ISSN: 0067-2904

Synthesis, Characterization, and Antimicrobial Potentials of Some Flavonoid-Metal Complexes from *Chromolaena Odorata*

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Received: 3/5/2019 Accepted: 15/3/2020

Abstract

Flavonoid-metal complexes from the medicinal plant, *Chromolaena Odorata* were synthesized in this study using a standard method. Flavonoid extracts complexes, namely Mn-flavonoid complex, Co-flavonoid complex, Zn-flavonoid complex, and Cd-flavonoid complex were characterized using Fourier-Transform Infrared spectroscopy technique (FT-IR). Based on IR data, it was observed that the complexes shifted to lower frequencieswhen compared with the extract, indicating the interaction of the C=O and O-H groups during the complex formation. It was observed that the complexes were synthesized at a certain condition, which is acidic,with pH values ranging from 2.11 to 3.68. The conductance values (Λ_m) of the complexes were found to be in the range of 7-15 Ω^{-1} cm^2 mol^{-1}, which indicates that the complexes are non-electrolytes. The synthesized flavonoid-metal complexes and the extract were assayed for antibacterial activity against several pathogenic bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*) and fungi (*Aspergillus niger*, *Blastomyces dermatitidis*, *Candida albicans*, *Cryptococcus gattii*) by measuring the zone of inhibition. The complexes were active and highly antibacterial to all organisms when compared with extract.

Keywords: Flavonoid, Metal Complexes, Extract, Microorganisms, Synthesis,*Chromolaena Odorata*.

Introduction

Flavonoids can be referred to as one group of the natural products which are present in plant parts, such as seeds, bark and flowers. Al-Obeidi *et al.* (2016)[1] reported that flavonoids can be extracted from the petals of plants. In addition, flavonoids exhibited different pharmacological effects such as antimicrobial and anti-oxidant ones [2]. The anti-oxidant activity of flavonoids depends on theirability to scavenge free radicals that may cause DNA damage [3]. Flavonoids have great beneficial effects on human health and more efforts are made to isolate their constituents [4]. They also support the cardiovascular and nervous systems along with detoxification of potentially tissue-damaging molecules [5, 6]. They behave as chemical messengers and physiological regulators. They are polyphenolic compounds synthesized in the roots of the plants andfound in human diet such as fruit and vegetable which contain high levels of flavonoids [7]. The aim of this present research is to synthesize flavonoid metal complexes which will be more effective than their parent flavonoid extraction.

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Methodology

Apparatus and Materials

The leaves used for this research belong to *Chromolaena Odorata* which was collected in Ilorin, Kwara state Nigeria. The solvent (ethanol) and metal ions ($MnCl_2$, $CoCl_2$, $CdCl_2$, and $ZnCl_2$) were used without any further purification. They were obtained from Sigma Aldrich Company, United States of America**.** The apparatus used were hotplate, conical flask and beakers.

Preparation of the plant leaves

*Chromolaena Odorata (*is a perennial plant with many stemmed shrub. It can be reproduce from the root part) used in this research was obtained from a house garden in Tanke, Ilorin, Kwara state, Nigeria. The plant leaves were freed from sand and dirt which might be present. They were washed thoroughly with clean tapwater followed by distilled water, then allowed to air dry for days. Later, they were grinded using mortar and pestle to obtain a fine powder. The pulverized sample was stored in a sample bottle and labeled for further analysis.

Flavonoid extraction from plant leaves

The extraction procedure described by Maitera *et al*. (2018) was adopted [8]. Ten grams of the dried leaves powder wasweighed and extracted using 75% (10 ml) ethanol for about 1 hour at a temperature of about 65 °C. The aqueous extractwas then filtered into a conical flask and evaporated to dryness. The dried samples were weighed and marked.

Synthesis of flavonoid-metal complexes

The metal ions $MnCl_2$, $CoCl_2$, $CdCl_2$, and $ZnCl_2$ (0.20 g/mol, 0.24 g/mol, 0.18g/mol and 0.24 g/mol, respectively) were weighed in a desiccator and dissolved in distilled water. Solutions of the metals (15 ml) were added slowly to the solution of the extracted flavonoid (15 ml). The mixed solution was thoroughly stirred on hot plate for 45 min at 75 °C. Formation of precipitates was observed and the pH was determined. The formed precipitates were filtered in a vacuum system, washed, and dried.

Characterization

The melting point of the plant extract-metal complexes were recorded on Gallenkamp melting point apparatus (Melting point SMP 10). Conductivity measurements were performed using Jenway 4510 conductivity meter at the Department of Chemistry, University of Ilorin, Ilorin, Nigeria. The FT-IR results were recorded in KBr pellets within the range of 400-4000 cm^{-1} on Buck Scientific M500 IR spectrometer at the Redeemer University, Sango-ota, Ogun state, Nigeria. The pH of the complexes was determined on Jenway model 3510 bentop pH meter. The isolated organisms: *Escherichia Coli, Klebsiella Pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger, Blastomyces*, *Candida Albicans* and *Cryptococcus gattii* were used for screening the antimicrobial activity of the complexes at the Department of Microbiology, University of Ilorin, Ilorin, Nigeria.

Antimicrobial analysis

Antimicrobial activities of the flavonoid extract and its complexes were measured according to Mohammed *et al.* (2014) [9] method. Seven grams of nutrient agar was weighed into a 250 ml conical flask containing distilled water. It was thoroughly mixed and heated for about 15 min. to ensure complete dissolution, and then sterilized for 24 hours in an autoclave at 121 °C. The sterilized agar was transferred into sterilized petri dishes to solidify. A 1 cm^3 hole was made in the plate. Solutions of the extract and its complexes were prepared by adding 5 g of the compounds to 1 L of ethanol at a concentration of 5mg/ml, which were poured into the hole in the petri dish and kept inthe incubator for a day. Zones of inhibition were then evaluated.

Results and Discussion

The chemistry of the compounds

The reaction products between the flavonoid and the transition metal (II) ions along withtheir physicochemical properties are presented in Table-1. It was observed that $ZnCl_2$ complex has the highest pH value being formed at an acidic condition while, $CdCl_2$ complex has the lowest pH value. The $MnCl_2$, $CoCl_2$ and $CdCl_2$ complexes have pH values lower than 3.0 which may be due to the presence of flavonoids in their interactingform. All the formed complexes were colored and stable in air. The melting points of the complexes were higher than that of the extract (within 116-141^oC). In addition, $ZnCl_2$ complexes showed the highest conductivity (15 Ω^{-1} cm^2 mol^{-1}) that is possibly because of un-dissociated complexes in the solvent (DMSO). The complexes are non-electrolytic in nature. It wasreported in previous works that DMSO andDMF are among the solvents used for the determination of conductivity. The magnetic moment of the Mn(II), Co(II), Cd(II) and Zn(II)

complexes are 3.42 B.M., 3.18 B.M., 3.76 B.M. and 3.15 B.M, respectively. The magnetic susceptibilities are independent of their field strength which are identified by their magnetism contribution.

Table 1-Physicochemical properties of the flavonoid extract and its complexes

Extract/ Complexes	**Conductivity Ω^{-1} cm^2 mol^{-1}**	**Melting point (^{o}C)**	**pH**	**μ_{eff}(B.M)**
Mn-flavonoid complex	7	141	2.96	3.42
Co-flavonoid complex	11	120	2.45	3.18
Cd-flavonoid complex	9	123	2.11	3.76
Zn-flavonoid complex	15	116	3.68	3.15

Footnote: μ_{eff} = Magnetic momen

Infrared analysis

The **infrared**(IR) data of the extract and complexes are presented in Table- 2. It was observed that the IR spectrum of the extract indicates a frequency at 1694 cm^{-1} which is attributed to C=O. This frequency was shifted to a lower one,within the range 1633 – 1656 cm^{-1}, in all the complexes. This indicates that an interaction occurs at the carbonyl group ν (C=O) with the central metal ions. The OH group which is within 3533 – 3585 cm^{-1} showed the presence of hydroxyl group and water (Figure- 1). The presence of water/ moisture gave a broad band that appeared in the regions 3533 – 3585 cm^{-1}; this can be due to ν(O-H) stretching and ν(O-H) rocking vibrations, respectively. This further supports the presence of non-ligands assignable to the rocking mode of water [10] and confirms the formation of the complexes [11].

Table 2-FTIR spectral data of the flavonoid and its metal complexes

Extract /complexes	**C=O stretch (cm^{-1})**	**C-H Alkane stretch (cm^{-1})**	**C-O alcohol stretch (cm^{-1})**	**O-H alcohol stretch (cm^{-1})**
Extract (Flavonoid)	1694	2911	1019	3510
Mn-flavonoid complex	1656	2938	1066	3585
Co-flavonoid complex	1649	2946	1058	3533
Cd-flavonoid complex	1653	2958	1063	3558
Zn-flavonoid complex	1633	2934	1051	3536

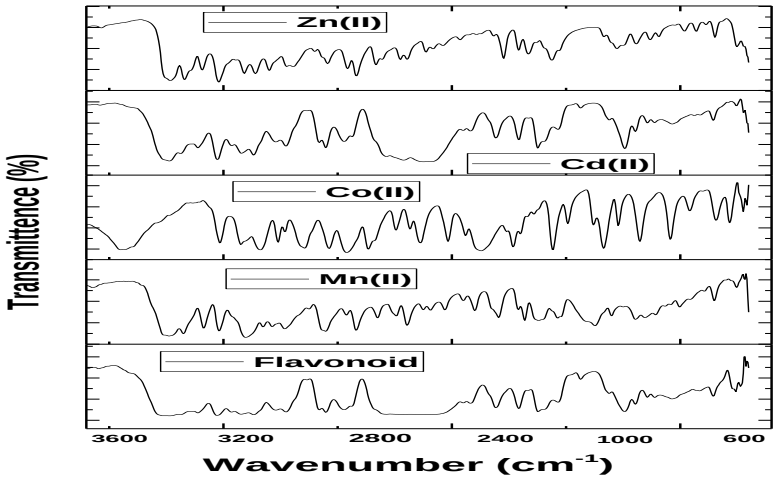


Figure 1-FT-IR spectra of the flavonoid and its metal complexes

Antimicrobial activities of the compounds

The extract and its complexes were screened against some selected organisms, as presented in Tables-(3 and 4). It was observed that the flavonoid extract was not effective against *Klebsiella pneumonia* and *Staphylococcus aureus*. The complexes were active and highly antibacterial to all organisms when compared with extract. DMSO that acted as control did not show any effects on the organisms. The antifungal activitiesof the extract and its complexes are presented. The results indicate that the complexes were more harmful than their extract against the organisms at certain conditions. The complexes showed more antifungal activities,which can be as a result of the effects of the metal on normal cellular process such as DNA replication [12,13]. This may be due to the solubility of the extract in the solvents [14]. A previous work studied the antibacterial activity of flavonoids which havethe ability to form complexes [15]. It was observed that the complexes showed larger zones of inhibitory after interacting with the flavonoid extract.

Table 3-Antibacterial activities of the flavonoid extract and its complexes

Extract /Complexes	***Escherichia coli***	***Klebsiella pneumonia***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
		Zone of Inhibition (mm)		
Mn-flavonoid	25	17	10	18
Co-flavonoid	28	15	31	16
Cd-flavonoid	14	21	18	23
Zn-flavonoid	19	11	15	20
Extract (Flavonoid)	02	13	01	01
Control (DMSO)	-	-	-	05

Table 4-Antifungal activities of the flavonoid extract and its complexes

Extract/Complexes	***Aspergillus niger***	***Blastomyces dermatitidis***	***Candida albicans***	***Cryptococcus gattii***
		Zone of Inhibition (mm)		
Mn-flavonoid complex	18	24	14	14
Co-flavonoid complex	13	19	12	24
Cd-flavonoid complex	25	27	18	17
Zn-flavonoid complex	23	13	23	13
Extract (Flavonoid)	9	12	9	11
Control (DMSO)	-	-	-	12

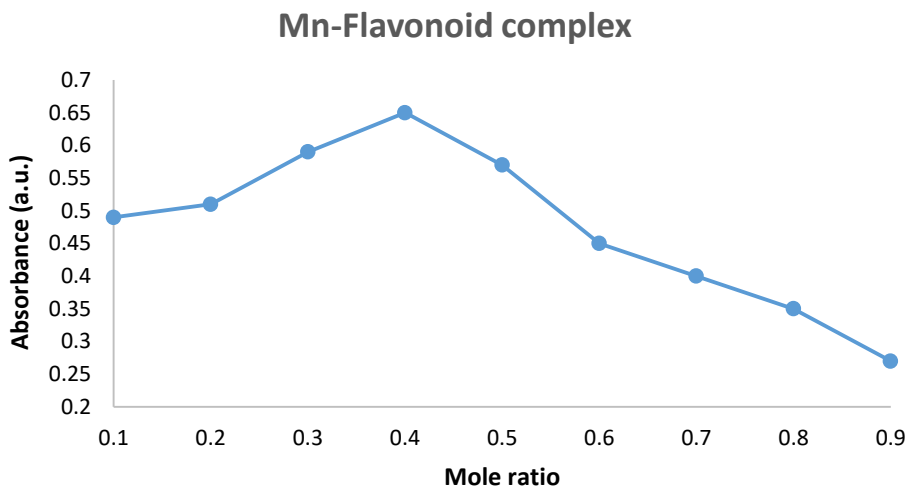
Job's method of continual variation

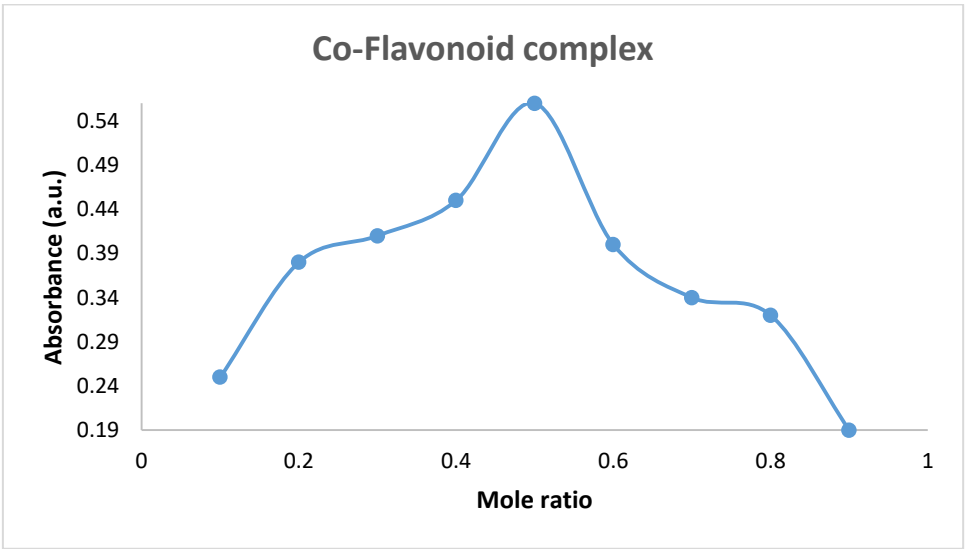
Job's method of continual variation was used to determine the stoichiometric ratio between the metals and the extract, as presented in Tabl- 5 [16]. Absorbance against mole fraction was plotted in each of the complexes (Figure-2). This indicated a curve with maximum absorbance as a result of

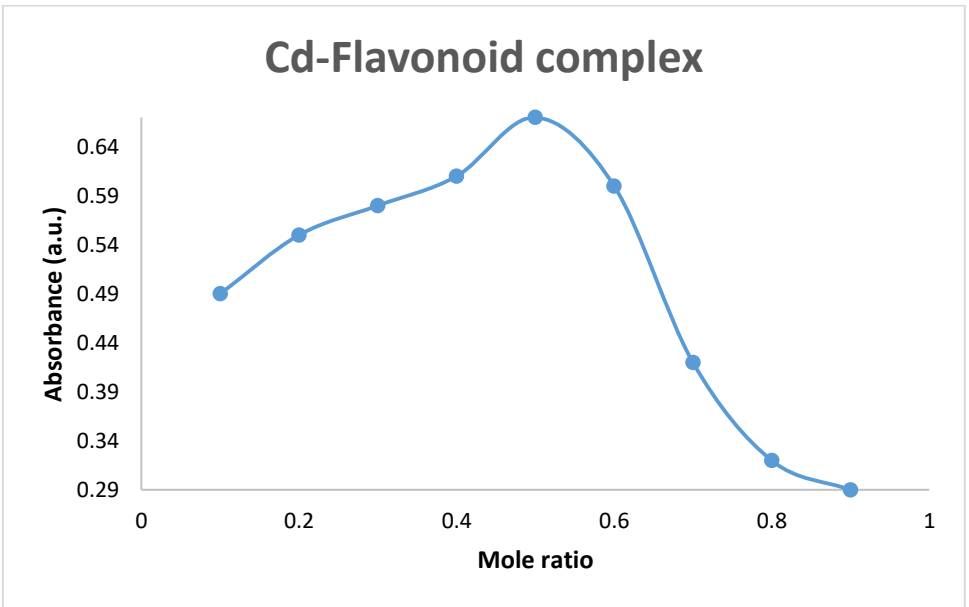
ligand mole fractions and interactions at 1:1 metal -ligand ratio in all complexes [17]. The maximum values of Mn(II), Co(II), Cd(II) and Zn(II) on the Job's plot were 0.65, 0.56, 0.67 and 0.45 respectively. It is observed that in order to get an estimate stoichiometry, the sum total of the flavonoid extract and its complexes must be related to the dissociation constant. The maximum number on the Job's plot corresponds to the stoichiometry of the extract and the complexes if there are high concentrations [17, 18]. This helps to understand the equilibrium constant of the complexes.

Table 5-Results of Job's method of continuous variation for flavonoid-metal complexes

Complexes	Ligand – Metal ratio	Mole fraction	Absorbance (a.u.)
Mn-Flavonoid complex	1:9	0.1	0.49
	2:8	0.2	0.51
	3:7	0.3	0.59
	4:6	0.4	0.65
	5:5	0.5	0.57
	6:4	0.6	0.45
	7:3	0.7	0.40
	8:2	0.8	0.35
	9:1	0.9	0.27
Co-Flavonoid complex	1:9	0.1	0.25
	2:8	0.2	0.38
	3:7	0.3	0.41
	4:6	0.4	0.45
	5:5	0.5	0.56
	6:4	0.6	0.40
	7:3	0.7	0.34
	8:2	0.8	0.32
	9:1	0.9	0.19
Cd-Flavonoid complex	1:9	0.1	0.49
	2:8	0.2	0.55
	3:7	0.3	0.58
	4:6	0.4	0.61
	5:5	0.5	0.67
	6:4	0.6	0.60
	7:3	0.7	0.42
	8:2	0.8	0.32
	9:1	0.9	0.29
Zn-Flavonoid complex	1:9	0.1	0.23
	2:8	0.2	0.37
	3:7	0.3	0.39
	4:6	0.4	0.45
	5:5	0.5	0.34
	6:4	0.6	0.30
	7:3	0.7	0.26
	8:2	0.8	0.21
	9:1	0.9	0.17


Mn-Flavonoid complex
Absorbance (a.u.)
0.7
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Mole ratio


Co-Flavonoid complex
Absorbance (a.u.)
0.54
0.49
0.44
0.39
0.34
0.29
0.24
0.19
0
0.2
0.4
0.6
0.8
1
Mole ratio


Cd-Flavonoid complex
Absorbance (a.u.)
0.64
0.59
0.54
0.49
0.44
0.39
0.34
0.29
0
0.2
0.4
0.6
0.8
1
Mole ratio

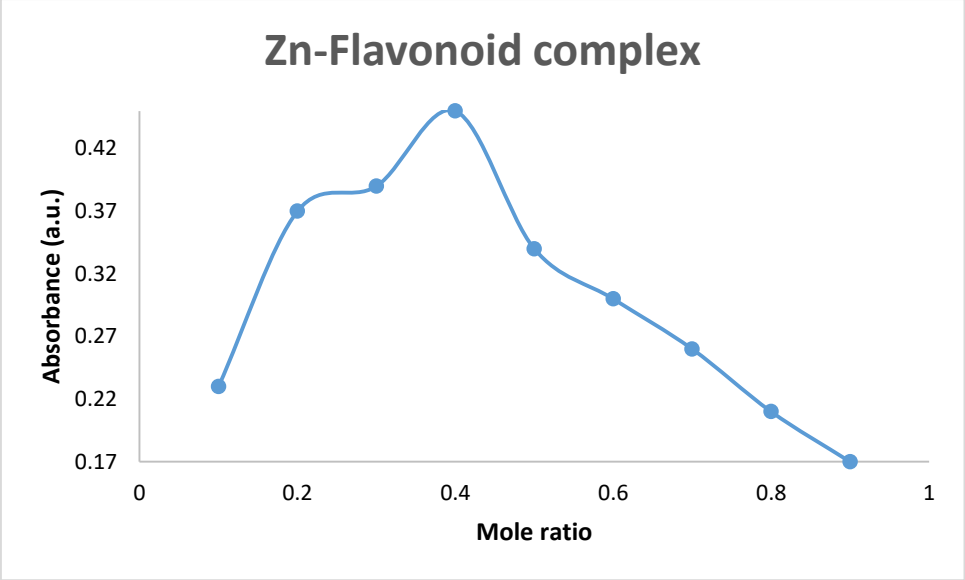


Figure 2-Curves showing the results of Job's method of continuous variation for flavonoid-metal complexes.

Conclusions

This study confirms the preparation of metal complexes using the flavonoid extracts from the medicinal plant *Chromolaena odorata*. The results confirmed the interactions between the metal ions and the flavonoid. The bands of ν(C=O) and ν(O-H) of the flavonoid were shifted to lower frequencies in all complexes. It was observed that the complexes were formed at an acidic condition of a pH value that is lower than 4. According to the results, the metal ions help to increase the activity of flavonoid complexes, as exemplified by the anti -microbiological study. Cd- complex was found to be the most effective among the other complexes against *Blastomyces dermatitidis*.

Conflict of interest

The authors have no conflict of interest to declare.

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