Synthesis of Metal (II) Complexes of Co, Cu and Ni with Organic Ligand (Maleic Acid) and Their Antioxidant Activities

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ABSTRACT

Complexes of Co, Ni and Cu with maleic acid as ligand were synthesized using reported procedure and characterized using UV spectrometry. The purity of the complexes was monitored and analyzed using solubility, melting point test and the complexes were evaluated for anti-oxidant activities against 1,1-diphenyl-2-picrylhydraxyl(DPPH) free radicals. All the complexes are of good yield, they are of different colors, partially soluble in organic solvent and soluble in DMF. The result was analyzed and presented as mean± SEM. The complex of Co, Ni and Cu at the concentration of 100ug/ml, 50ug/ml, 25ug/ml and 12.5ug/ml respectively shows less scavenging activity compared to the standard (vitamin C) at all concentration. The EC_{50} result shows that all the complexes have less activity compared to the standard (Vit C.) but still showing notable antioxidant capacity. On the bases of the above studies, an octahedral has been proposed for the complexes.

Key words: meliac acid, Co(II), Ni(II), Cu(II), Metal complexes, DPPH, Antioxidant activity, coordination chemistry.

INTRODUCTION

Free radicals and reactive oxygen species (ROS) are unstable molecules that can cause oxidative damage to biological macromolecules such as lipids, proteins, and DNA. This oxidative stress is linked to the pathogenesis of numerous chronic diseases, including cancer, cardiovascular disorders, neurodegenerative diseases, and diabetes mellitus (Halliwell & Gutteridge, 2015; Valko *et al.*, 2007). Antioxidants are agents capable of neutralizing these free radicals, thereby preventing or reducing the damage they cause. The need for effective and safe antioxidants has prompted the exploration of various chemical compounds, including metal-based complexes.

Coordination compounds, especially those involving transition metals, have attracted attention in pharmaceutical and medicinal chemistry due to their structural diversity, redox potential, and ability to interact with biological targets (Beraldo & Gambino, 2004; Chitrapriya & Joseph, 2014). Metal complexation often enhances the biological activity of organic ligands, including their antioxidant capacity, by altering electron distribution and enabling redox cycling (Abu-Dief & Mohamed, 2015). Among first-row transition metals, cobalt (Co), nickel (Ni), and copper (Cu) form stable complexes with diverse ligands and exhibit a range of biological activities (Karthikayen, 1992; Lee, 2009).

Maleic acid is a naturally occurring dicarboxylic acid with the ability to coordinate metal ions through its carboxylate groups. It has been used in the synthesis of coordination complexes with known applications in pharmaceuticals, agriculture, and materials chemistry (Khan & Abourashed, 2010; Shennara *et al.*, 2014). When chelated with metal ions, maleic acid can exhibit improved physicochemical properties, including solubility and biological activity. Some metal-maleate complexes have demonstrated antimicrobial, anticancer, or antioxidant properties (Turan *et al.*, 2016).

In this study, we synthesized and characterized Co(II), Ni(II), and Cu(II) complexes of maleic acid and evaluated their antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The DPPH method is widely used for assessing antioxidant potential due to its simplicity, sensitivity, and reproducibility (Noipa *et al.*, 2011). We hypothesize that metal coordination will enhance the antioxidant activity of maleic acid by stabilizing its radical form and facilitating hydrogen donation.

Properties of coordination compound

Magnetism

Electron impairment makes metal complexes magnetic. Unpaired electrons occur when considering only nonmetal complexes, either because electron pairing is unstable or because the complex contains an odd number of electrons. Therefore, regardless of ligand geometry or type, monomeric Ti (III) species all have one "d-electron" and must be (para) magnetic. .Ti (ll) with two d-electron forms some complexes that have two unpaired electrons and others with none. This effects is illustrated by the compounds $TXi_2[(CH_3)_2PCH_2CH_2P(CH_3)_2]$. when X=CL, the complex is paramagnetic (high-spin configuration), whereas $X=CH_3$, it is diamagnetic (low-spin configuration). It is important to realized that ligand provide an important means of adjusting the ground state properties. Some methods verifying the presence of complex ions include studying it chemical behavior. This can be achieved by observing the compound's color, solubility, melting point, absorption spectrum, magnetic properties etc. The characteristics of individual atoms are distinct from those of complex compounds. Coordination compounds change the characteristics of the metal and the ligand (Martell & Calvin, 1952).

Bonding in coordination compound

The term "ligand" refers to the ions or molecules that surround the center atom. Ligands are referred to as coordinated to the atom when they are typically joined to it by a coordinate covalent connection, which donates electrons from an ion pair into an empty metal orbital. There are also organic ligands such as alkenes whose pi bond can coordinate to empty metals. Orbital complex can form many compound by bounding with order complex ions in multiple ratios. This let many combinations of coordination compounds. The structures have isomer, which can change their interaction with either chemical agent. Metal tetrahedral and octahedral structures are used to study the binding between metal and ligand. Usually, two interactions are included while discussing metal-ligand connections. Lewis base and Lewis acid (Schmidbaur *et al.,* 2013).

Geometry

The coordination number, or the number of ligands attached to the metal (more precisely, the number of donor atoms), is the first characteristic of a structure in coordination chemistry. Usually, the number of ligands attached can be counted, but occasionally even this becomes unclear. Although a coordination number of two to nine is typical, lanthanide and actinide compounds can have a considerable number of ligands. Metal ions may have more than one coordinate number. Typically, the chemistry of transitional and metal complexes is dominated by interaction between s and p molecular orbital of the ligands and the d-orbital of the ion. The s, p, and d-orbital of the metal can accommodate 18 elections. The maximum coordination number for a certain metal to the electron configuration of the metal ions and to the ratio of the size of ligand and the metal ions .Large metals and small ligands lead to high coordination number, example $[Mo(CN)_8]^{4-,}$ small metal with large ligands lead to low coordination number, example $PT[P(Cme_3)]^2$.Due to their large size, lanthanide, actinides and early transition metal tend to have high coordination numbers .Different ligand structural arrangements result from the coordination number. The following is the geometry that is most frequently seen: Pentagonal bipyrimidal (seven-coordination), Octahedral (six-coordination), Tetrahedral (four-coordination), Trigonal (three-coordination), and Linear (for two-coordination) (Khaled *et al.*, 2014).

Maleic Acid

Almost every area of industrial chemistry uses fumaric acid (FA) and maleic acid (MA), two crucial chemical intermediates. An essential raw material for the production of surface coatings, copolymers, lubricant additives, unsaturated polyester resins, plasticizers, and agricultural chemicals is maleic acid (Klaus, 2008).

Maleic acid is a dicarboxylic acid that occurs in nature as L-malic acid. Another optically active isomer is D-malic acid which can be synthesized as the racemic mixture of D L-malic acid. Malic acid is commonly referred to as ‘apple acid’ because of its high concentration in apples (Malusdomestica). Other natural sources of malic acid include acerola (Malpighiae marginata), alfalfa (Medicago sativa), angelica (Angelica archangelica), blackhaw (Viburnum prunifolium), blood root (Sanguinaria Canadensis), cherries and others tone fruit (Prunus spp.), cranberries (Vacciniumma crocarpon), horsetail (Equisetu marvense), jujube (Ziziphus jujube), marigolds (Tagates spp.), rosehips (Rosa canina), schisandra (Schisandra chinensis), and tamarind (Tamarindus indica), (Khan and Abourashed, 2010). Malic acid is produced in the metabolic cycles of humans, plants, and animals. In the Krebs and glycosylate cycles, malic acid provides cells with the carbon skeleton and energy necessary for amino acid formation. There are two enantiomers, the L-, which sometimes is referred to as S-, and the D-,which is sometimes referred to as R-.Racemic DL-malic acid was first synthesized in 1923 (Mckenzie, 1923).

Biological production of L-malic acid from bacteria, including Lactobacillus L-malic acid is produced by the fermentation of fumaric acid. Fumaric acid can be produced by the fermentation from glucose. Yeast (Aureo basidium pullulans) can also be used to produce L-malic acid by fermentation (Zou *et al.*, 2013). DL-Malic acid can be commercially produced by several different synthetic processes. Most involve either maleic anhydride or fumaric acid hydrated at high temperatures (Felthouse *et al.*, 2000). Maleic anhydride is converted to maleic acid, which in turn is converted to malic acid. Another process involves the mixing of maleic acid, fumaric acid, and sodium hydrogen maleate in an aqueous solution (Ramsey and Schultz, 1993).

Foods and drinks include malic acid as an ingredient. Because little children lack the ability to metabolize D-form, the use of the less common D-recemic forms in infant meals is restricted. It functions as a chelating and buffering agent (Merck, 2015).

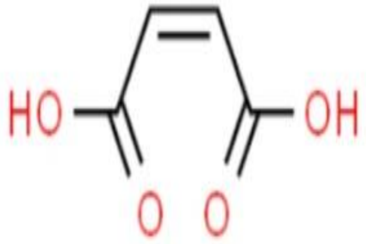

Fig.1 showing maleic acid

Complexes of Maleic acid

The intricate chemical processes of an aparticular metal's solubility, binding, and complexation with the elements of the aquatic phase of the environment determine its bioavailability (West *et al*., 1991).

Certain negative effects may lessen after complexation, and the metal complexes may be more active than the free ligands. Furthermore, the complexes may have bioactivities that the free ligand does not. Binding to a metal ion in vivo or using the metal complex as a carrier to activate the ligand as the cytotoxic agent are two possible mechanisms of action. Additionally, coordination could result in a notable decrease in medication resistance (Beraldo *et al*., 2004).

In biological fluids, the metal ions exist in non-exchangeable form as metalloproteins or loosely bound to some biological ligands as in metal-activated proteins. The loosely bound metal ions are in equilibrium with similar metal ions present in the bio-fluids. These simultaneous equilibria involving a variety of metal ions and ligands are important in biological fluids (May *et al*., 1977). Hence, the chemical speciation of ligands with metal ions has been studied in this laborator. (Ramanaiah *et* al 2013). Maleic acid is a dicarboxylic acid. It is the cis isomer of butenedioic acid, whereas fumaric acid is the trans isomer. It is soluble in water and moderately toxic. Inhalation causes irritating of nose and throat. Contact with eyes or skin causes irritation. It is used to make other chemicals and for dyeing and finishing naturally occurring fibers. Speciation profoundly influences both the toxicity and bioavailability of an element. The speciation studies of toxic metal ion complexes are useful for understanding the role played by active site cavities in biological molecules and the binding behavior of protein residues with the metal ions and helpful to understand the interaction with other ligands commonly exist in biological fluids. Chemical specification of metals is important for the understanding of their distribution, mobility, bioavailability, toxicity, and for setting environmental quality standards. Bioavailability of a particular metal depends on its complex chemical reactions of dissolution, binding, and complexation with the constituents of the environmental aquatic media. Studies have been done on the complex formation of maleic acid with Pb (II) and Cd (II) as a good example in modeling the bonding modes of dicarboxylic acids to toxic metal ions in mixtures containing ethylene glycol (EG) and water in order to uncover the effects of solvents on equilibrium processes involving charged species. The neurological, reproductive, renal, and hematological systems of both humans and animals are negatively impacted by heavy metals like lead, cadmium, and mercury. The central nervous system is toxically affected by organo-lead compounds (Chang *et al*., 1990)

Antioxidants activities of maleic acid complexes

Because fruits and vegetables include a variety of antioxidants and other useful substances, increasing consumption of these foods has been suggested as a crucial part of a balanced diet for lowering risks and preventing diseases (Genkinger *et al.*, 2004). This is also true of white button mushrooms (Agaricus busporum), which are used as functional foods and medicines in addition to being used as food because of their high protein and mineral content and low levels of starch and cholesterol. It is also thought to be a significant source of a number of antioxidant compounds, including ascorbic acid, carotenoids, flavonoids, tocopherols, and phenol (Wani *et al.*, 2010). Although button mushroom is reported to possess considerably higher levels of antioxidant properties, from the point of view of post-harvest physiology, mushroom is one of the most sensitive agricultural crops after harvesting and these antioxidant properties of button mushroom tend to decrease during post-harvest storage with the passage of time (Jahangir *et al.*, 2011). In order to increase metal solubilization, there has been a greater focus on the generation of organic acids by plant-associated microorganisms, such as oxalic acid, maleic acid, tartaric acid, succinic acid, and formic acid (Mahmud *et al.*, 2017).

In Brassica juncea plants under Cr stress, maleic acid (MA) increased the amount of non-enzymatic antioxidants (AsA, GSH) and the activities of enzymatic antioxidants (SOD, CAT, APOX, MDHAR, DHAR, GR, and GPOX). This further improved the uptake of Cr in the roots, but at lower concentrations of Cr it slightly reduced the translocation of Cr from roots to shoots, and at higher concentrations it significantly reduced it. MA enhanced the plants' growth, biomass, hydration status, and chlorophyll content while reducing the oxidative damage brought on by Cr (Mahmud *et al.*, 2017). In-vitro studies with soil microbes have elucidated to some level, in which the concentration of organic acid influx is directly normalized by the external concentration (Jones *et al.*, 1996). The rhizosphere's soil characteristics (sorption, biodegradation, buffering capacity, and metal complexation) might alter the organic acid profile, making it difficult to forecast how the acid will behave (Rajkumar *et al.*2012).

Cobalt

Cobalt is the main component of vitamin B12 (cobalamin), which serves as a co-enzyme in protein synthesis. It is located in the third period of the periodic table, between iron and nickel (Amudat, 2010). Cobalt is a white lustrous metal, having a grayish tinge. It has a melting point of 1497 ^{o}C, boiling point of 2880 ^{o}C and has a density of 8.90 g/cm3. The electrical conductivity is 10.8 $\Omega^{-1}m^{-1}\Delta$Ho sublime at 298K, is 425 KJ/mol, S298 is 30.04 J/kmol. Its atomic mass is 58.933, metallic atomic radius, is 0.125 nm, and valence electrons [Ar] $3d^{7}4s^{2}$ (Karthikyan, 1992).

The oxidation states of cobalt are +1, +2, +3, and +4. These two are the most popular: +2 and +3. The cobalt (II) ion is octahedrally coordinated in the structures of all anhydrous halides. Many complexes of different stereochemical kinds are formed by divalent cobalt. The most prevalent ones are octagonal and tetrahedral, but there are also several square planar and five-coordinated ones. No other transition metal ion generates as many tetrahedral complexes as cobalt (II), for example. where X = C1-, Br-, OH-, and SCN- in [CoX4]2- (Amudat, 2010). Cobalt (II) forms numerous complexes mostly either octahedral or tetrahedral. Five coordinate and square planar complexes are also known. Tetrahedral complexes of cobalt (II) are more in number than for other transition metal ions. This is mainly due to the fact that, for the d^7 ion, ligand stabilization energies disfavour the tetrahedral configuration relative to the octahedral one to a smaller extent than for any other configuration. Magnetic moment values for the high spin octahedral complexes lie between 4.7 and 5.2 BM. Because of the intrinsic orbital angular momentum in the octahedral ground state, there is considerable orbital contribution to the magnetic moment (Karthikyan, 1992). Low spin cobalt (II) octahedral complexes are rare. These species posses $t_{2g}{}^6$ eg^1 configuration and because of the presence of an electron in antibonding e*g orbitals they would be unstable. Furthermore, the complexes are subject to strong Jahn-Teller distortions. Thus they tend to lose ligands and form low spin four or five coordinate species (Lee, 2009).

Square planar complexes are low spin with magnetic moments of 2.2-2.7 BM. Their spectra are complex and neither magnetic nor spectral properties of such compounds have been studied in detail. Five coordinate high spin (with three unpaired electrons) and low spin (with one unpaired electron) complexes are found to have either trigonal bipyramidal or square pyramidal structures. The strength of the ligand field, which determines the magnitude of the splitting, is important in determining the spin state (Lee, 2009).

Cobalt (II) has a d^7 configuration and the ground state configuration in an octahedral ligand field is t_2g^5 eg^2 in weak field or t_2g^6 eg^1 in strong field. Octahedral coordinated cobalt (II) should have three spin-allowed d-d transitions. The visible absorption spectra of cobalt (III) complexes consist of transitions from A_2g ground state to other singlet states. It is possible to observe spin-allowed, d-d bands in the visible region of the spectra of low-spin. Thus, the strong field ligands that do not cause the low-energy charge-transfer bands, often dictate the spectra of low-spin complexes. Consequently, the two absorption bands found in the visible spectra of regular octahedral cobalt (II) complexes represent transition to the upper states T_1g and T_2g i.e. $A_2g \rightarrow T_1g$ and $A_2g \rightarrow T_2g$ (Karthikyan, 1992).

The complexes of Co (III) are numerous. Almost all Co (III) complexes are octahedral, though a few tetrahedral, square planar and square antiprismatic complexes are known. Six coordinate complexes of cobalt (III) which make up over 99% of known cobalt (III) complexes are invariably low spin and diamagnetic (with ${}^1A_{Ig}$ ground state term) (Karthikyan, 1992). Animals must have trace amounts of cobalt in their food, but higher concentrations are dangerous. In certain enzymes, cobalt plays a crucial biological role. One cobalt molecule that is extracted from the liver is vitamin B12. Consuming more raw liver has proven to be a successful treatment for pernicious anemia (Golding, 1983; Lee, 2009).

Nickel

Nickel has a silvery shine and is a bright white metal. It has a density of 8.91 g/cm3 and melts at 1455 oC. Nickel has an atomic mass of 58.71, a boiling point of 2840 0C, and valence electrons [Ar] $3d^8 4s^2$. 14 Ω-1m-1 is the electrical conductivity. It has more chemical activity than cobalt or iron. Like iron and cobalt, nickel is an element in group VIIIB. (Karthikyan, 1992).

Nickel has oxidation states of +2 and +3. Nickel (II) forms a large number of complexes encompassing coordination numbers 4,5 and 6. An example of 6-coordinates are $[Ni(H_2O)_6]^{2+}$, $[Ni(NH_3)_6]^{2+}$, $[Ni(en)_3]^{2+}$ etc. $[Ni(CN)_5]^{3-}$ is an example of 5-coordinate, and exists as a trigonal bipyramidal. Some of the 4-coordinates are square planar, an example is $(Ni(CN)_4)_2$- tetracyanonickelate (II) (Amudat, 2010). Nickel (II) has a d^8 configuration. Octahedral Ni^{2+} complexes showed three spin-allowed transitions, which are $3A_2g \rightarrow 3T_2g$, $3A_2g \rightarrow 3TIg(P)$, $3A_2g \rightarrow 3TI\ g\ (F)$, with the presence of two unpaired electrons, given a magnetic moment of two 2.9 – 3.4 BM. For regular tetrahedral complexes, the magnetic moment values are in the range 3.5-4.0 BM and for the more distorted ones the moments are in the range 3.0-3.5 BM (Karthikyan,1992). The +2 oxidation state is undoubtedly the most prolific oxidation state for nickel. The primary stereochemistries of Ni (II) include octahedral, tetrahedral, square planar, square pyramidal, and trigonal bipyramidal. Its coordination number hardly ever rises over 5. Depending on the size of the orbital contribution, the magnetic moments of octahedral nickel (II) complexes, which contain two unpaired electrons, vary from 2.9 to 3.4 BM. The most prevalent of the five coordinate complexes of Ni (II) is square planar stereochemistry (Golding, 1983; Lee, 2009; Amudat, 2010).

Copper

In nature, copper is found in large quantities as metals in carbonates (CuCO3), sulphides (CuS), and chlorides (CuCl2). Copper is frequently found in the oxidation states +1 and +2. As a result, copper (II) compounds are typically paramagnetic and colorful. CuSO4, CuCl2, and CuO are a few examples. The active ingredient in Fehling's and Benedict's solutions, which are the traditional test solutions for reducing sugars, is copper (II) sulphate. In water treatment, Cupper (II) sulphate is employed as an algaecide (Golding, 1983; Lee, 2009).

The majority of Cu (I) compounds can be readily oxidized to Cu (II) compounds, but it is more challenging to further oxidize to Cu (III). If placed in a cubic symmetry environment, Copper (II)'s d^9 configuration with one unpaired electron exposes it to John-Teller distortion, which has a significant impact on all of its stereochemistry. It is impossible to distinguish between square coordination and tetragonal deformed octahedral coordination. The Cu^{2+} ion is generally found in an environment with rather low symmetry, making it somewhat difficult to analyze the spectra and magnetic characteristics in detail. The magnetic moments of Cu (II) complexes are in the range 1.75-2.20 BM regardless of stereochemistry and independently of temperature except at extremely low temperature. Majority of the complex exhibits single absorption band in the region 11,000-16,000 cm^{-1}. The d^9 ion is characterized by large distortion from octahedral symmetry and the band is unsymmetrical being the result of a number of transitions which are by no means easy to assign unambiguously. An orbital contribution to the magnetic moment is implied by the T ground term of the tetrahedrally coordinated ion, and a value greater than the spin-only value (1.73 BM) is produced (Karthikyan, 1992).

All the copper (II) complexes are either blue or green. These colors are due to the presence of bands at 600-900 nm region of the spectrum. $Cu(H_2O)_6^{2+}$ is the most familiar complex of copper. It possesses a tetragonal distorted octahedral structure in which two of the water molecules are situated further from the copper ion than the other four. An example of tetrahedral complex is $CuCl_4^{2-}$, which is orange in color in the tetrahedral form but yellow when square planar (Golding, 1983; Lee, 2009).

In the chemical and medicinal sciences, copper is extremely important. Among transition metals, it is the third most important element. Both plants and the human body need it in varying amounts. After iron (4 g) and zinc (2 g), copper is the third largest transition metal in human bodies (approximately 100 mg in adults) (Amudat, 2010).

Even though larger concentrations of copper are harmful, humans still need to consume roughly 4-5 mg of copper every day. Animals that lack this metal are able to use the iron that is stored in their livers. Various oxidases and blue proteins, such as amine oxidases, ascorbate oxidases, cytochrome oxidases, and galactose oxidases, are examples of copper-binding enzymes or metalloproteinases found in animals (Golding, 1983; Lee, 2009). Protonation and stability constants of complexes they form with different metal ions, and copper in particular, are among these characteristics. Crucially, the determination of the bio-ligands' protonation constants and stability constants with different metal ions in media akin to those of biological systems is necessary for the clarification and comprehension of the many events in biological systems. It is often accepted in this context that aqueous solution can serve as a representation of "in vivo" media. Non-aqueous media have been developed as an alternative since biological media have lipophilic characteristics, although it has been demonstrated in recent years that aqueous media are not entirely appropriate for in vivo biological interactions (Amudat, 2010).

MATERIALS AND METHOD

Materials

Nickel (II) chloride,Ferric Chloride, Cobolt (II), Sodium hydroxide (NaOH). Ligand molecule (Maleic acid). Solvent: dimethyl formamide (DMF), ethanol and chlorofoam. Beaker, test-tube, magnetic stirrer conical flask, measuring cylinder, spatula, weighing balance, filter paper, funnel, desiccators, melting point apparatus, thermometer, UV- spectrophotometer

Method

All glass wares used in this work were washed with detergent after sucking in Conc. HNO_3 rinsed with distilled and dried in an oven. Weighing was conducted using electrical analytical balance model J A 203H. Melting point was determined using gallen kemp melting point apparatus. Electronic spectra of metal were recorded using Perkin-Elmer lambda 35 UV- Vis spectrometer. Percentage of metal was determined using AAS. Percentage composition of carbon, hydrogen and oxygen for the complexes and ligand were determined using micro analytical techniques.

Synthesis of cobalt (ii) complexes

The complexes were prepared following reported procedure (Nadira *et al.*, 2010; Ogunniran *et al.*, 2008). Ibrahim *et al* 1995). Maleic acid (0.416g, 161.1g/Mol) was dissolved in 10ml of ethanol and stirred until homogeneous solution is obtained. To this miscible solution ($Co(NO_2)_2.2H_2O$) (1.168g) dissolved in 10ml of ethanol was added dropped twice with constant stirring. The resulting mixture was stirred for three hours using magnetic stirrer at room temperature. The complexes were precipitated by adjusting the pH to 6-8 using buffer 7 by adding very dilute NaOH solution (5ml, 0.001M), drop wise and stirred using magnetic stirrer for 5-10 minutes, after which the solid complexes formed were poured into a beaker and left to stand for 24 hours. The precipitate was filtered and washed with equal volume of water and ethanol and dried over $CaCl_2$ in desecrator.

Equation for reaction is presented as

$C_4H_4O_4 + Co(NO_3)_2.6H_2O \rightarrow [Co(C_4H_4O_4).H_2O] + NO_3$

Synthesis of Nickel (II) Chloride

The complexes were prepared following reported procedure (Nadira *et al.*, 2010; Ogunniran *et al* 2008). Ibrahim *et al* 1995). Maleic acid (0.416g, 161.1g/Mol) was dissolved in 10ml of ethanol and stirred until homogeneous solution is obtained. To this miscible solution ($NiCl_2.2H_2O$) dissolved in ethanol 10ml was added dropped twice with constant stirring. The resulting mixture was stirred for three hours using magnetic stirrer at room temperature. The complexes were precipitated by adjusting the pH to 6-8 using buffer 7 by adding very dilute NaOH solution (5ml, 0.001M), drop wise and stirred using magnetic stirrer for 5-10 minutes, after which the solid complexes formed were poured into a beaker and left to stand for 24 hours. The precipitate was filtered, washed with equal volume of water and ethanol and dried over $CaCl_2$ in desecrator.

Equation for reaction is presented as

$NiCl_2.2H_2O + C_4H_4O_4 \rightarrow [Ni(C_4H_4O_4).2H_2O] + Cl_2$

Synthesis of Copper Sulphate

The complexes were prepared following reported procedure (Nadira *et al* 2010; Ogunniran *et al* 2008). Ibrahim *et al* 1995). Maleic acid (0.416g, 161.1g/Mol) was dissolved in 10ml of ethanol and prepared in 100ml three necked round bottom flask provides with electromagnetic stirrer and stirred until homogeneous solution is obtained. To this miscible solution ($CuSO_4.2H_20$) (2.988g) dissolved in ethanol 10ml was added dropped twice with constant stirring. The resulting mixture was stirred for three hours using magnetic stirrer at room temperature. The complexes were precipitated by adjusting the pH to 6-8 using buffer 7 by adding very dilute NaOH solution (5ml, 0.001M), drop wise and stirred using magnetic stirrer for 5-10 minutes, after which the solid complexes formed were poured into a beaker and left to stand for 24 hours. The precipitate was filtered, washed with equal volume of water and ethanol and dried over $CaCl_2$ in desecrator.

Equation for reaction is presented as

$C_4H_4O_4 + CuSO_4 + 2H_2O \rightarrow [Cu(C_4H_4O_4).2H_2O] + SO_4$

Analysis of purity

Determination of melting point

The melting points of the synthesized complexes were determined gallenkemp melting point apparatus fitted with a thermometer at Pharmaceutical Chemistry Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Maiduguri along Bama road Maiduguri, Borno State, Nigeria.

Solubility

The solubility of complexes was determined in some polar and non-polar solvent such as methanol, ethanol, chloroform and dimethyl sulformide (DMF)

Diphenyl-2-pickryl hydrazyl (DPPH).

The DPPH free radical scavenging potential of the ligands and their complexes relating to the standard i.e. ascorbic acid (VIT.C) was determined using reported procedure as described by Khan *et al* 2013. In the assay, ligands and their complexes at different concentrations (6.25µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml,) dissolved in ethanol were prepared in a test tube using serial dilution method and 2ml of freshly prepared 0.004mg DPPH in ethanol is added to each concentration the resulting mixture was allowed to stay in a dark at room temperature, after which, absorbance of the mixture was taken at 517nm using UV spectrophotometer. The absorbent was decreased by conversion of DPPH to the more stable DPPH molecule which serve as indication of the capacity of antioxidant compounds to donates H ion (Noipa, 2011).All measurement was taken in triplicates DPPH radical scavenging activities was calculated using the formula :

Scavenging activity (%) = $\frac{\text{Ac} - \text{As} \times 100}{Ac}$

Where Ac =Absorbance control and As = Absorbance of sample

Determination of EC_{50}

The EC_{50} value required for 50% of the DPPH free radicals scavenging by the complexes were determined from, a series of dose response data, (sample and DPPH free radical scavenging (%)). Using an X and Y plot fitted with a linear regretion line and the EC_{50} was estimated using the following relationship

$$EC_{50} = \frac{50-C}{M}$$

Where C rep. the intercept

M rep. the gradient of the line

RESULTS, DISCUSSION AND CONCLUSION

Results

Physical properties of the ligands and their and their complexes

The physical properties of the complexes were analyzed by physical observation, computation and instrumentation such as melting point determination, determination of percentage yield, color identification and molecular weight determination. The results were presented in table 4.1.

Table 4.1 Analytical Data and other Physical Properties of Maleic Acid and Complexes.

Empherical formula	Molecular Weight (g)	Color	Melting Point °C	Actual yield (g)	%Yield
$[Co(C_4H_4O_4).2H_2O]$	317.0	Ash	178	0.128	66.3
$[Ni(C_4H_4O_4).2H_2O]$	263.8	Grey	180	0.300	41.1
$[Cu(C_4H_4O_4).2H_2O]$	293.7	Light green	165	0.100	50.5

Physical Properties: The complexes were colored solids with melting points in the range of 165–180 °C, indicating stability. Yields were moderate (41.1–66.3%).

Solubility

The test of solubility of the ligands and their complexes were carried out in different solvent to know the best solvent to be used for purification and characterizations of the complexes, the result were represented in table 4.2.

Table 4.2: Solubility of the ligands and complexes in different solvent.

Complexes	Ethanol	methanol	chloroform	DMF
$[Co(C_4H_4O_4).2H_2O]$	S	IS	SS	SS
$[Ni(C_4H_4O_4).2H_2O]$	SS	SS	SS	S
$[Cu(C_4H_4O_4).2H_2O]$	S	SS	SS	SS
$C_4H_4O_4$	S	S	IS	SS

Key : Soluble (S), Slightly soluble (SS), Insoluble (IS), Dimethylformide (DMF).

Solubility: Complexes were soluble in ethanol and partially soluble in methanol, chloroform, and DMF.

DPPH (Diphenyl-2-pickryl hydrazyl)

The % scavenging activity against DPPH free radicals of the ligands and their complexes were determined and presented in table 4.3

Table 4.3: DPPH Radical scavenging activities (%) of complexes, ligands and standards

Conc (µg/ml)	Co complexes ± SEM	$NiCl_2$ complexes ± SEM	Ascorbic Acid (Vit C)
100	70.6 ± 0.09	68.8 ± 22	96.32 ± 0.1
50	60.4 ± 0.12	65.4 ± 0.08	96.78 ± 80
25	66.2 ± 0.08	64.6 ± 0.17	96.70 ± 12
12.5	63.5 ± 0.12	64.5 ± 0.05	96.52 ± 06

Antioxidant Activity: The complexes showed moderate scavenging activity but consistently less than Vitamin C across all concentrations..

EC_{50}

The EC_{50} results determined from linear regression analysis of the ligands and their complex were presented in table 4.4

Table 4.4 EC_{50} result of the complexes, ligand and standard

Compound	EC_{50}
Cobalt complexes	1.31
Nickel Complexes	1.14
Ascorbic acid	0.55

EC50 values (Co = 1.31, Ni = 1.14,) were higher than that of Vitamin C (0.55 µg/ml), confirming that Vitamin C had superior antioxidant activity

Table 4.5: showing ligand and complexes

Complex	Empherical Formula	% C	%H	%Metal
$C_4H_4O_4$	-	41.34	3.45	-
$[Co(C_4H_4O_4).2H_2O]$	$C_4H_8O_6Co$	24.86	4.14	30.5
$[Ni(C_4H_4O_4).2H_2O]$	$C_4H_8O_6Ni$	24.94	4.25	30.4
$[Cu(C_4H_4O_4).2H_2O]$	$C_4H_8O_6Cu$	24.29	4.05	32.16

Where: Hydrogen (H), Carbon (C).

Electronic absorption spectra

The electronic spectra of the ligand and their corresponding complexes were analyzed and presented in table 4.6 between the wavelength 200-800 nm.

Table 4.6 Electronic abortion spectra (UV) of Maleic acid and complexes.

Complexes	**Absorption (nm)**	**CM^{-1}**	**Proposed geometry**
$C_4H_4O_4$	250 370		
$[Co(C_4H_4O_4).2H_2O]$	440 490 590	25641 20408 16949	Octahedral
$[Ni(C_4H_4O_4).2H_2O]$	380 530 670	26316 18869 14925	Octahedral

[Cu($C_4H_4O_4$).2H_2O]	340 470 490	29412 21276 20408	Square Pyramidal

(Khaled *et al.*, 2014)

DISCUSSION

The result of physical properties of the complexes showed that all our complexes are colored, have high melting points (165,178 and 180) °C which indicates the strong bonding between the ligand and the metals. The percentage yields of the complexes were also calculated in which Co have the highest yields 63.3%, followed by Cu and Ni with yields of 50.5% and 41.1% respectively which are of good yields. From the solubility results obtained (table 4.2), it indicates that all the complexes are soluble in ethanol while slightly soluble in methanol, chloroform and DMF. The ligand is soluble in ethanol, methanol and insoluble in chloroform while slightly soluble in DMF. The antioxidant study (table 4.3), shows that the complexes of Co and Ni at conc of 100 μg/ml, 50 μg/ml, 25 μg/ml and 12.5 μg/ml have less scavenging activities compared to the Vitamin C which has high scavenging activities at all the concentration (96.78) as reported by this literature (Harinath *et* al., 2015). Table 4.4 shows that the EC_{50} of the complexes Co and Ni with EC_{50} (1.14 and 1.31) has weaker antioxidants activities compared to the Vitamin C which serve as a standard with EC_{50} of 0.55 but still showing notable antioxidant capacity. The percentage of carbon, hydrogen and metal was calculated, the percentage of carbon and hydrogen has been observed to reduce compared to the initials because some hydrogen have been lost during formation of the complexes as well as carbon while there is slightly increase the percentage of the metals from its initials.as shown in (table 4.6)

Table 4.6 show the various absorption Spectra the complexes and bands which was calculated and was used in the proposing the geometry of the complexes that was obtained as compared with some literatures Khaled *et al.*, (2014), Nevin *et al.*, (2016).

CONCLUSION

All the complexes are colored and possess high melting point which indicate good thermal stability of the complexes and strong metal – ligand bonding. They show good percentage yields (41.1%, 50.5%, and 66.3%). The complexes are soluble in ethanol and partially soluble in common organic solvents such as, methanol, chloroform and dimethylformamide (DMF).

The elemental analysis of the metal complexes is consistent with their general formula as 1:2, ligand: metal ratio. This is quite in agreement with the proposed geometry of the complexes which also indicate the presences of complexes.

RECOMMENDATION

Future studies should include detailed mechanistic investigations using multiple antioxidant assays and cell-based models to clarify how the complexes exert their activity. Cytotoxicity testing on normal and cancer cell lines, along with in vivo validation of safety and bioavailability, will be essential before proposing biomedical applications.

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