 hours. The activity was measured by turbidity for inhibition and growth of the bactericidal.

(iv) Antimicrobial Assay

20ml of media was poured in petriplates and allowed for solidification. The microbial culture was made using sterile cotton swab and labeled. The wells were made in the media with the help of a cork borer with centers of at least 24mm. The recommended concentration of 50ml of the test sample and 100mg/ml of water was introduced in the respective wells. Other wells were supplemented with reference antibacterial drug (ciprofloxacin).

The agar plates were incubated aerobically at 37^0c for 24 hours. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared with that of the drug, ciprofloxacin.

III. RESULTS AND DISCUSSION

Table 1: Results of the UV-Visible spectrophotometry

Compound	Λmax(nm)	Absorbance	E
$K_3[Fe(C_2O_4)_3].3H_2O$	212	1.385	340

Table 2: Results of the FT-IR spectrophotometry

Compound	$\upsilon_{(C=O)\ str}(cm^{-1})$	$\upsilon_{(C-O)str.}(cm^{-1})$	$\upsilon_{(C-C)str.}(cm^{-1})$	$\upsilon_{(Fe-O)str.}(cm^{-1})$
$K_3[Fe(C_2O_4)_3].3H_2O$	1642.28 (s)	1254.04 (s)	1377.44-1462.38	531.09-889.07

Table 3: Results of the Antibacterial Activity

Organisms	**Zones of Diameter (mm)/ Concentration of Extract (mg/ml)**					**Extracts**	**Control**
	400	**300**	**200**	**100**	**50**		
E.coli	00	00	00	00	00	Fe	28
S. aureus	26	22	17	12	00	Fe	30
B. subtillus	18	12	08	00	00	Fe	30

Control: Ciprofloxacin 10mg/ml for bacterial isolates.

Table 4: Results of the Minimum Inhibitory Concentration

Organisms	**Concentration of extract (mg/ml)**						**Extract**	**MIC (Mg/ml)**
	100	**50**	**25**	**12.5**	**6.25**	**3.125**		
E. coli	+	+	+	+	+	+	Fe	0
S. aureus	-	-	+	+	+	+	Fe	50
B. subtillus	-	+	+	+	+	+	Fe	100

Key: Visible turbidity (Growth) (+), No visible turbidity (No growth) (-)

The synthesized complex, $K_3[Fe(C_2O_4)_3].3H_2O$ (molecular mass = 491, density = $2.13gcm^{-3}$ and melting point =230^oC) is a green octahedral complex. The UV-Visible data of the complex gave a λmax of 212nm with an absorbance of 1.385 in water (Table 1). The IR-spectra (Table 2) shows strong bands attributed to $C=O_{(str)}$ and $C-O_{(str)}$ at $1642.28cm^{-1}$ and $1254.04cm^{-1}$ respectively. Strong bands attributed to C-C also occurs in the region of $1377.44cm^{-1}$-$1462.38cm^{-1}$ while medium intensity bands attributed to Fe-O occurred at $531.09cm^{-1}$ – $889.07cm^{-1}$.

The antibacterial activity of the complex was tested using three (3) bacterial pathogens namely: Escherichia coli, Staphilococus aureus and Bacillus subtillus. A commercial drug, ciprofloxacin was used as control. The results in Table 3 show that E.coli was not sensitive to the complex within the range of concentration used. However, S. aureus and S. subtillus were sensitive to the complex, as their growths were inhibited within 50 – 400 mg/ml and 200 - 400mg/ml respectively.

Table 4 (Results of minimum inhibitory concentration) shows that the complex did not inhibit the growth of E. coli within the range of concentration used. However, the growth of S. aureus and B. subtillus were inhibited within 50 – 100 mg/ml and 50 mg/ml respectively.

IV. CONCLUSION

The results of the antimicrobial analysis showed that escherichia coli was not sensitive to the complex. However, staphilococus aureus and bacillus subtillus were sensitive to the complex at a concentration of 100 - 400mg/ml and 200 – 400 mg/ml respectively. The results of minimum inhibitory analysis showed that the complex could not inhibit the growth of escherichia coli, but it inhibited and destroyed staphilococus aureus and bacillus subtillus at some range of concentration. The results of this study has revealed that the complex, potassium trisoxalatoferrate(III) trihydrate has some medicinal potentials as likely antibacterial agents.

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