SYNTHESIS, MAGNETIC AND SPECTRAL PROPERTIES, ANTIOXIDANT AND *IN-VITRO* ANTI-INFLAMMATORY ACTIVITIES OF SOME HETEROLEPTIC ASPIRIN-ASCORBIC ACID METAL COMPLEXES

Supported by tetfund

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Received: January 18, 2021 **Accepted:** March 28, 2021

Abstract: Synthesis of Cu(II), Co(II), Ni(II), Mn(II) and Zn(II) complexes with mixed ligands Aspirin (asp) and Ascorbic acid (asc) has been carried out. The complexes were characterized by determination of physicochemical properties such as melting point, solubility, conductance, elemental analyses, magnetic susceptibility and spectroscopic analyses(UV-Visible and Infrared). The electronic spectra data are characteristic of octahedral geometry except [Zn(asp)(asc)], which is tetrahedral. The coordination to the metal ion was through the carboxylate oxygen atoms in aspirin while the carbonyl oxygen and hydroxyl oxygen of ascorbic acid serve as coordination atoms. The antioxidant studies revealed that Ni(II) and Zn(II) complexes showed the best activities with 84.2 and 89.0 percentage inhibitions respectively, which are comparable to that of the standard (ascorbic acid) with 87.0 percent inhibition. Also, the result of anti-inflammatory activity using egg albumin denaturation shows that the Co(II) and Mn(II) complexes had the best activity of 70.6 and 62.0 percentage inhibition of protein denaturation and compared favorably with the standard drug(aspirin). The derived metal complexes displayed considerable antioxidant and anti-inflammatory activities.

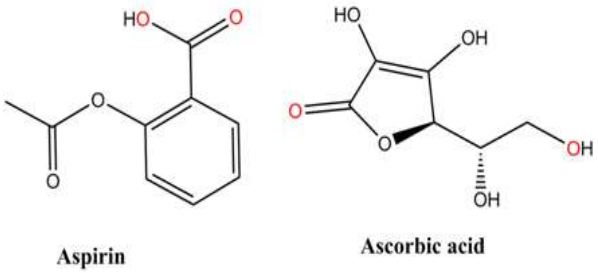
Keywords: Metal complexes, NSAIDs, aspirin, anti-inflammatory, ascorbic acid, antioxidant

Introduction

Inflammation is classically described as a response to infection or injury. Inflammation has been identified to be one of the primary ways that human body or the human tissue specifically respond to some irritations, injuries, and most times infections; some of the characteristics features are warmth swelling, pain and most times redness (Nathan and Ding, 2010). Inflammation is considered a normal protective response as a part of host defense system during tissue injury. Inflammatory responses involve release of chemo-attractant mediators and chemo-activators, enzyme activation, tissue break down and repair. However, inflammation may also be responsible for serious fatal situation. Non-steroidal anti-inflammatory drugs (NSAIDs) over the years have been used for many purposes but majorly as analgesic, anti-inflammatory, and more recently in the case of aspirin, antithrombotic treatments (Sen *et al.*, 2012).

Antioxidant can be defined as any material or substance when present in low concentration can delay or prevent the oxidation, and inhibit the free radical effect or damage to cells. Antioxidants when present in the body may offer protection against cancer, Alzheimer's and Parkinson's diseases (Halliwell and Gutteridge, 1984). Vitamins are essential for the normal growth and development of multicellular organisms. Once growth and development are completed, vitamins remain essential nutrients for the healthy maintenance of cells, tissues and organ that make up multicellular organisms. Ascorbic acid, also known as vitamin C, is involved in many physiological functions in living organisms. Its role in the synthesis of collagen in connective tissues is well known (Aguirre and May, 2008). Vitamin C is one of the potent reducing agents and scavengers of free radicals in biological systems, working as a scavenger of oxidizing free radicals and harmful oxygen-derived species, such as hydroxyl radical, hydrogen peroxide (H_2O_2), and singlet oxygen (Arrigoni and De Tulio, 2002).

Aspirin is a derivative of salicylic acid (acetylsalicylic acid). It has analgesic, anti-inflammatory and antipyretic actions and inhibits prostaglandins synthase (Lawal and Obaleye, 2007). It is often used to treat body joint pain, fever and inflammation, and sometimes to avert or treat strokes, chest pain and heart attacks (Burke *et al.*, 2006). Aspirin is also an antioxidant that functions via its ability to scavenge OH radicals. Its OH radical scavenging rate is faster than that of ascorbate, glutathione and cysteine (Fig. 1). However, aspirin is not a good scavenger for O^{2-} or H_2O_2. This antioxidant property explains some of its various physiological and pharmacological actions (Shi *et al*., 1999).



Aspirin Ascorbic acid

Fig. 1: Structure of aspirin and ascorbic acid

Metal complexes play an important role in continuous quest for drug discovery and development (Lawal and Obaleye, 2007). One of the principal research focuses of present day synthetic inorganic coordination chemists is the development of alternative drugs to fight diseases. This has led to a lot of researches on drug metal complexes (Adediji *et al*., 2009 and Obaleye *et al.*, 2019). Various researches have been carried out onthe complexation of anti-inflammatory drugs with metal ions. A coordinated metal ion with NSAIDs offers advantages over thefree drug themselves. The metal complexes of NSAIDs offer a wide range of improved biological activities quite often absent to the parent NSAIDs ligands (Banti and Hadjikakou, 2016).

Literature search revealed various works reported on metal complexes of aspirin, ascorbic acid in combination with other drugs as ligands. However, mixed ligand metal complex derived from ascorbic acid and aspirin have not yet been

reported (Weder *et al.*, 2002; Lawal and Obaleye, 2007; Osowole *et al.*, 2015a; Olanrewaju *et al.*, 2015; Lawal *et al.*, 2017). Consequently, our aim is to prepare new compounds derived from aspirin and ascorbic acid and investigates their potential antioxidant and *in-vitro* anti-inflammatory properties.

Material and Methods

Experimental

Materials and physical measurements

Aspirin and Ascorbic Acid are products of sigma Aldrich (USA) and were obtained as gifts from Rajrab Pharmaceuticals Limited, Ilorin. Nickel(II) chloride hexahydrate, Copper(II) chloride dihydrate, Cobalt(II) chloride hexahydrate, Manganese(II) sulphate monohydrate and Zinc(II) sulphateheptahydrate, methanol, dimethylsulphoxide(DMSO), dimethylformamide (DMF), distilled water, ammonia were employed in this work. All the chemicals are of analytical grade and were used as purchased from the manufacturer without further purification. Melting points were recorded using Optimelt Automated Melting Point System (SRS). The Uv/Vis spectra of the complexes in DMSO were recorded on a DU-730 Beckman Coulter UV/Vis spectrophotometer with a quartz cell of path length 1 cm. The infra-red spectra were recorded on a Shimadzu FTIR spectrophotometer using KBr pellets in the range 4000 – 500 cm^{-1}. Conductivities in 1 mMDMSO were determined using HANNA H1763100 conductivity meter with cell constant 0.868 cm^{-1}. The elemental analyses (CHN) were performed on a Perkin-Elmer CHN Analyzer. Magnetic susceptibility measurement was done using a Sherwood Scientific Magnetic Susceptibility Balance. The *in-vitro* anti-inflammatory activity screening was carried out using protein denaturation by egg albumin assay (Sen *et al.*, 2015). Antioxidant activity was carried out by investigating their ability to scavenge the free radicals of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Adesegun *et al.*, 2008).

Method

The metal complexes were prepared according to the reported literature (Olanrewaju *et al.*, 2016). Aqueous solution (15 mL) of ascorbic acid (4.16 mmoles, 0.73 g) was mixed with 15 mL of aspirin (4.16 mmoles, 0.75 g) in methanol. The mixture was stirred for 5 min at room temperature. 4.16 mmoles each of the metal salt solution was added in a dropwise manner to the stirring ligands. After 30 min, 8-9 drops of concentrated ammonia was added to the solution to adjust the pH. The mixture was further stirred for 3 h and then concentrated by slow evaporation of solvents for 14 days. The precipitate obtained was filtered, washed with methanol and distilled water, dried under vacuum and stored in sample bottles for further analysis.

Results and Discussion

The synthetic route of the complexes is presented in Fig. 2. The physicochemical properties are presented in Table 1.

The complexes are air stable, insoluble in distilled water and most organic solvents like ethanol, methanol and acetone but soluble in polar coordinating solvents like DMF and DMSO. Conductivity values in DMSO range from 8.7 to 42.0 µs/cm suggesting their non-electrolytic nature in solution (Babamale *et al.*, 2016; Rajee *et al.*, 2020). Percentage yield falls between the range of 36 – 74%. The percentage composition of carbon and hydrogen were determined to establish the composition of the coordination compounds and are as presented in Table 1. The values were in close agreement with the calculated values which further confirms their stoichiometry.

Table 1: Physicochemical properties of the ligands and complexes

Ligands/Complexes	Formula mass	Colour	M.p (°C)	µeff (B.M)	Λ_m (µs/cm)	Elemental Analysis Found (calculated)		
						% C	% H	%N
asp	180.16	White	143.40	_	_	(60.00)	(4.48)	_
asc	176.12	White	178.70	_	_	(40.92)	(4.58)	_
$[Cu(asp)(asc)(H_2O)_2]$	453.84	Bluish green	158.80	1.37	8.7	39.94 (39.70)	3.91 (4.00)	_
$[Co(asp)(asc)(H_2O)_2]$	449.23	Black	228.70	7.02	17.0	40.17 (40.11)	4.04 (4.04)	_
$[Ni(asp)(asc)(H_2O)_2]$	448.99	Light green	237.10	4.30	42.0	40.20 (40.13)	4.18 (4.04)	_
$[Mn(asp)(asc)(SO_4)(H_2O)]$	523.28	Golden yellow	217.40	2.89	11.5	34.21 (34.43)	3.05 (3.08)	_
[Zn(asp)(asc)]	419.65	White	157.20	0.36	13.4	42.93 (42.93)	3.35 (3.36)	_

M.p =melting point; Λ_m= molar conductivity

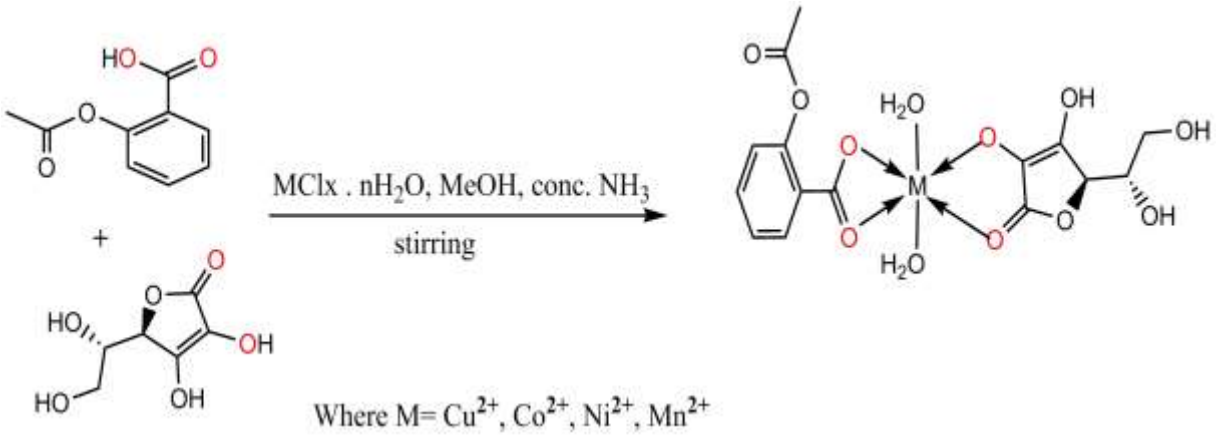


Fig. 2: Synthetic route for the preparation of the metal complexes

Table 2: Infra-red spectra data of the ligands and metal (II) complexes (cm^{-1})

Ligands/Complexes	υ (OH)	υ (C=O)	υ (C–O)	$\upsilon_s(COO^-)$	$\upsilon_{as}(COO^-)$	υ (M-O)
asp	3489 b	1751 s	–	1309 m	1456 sh	–
$C_9H_8O_4$		1685 s				
asc	3411 b	1755 s	1026-1120	–	–	–
$C_6H_8O_6$	3526 m	1674 b				
[Cu(asp)(asc)(H_2O)$_2$]	3240 m	1659 s	1031	1324 m	1445 sh	465
$C_{15}H_{18}CuO_{12}$	3419 b	1580 s				
[Co(asp)(asc)(H_2O)$_2$]	3370 b	1636 s	1044	1316 m	1359 sh	490
$C_{15}H_{18}CoO_{12}$						
[Ni(asp)(asc)(H_2O)$_2$]	3393 b	1622 s	1120	1317 m	1360 sh	487
$C_{15}H_{18}NiO_{12}$						
[Mn(asp)(asc)(SO_4)(H_2O)]	3388 m	1629 s	1111	1315 m	1362 sh	497
$C_{15}H_{16}MnO_{15}S$						
[Zn(asp)(asc)]	3238 m	1750 s	1030	1324 m	1444 sh	464
$C_{15}H_{14}O_{10}Zn$		1659 s				

b = broad, m = medium, s = strong, sh = sharp

Infrared spectra

The selected infrared absorption band of the ligands and metal complexes is presented in Table 2. The FT-IR spectra of the ligands were compared with those of the compounds and then carefully assigned. The broad and medium bandsat 3411 and 3489 cm^{-1} were assigned to the ν(OH) of ascorbic acid and aspirin, respectively (Osowole *et al.*, 2015a; Osowole *et al.*, 2015b; Olanrewaju *et al.*, 2016). These bands were shifted in the metal complexes to new bands in the range 3238-3393 cm^{-1} due to the involvement of their hydroxyl oxygen atom in the coordination to the metal ion (Aguirre and May, 2008; Osowole *et al.*, 2015). The ν(C=O) bands of aspirin and ascorbic acid were in the range 1685-1751 and 1674-1755 cm^{-1}, respectively. These bands shifted to 1613-1659 cm^{-1} in the metal (II) complexes due to coordination (Lawal and Obaleye, 2007; Williams and Fleming, 1980).

In metal complexes bearing the carboxylate group, a characteristic of their IR spectra is the frequency of the symmetric (υ_s) and asymmetric (υ_{as}) stretching vibrations of (COO^-) group. The difference $\Delta\nu$=[(υ_{as}) -(υ_s)] value gives an insight as to the type of coordination mode exhibited by the carboxylate group; monodentate, ionic, bidendate (chelating). For the reported complexes, $\Delta\nu$ values falls between (43 – 121 cm^{-1}) which is lower than 147 cm^{-1} for the free aspirin. This result is in agreement with values for bidentate coordination modes (Nakamoto, 2009; Ali *et al.*, 2016; Dimiza *et al.*, 2018). The appearance of new bands (conspicuously absent in the free ligands) in the metal complexes around 464-497 cm^{-1} further confirmed coordination sites as they were assigned the M-O and M-S.

Electronic spectra

The spectra were recorded in DMF and are presented in Table 3. The UV-visible spectrum of aspirin shows a peak at 300 nm duly assigned to the n-π* electronic transition. The spectrum of ascorbic acid exhibit absorption peak at 210 nm, which is assigned to (π-π*) electronic transition. These transitions have been attributed to intra ligand transfer. The UV-vis spectrum of Co(II) and Ni(II) complexes at 635 and 500 nm, respectively was assigned d-d transition, in an octahedral geometry (Anacona and Rodriguez, 2004; Köse *et al.*, 2007). The Cu(II) complex has absorption peak at 220 nm assigned to (π-π) transition. However the Zn(II) complex has absorptions at 210, 220 nm which could be attributed to ligand to metal (L→M) charge transfer transition which is compatible with tetrahedral structure for Zn(II) complex (Mishra and Gupta, 2011).

Table 3: Electronic spectra data of the ligands and metal (II) complexes

Ligands/Complexes	Wavelength (nm)	Wave number (cm^{-1})	Absorbance	Assignment
asp	300	33,333	3.6129	n-π*
asc	210	47,619	2.3010	π-π*
[Cu(asp)(asc)(H_2O)$_2$]	220	45,454	3.1251	π-π*
[Co(asp)(asc)(H_2O)$_2$]	635	15,748	0.1662	$_4T_{1g}(F)\rightarrow{}^4E_{2g}(F)$
[Ni(asp)(asc)(H_2O)$_2$]	500	20,000	0.1711	$^3A_{2g}\rightarrow{}^3T_{1g}(F)$
[Mn(asp)(asc)(SO_4)(H_2O)]	350	28,571	2.6480	n-π*
[Zn(asp)(asc)]	210	47,619	3.5524	n-π*

Table 4: Antioxidant activity data of selected metal complexes at various concentrations (µg/ml)

Concentration (mg/mL)	% inhibition				
	asc	asp	[Cu(asp)(asc)(H_2O)$_2$]	[Ni(asp)(asc)(H_2O)$_2$]	[Zn(asp)(asc)]
25	52.00	6.00	23.00	24.80	22.00
50	61.00	9.00	30.00	41.00	34.00
100	71.00	23.00	40.00	50.00	48.00
200	76.00	34.00	48.00	65.00	64.00
400	83.00	43.00	52.00	77.57	81.00
800	87.00	47.00	66.00	84.20	89.00

Antioxidant activity

The antioxidant (free radical scavenging activity) was investigated using 1,1-diphenyl-picrylhydrazyl (DPPH) method. The DPPH acts by accepting an electron from test compounds, and gets converted into a stable molecule. The percentage inhibition have been evaluated using Equation (1). From the results obtained in this study, it was observed that Zn(II) and Cu(II) complexes had highest percentage inhibition of 89 and 84%, respectively which was higher than, and comparable to that of standard ascorbic acid with activity of 87% at the concentration of 800 µg/mL (Table 4). The scavenging ability of the metal complexes decreases in the order Zn>Ni> Cu. Thus these compounds have potential as suppressors of neurodegeneration diseases suggesting them as potential therapeutic agents for chronic ailments reduction.

Equation (1): % free radical scavenging activity $= \frac{(AC) - (As)}{(AC)} x\ 100$

Where AC = the absorbance of control (blank); As = the absorbance of the test sample

Table 5: Anti-inflammatory activity data of the metal complexes

Compounds	Absorbance	% inhibition
$[Cu(asp)(asc)(H_2O)_2]$	0.074	27.58
$[Co(asp)(asc)(H_2O)_2]$	0.099	70.60
$[Ni(asp)(asc)(H_2O)_2]$	0.083	43.10
$[Mn(asp)(asc)(SO_4)(H_2O)]$	0.094	62.00
[Zn(asp)(asc)]	0.090	55.17
Standard (Aspirin)	0.075	29.30

Inhibition of protein denaturation

Anti-inflammatory activity screening of the complexes and their parent ligands was done. The study was carried out *in-vitro* using egg albumin denaturation assay. A 5.0 mL of solution is prepared containing 0.2 mL of egg albumin (collected from fresh hen's egg) mixed with 2.8 mL phosphate-buffered saline (PBS, pH 6.4) and 2.0 mL each of test compounds (200 ppm) were taken in test tubes. A 2.0 mL of distilled water was used as control. The mixtures were heated in water bath at 37ºC for 15 min and the temperature gradually increased up to 70ºC while the samples are retained for a further 5 min. The samples are then allowed to cool down to room temperature and absorbance measured at 660 nm with the use of a UV-Vis spectrophotometer (Chandra *et al*., 2012). Aspirin was used as standard drug and was treated similarly to determine their absorbance. The values were recorded accordingly. Percentage inhibition of protein denaturation was calculated by using the formula in equation (2).

Equation (2): % inhibition of protein denaturation $= 100 \times (\frac{Vt}{Vc}) - 1$

Where: Vt = absorbance of test sample; Vc = absorbance of control

Conclusion

Cu(II), Co(II), Ni(II), Mn(II) and Zn(II) complexes of mixed drugs aspirin and ascorbic acid have been synthesized and characterized. All the metal complexes adopted 6-coordinate octahedral geometry except for the Zn(II) complex which is tetrahedral. The *in-vitro* anti-inflammatory activity of the complexes was moderate compared to the standard drug Aspirin.

Also the antioxidant studies showed that the Zn(II) and Ni(II) complexes had the best antioxidant activities, suggesting them as potential agents for chronic ailments reduction and treatment of degenerated diseases. Further work could be extended towards studying the anticancer potential and toxicity profile of the prepared complexes.

Acknowledgement

The authors express their thanks to the Department of Pure and Industrial Chemistry, Kogi State University Anyigba, for research chemicals/facilities.

Conflict of Interest

The authors have declared that there is no conflict of interest in carrying out this research.

References

Adediji JF, Olayinka ET, Adebayo MA & Babatunde O 2009. Antimalarial mixed ligand metal complexes:Synthesis, physicochemical and biological activities. *Int. J. Phys. Sci.*, 4(9): 529-534.

Adesegun SA, Elechi NA & Coker HAB 2008. Antioxidant activities of methanolic extract of *Sapiumelliticum*. *Pak. J. Biol. Sci.*, 11: 453-457.

Aguirre R & May JM 2008. Inflammation in the vascular bed: Importance of vitamin C. *Pharmacological Ther.*, 119: 96-103.

Ali HA, Omar SN, Darawsheh MD & Fares H 2016. Synthesis, characterization and antimicrobial activity of zinc(II) ibuprofen complexes with nitrogen-based ligands. *J. Coord. Chem.*, 69(6): 1110-1122, DOI:10.1080/00958972.2016.1149819

Anacona JR & Rodriguez I2004. Synthesis and antibacterial activity of cephalexin metal complexes. *J. Coord. Chem.*, 57(15): 1263-1269.

Arrigoni O & De Tulio MC 2002. Ascorbic acid: much more than just an antioxidant. *Biochim. Biophys. Acta*, 1569: 1-9.

Babamale HF, Lawal A, Rajee OA & Oloyede EA 2016. Synthesis, characterization and biological activity studies of mixed paracetamol-ascorbic acid metal complexes. *J. Appl. Sci. Environ. Mgt.*, 20(4): 1157-1161.

Banti CN & Hadjikakou SK 2016. Non-steroidal anti-inflammatory drugs (NSAIDs) in metal complexes and their effect at the cellular level. *Eur. J. Inorg. Chem.*, 2016(9): 1-25.

Burke A, Smyth E & FitzGerald GA 2006. Analgesic antipyretic and antiinflammatory agents. In: Brunton LL, Lazo JS & Parker K (eds.). *Goodman and Gilman's the Pharmacological Basis of Therapeutics*, New York, McGraw-Hill, 11th Ed., pp. 671–716.

Chandra S, Dey P & Bhattacharya S 2012. Preliminary in-vitro assessment of anti-inflammatory property of Mikaniascandens flower extracts, *J. Adv. Pharm. Edu. Res.*, 2: 25-31.

Dimiza F, Raptopoulou CP, Psycharis V, Papadopoulos A & Psomas GL 2018. Manganese(II) complexes with the non-steroidal anti-inflammatory drugsnaproxen and mefenamic acid. Synthesis, structure, antioxidant capacity, interaction with albumins and DNA. *New J. Chem*., DOI:10.1039/C8NJ03226B

Halliwell B & Gutteridge JMC 1984.Oxygen toxicology, oxygen radicals, transition metals and disease. *Biochem. J.*, 219: 1-4.

Köse DA, Icbudak H & Necefoglu H 2007. Synthesis and Characterization of the Nicotinamide-acetylsalicylato complexes of Co (II), Ni (II), Cu (II), and Zn (II) studies. *J. Biol. & Chem.*, 10: 13.

Lawal A & Obaleye JA 2007. Synthesis, characterization and antibacterial activity of aspirin and paracetamol metal complexes. *Biokemistri.*, 19(1): 9-15.

Lawal M, Obaleye JA, Bamigboye MO, Nnabuike GG, Ayinla SO, Rajee AO, Babamale HF & Ajibola AA 2017. Mixed metal complexes of isoniazid and ascorbic acid: Chelation, characterization and antimicrobial activities. *Nig. J. Chem. Res.*, 22(1): 20-28.

Mishra AP & Gupta P 2011. Effect of chelation on therapeutic potential of drugs: Synthesis, structure, antimicrobial and insecticidal activity of 3d-metal complexes involving Schiff bases. *J. Chem. Pharm. Res.*, 3(2): 150-16.

Nakamoto, K 2009. *Infrared and Raman spectra of inorganic and coordination compounds, Part B: Applications in Coordination, Organometallic, and Bioinorganic Chemistry*, 6th Ed., Wiley: New Jersey.

Nathan C and Ding A 2010. Non-resolving inflammation. *Cell.,* 40: 871–882.

Obaleye JA, Atoyebi AA, Bernardus VB & Hosten EC 2019. Synthesis, X-ray crystallography, spectroscopic and *in-vitro* antimicrobial studies of a new Cu(II) complex of trichloroacetic acid and imidazole. *J. Mol. Struct.*, 1203: 127435.

Olanrewaju AA, Oni TI & Osowole AA 2016. Synthesis, characterization and antioxidant properties of some metal(II) complexes of mixed drugs-vitamin Bx and Aspirin. *Chem. Res. J.*, 1(4): 90-96.

Osowole AA, Malumi EO & Odutemu AE 2015. Synthesis, magnetic, spectral and antioxidant properties of some metal(II) mixed-drug complexes of Aspirin and Vitamin B_3. *Research and Reviews*: *J. Chem.*, 4(1): 60-65.

Osowole AA, Wakil SM & Okediran EQ 2015. Synthesis, characterization and antimicrobial activities of some metal(II) complexes of mixed-ligands- dimethyl dithiocarbamic and paraaminobenzoic acids. *Elixir Appl. Chem.*, 79: 30375-30378.

Rajee AO, Aliyu AA, Babamale HF, Lawal A, Ayinla SO, Osunniran WA & Obaleye JA 2020. Preparation, characterization and antibacterial activity of Mn(II), Cu(II) and Zn(II) complexes of methionine and 2,2-bipyridine co-ligands. *J. Kenya Chem. Soc.*, 13(1): 16-21.

Sen S, Chakraborty R, De B, Ganesh T, Raghavendra HG & Debnath S 2012. Analgesic and anti-inflammatory drugs: Apotential source of herbal medicine. *Int. J. Pharma. Sci. Res.*, 1: 32-44.

Sen S, Chakraborty R, Maramsa N, Basak M, Deka S & Dey BK 2015. *In-vitro* antiinflammatory activity of *Amaranthuscaudatus L*. leaves. *Indian J. Nat. Prod. Resou.* 6(4): 326n – 329.

Shi X, Ding M, Dong Z, Chen F, Ye J, Wang S, Leonard SS, Castranova V & Vallyathan V 1999. Antioxidant properties of aspirin: characterization of the ability of aspirin to inhibit silica-induced lipid peroxidation, DNA damage, NF-kappaB activation, and TNF-alpha production. *Mol. Cell Biochem*., 199(1-2): 93-102.

Weder JJE, Dillon CT, Hambley TW, Kennedy BJ, Lay PA, Biffin JR, Regtop HL & Davies NM 2002. Copper complexes of non-steroidal anti-inflammatory drugs: an opportunity yet to be realized. *Coord. Chem. Rev.*, 232: 95-126.

Williams HD & Fleming I 1980 Spectroscopic Methods in Organic Chemistry. 4th edition, McGraw Hill Bok Company Limited, London, Chapter two.