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<table>
<thead>
<tr>
<th></th>
<th>Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Synthesis, Spectral Characterisation and Antimicrobial Properties of Cu(II) and Fe(II) Complexes with Xanthone</td>
<td>Rabuyah Ni, Mohammad Isa Mohamadin, Vivien Jong Yi Mian</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Bioadsorption of Multiple Heavy Metal Ions by Rhizophora Apiculate sp. and Elaesis Guineensis sp.</td>
<td>M.B. Nicodemus Ujih, Mohammad Isa Mohamadin, Millaa-Armila Asli, Bebe Norlita Mohamed</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
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<td>Karimah Kassim, Muhamad Azwan Hamali</td>
<td>29</td>
</tr>
</tbody>
</table>
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Husnul Azan Tajurudin
Mohd Sharizal Mohd Sapingi
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The new complexes \([\text{CuL}_2(\text{H}_2\text{O})_2]\) and \([\text{FeL}_2(\text{CH}_3\text{O})_2]\) in which \(L = \beta\)-mangostin were synthesised and characterised. The structure of the ligand, \(\beta\)-mangostin was confirmed using NMR and the purity of ligand was determined using HPLC. Both \(\text{Cu(II)}\) and \(\text{Fe(II)}\) complexes were prepared by reaction between the ligand and the acetate of the metals in one-step reaction. The synthesised compounds have been characterised using UV-Visible, FTIR and CHNS analyser. Ligand and metal complexes were tested against bacteria to assess on their antimicrobial properties using Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) method. The elemental analysis and spectra data suggested octahedral geometry for both \(\text{Cu(II)}\) and \(\text{Fe(II)}\) complexes. The IR spectroscopy revealed that the chelation of \(\text{Cu}^{2+}\) and \(\text{Fe}^{2+}\) ion occurred with hydroxyl and carbonyl group at \(C_9\) and \(C_1\) respectively of \(\beta\)-mangostin. Both \(\text{Cu(II)}\) and \(\text{Fe(II)}\) complexes showed stronger inhibition against \(\text{Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae and Salmonella pneumonia}\) at concentration 900 mg/mL and \(\text{Escherichia coli}\) at 450 mg/mL compared to the ligand itself.

**Keywords:** \(\beta\)-mangostin, copper(II) complex, iron(II) complex, antimicrobial, xanthones
INTRODUCTION

Xanthones are naturally oxygenated heterocycles with $\gamma$-pyron moiety fused with two benzene rings (Figure 1). There has been strong interest of this class of compounds due to their unique chemical structures containing different types of substituent in different positions, which leads to a large variety of pharmacological activities [1, 2]. They have remarkable biological and pharmacological properties such as antibacterial, antioxidant, antiviral, anticancer, anti-inflammatory and antifungal. Consequently, researchers tend to isolate xanthone derivatives from natural product and also tend to synthesise these compounds as novel drug candidates [3]. The major secondary metabolites that can be isolated from *Garcinia mangostana* are $\alpha$-mangostin, $\beta$-mangostin, and $\gamma$-mangostin containing xanthone scaffold [4]. Simultaneously, synthetic and medicinal chemistry studies of xanthone derivatives have been performed [5]. In contrast, there are only a few reports that deal with complexation of metal ions with xanthone derivatives.

Coumarins and flavonoid as secondary metabolites have attracted more interest among researchers to check whether its metal complexes are more biologically effective than the ligand itself. Quercetin (Figure 2) and Morin (Figure 3) are examples of flavonoids forming metal complexes that have better antibacterial and cytotoxic properties as compared to its ligand. The metal complexes were formed via chelation of hydroxyl and carbonyl group of the ligands. The location of chelation is influenced by the anion used in metal, the ratio of starting material and pH value. Farhan [6] proposed that metal chelation with hydroxyl and carbonyl are at C$_5$ and C$_4$ respectively. The reaction condition was in the presence of ammonia at pH 7-8 and molar ratio 1:2 of copper(II) chloride and morin in ethanolic solution. While, Panhwar *et al.* [7] suggested that chelation with hydroxyl and carbonyl group at C$_3$ and C$_4$ respectively in equal molar of copper(II) sulphate and morin in methanol. Besides, Bukhari *et al.*, [8] proposed chelation also occurred in copper(II)-quercetin complex utilising 1:2 proportion of copper(II) sulphate and quercetin in methanol.

Up to this point, complexation having xanthones as a ligand only involves synthetic xanthones. The synthetic xanthone was prepared via reaction of dihydroxyxanthone with crown ether [9] and piperidinyl [10] respectively. The macrostructure of crown ether helps to stabilise the
Cu(II) complexes formed whereas piperidinyl structure contributes basicity properties to encourage formation of Cu(II) and Zn(II) complexes with simple oxygenated xanthones (Figure 4 and Figure 5).

In this work, the natural occurring xanthone, β-mangostin from Garcinia mangostana were used as ligand to synthesise novel antibacterial agents through complexation process involving copper and iron. Subsequently, β-mangostin, Cu(II) and Fe(II) complexes were characterised and tested for antimicrobial properties.

Figure 1: Xanthone

Figure 2: Quercetin
Figure 3: Morin

Figure 4: 1,8-dihydroxyxanthone

Figure 5: 1,6-dihydroxyxanthone
METHOD

Materials

All reagents and solvents used were analytical grade. Thin Layer Chromatography (TLC) analysis was performed using silica gel 60 F$_{254}$ (Merck), liquid vacuum column chromatography analysis was carried out on silica gel 60 F$_{254}$ (Merck) and gravity column chromatography using silica gel 60 (0.040 – 0.063 mm) (Merck). Cu(II) acetate and Fe(II) acetate were purchased from R & M whereas, nutrient agar and nutrient broth were brought from Bendosen.

Extraction, Isolation and Purification of β-mangostin

The barks sample of *Garcinia mangostana* was collected from Sarawak Forestry Department. The herbarium voucher specimens were kept at Universiti Teknologi MARA Sarawak. The stem barks sample was cut to smaller pieces and air dried at room temperature for few weeks. Then, it was grinded using heavy duty grinder at Sarawak Forestry Department. The air-dried powder of stem barks sample was soaked with chloroform for 48 hours at room temperature. Evaporation of solvents yielded 31.8 g of residues. The crude chloroform extract was isolated with hexane/chloroform, chloroform/ethyl acetate and ethyl acetate/methanol using Liquid Vacuum Chromatography to afford 27 fractions. Fractions 11 until 13 were further isolated using gravity column chromatography, eluted with hexane/chloroform and followed by chloroform/methanol solvent system gradient. Fractions which gave similar spots and same R$_f$ values on the TLC plates of β-mangostin were combined. The purity of β-mangostin was determined using Agilent HPLC Series 1260 Infinity.

Preparation of the Metal Complexes

The copper(II) complex was prepared by the addition of ligand to an ethanolic solution of copper(II) acetate in 1 : 2 ratio. Crystal ligand was formed when the procedure was repeated with iron(II) acetate. Therefore,
β-mangostin complex with iron(II) was prepared by 1 : 2 molar reaction of ligand and iron(II) acetate using methanol in one-pot reaction as described by Bukhari et al. [8]. The resulting complexes were characterised using spectroscopic techniques.

Instrumentation

Elemental analysis was performed using Elemental analyser, vario MICRO cube. The electronic spectra determinations were performed using Perkin-Elmer, Model Lambda 25. Infrared spectra were recorded in 4000-400 cm$^{-1}$ by Perkin-Elmer, Frontier FTIR spectrophotometer in KBr pellets. $^1$H and $^{13}$C NMR measurements were carried out by Bruker at 400 MHz. HPLC analysis for β-mangostin was performed by Agilent G1316A (150 mm x 4.6 mm, 5µm) column, the mobile phase was acetonitrile/water (80:20, v/v) mixture at room temperature with one mL min$^{-1}$ flow rate and 5µl injection loop. UV detector of HPLC was set at 320 nm since this wavelength was a selective wavelength for xanthone scaffold detection and only few other compounds can be ingested at this wavelength [11].

Antimicrobial Properties

β-mangostin, Cu(II) and Fe(II) complexes were evaluated for antimicrobial properties against five gram negative bacteria strains. Unlike gram positive bacteria, Gram-negative bacteria are more resistant against antibodies and most antibiotics because of their impermeable cell wall. Bacteria used were Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia and Salmonella pneumoniae. Antimicrobial activity was performed using Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) method. Streptomycin sulphate was used as the positive control for antimicrobial test.
RESULT AND DISCUSSION

Structural Elucidation of \( \beta \)-mangostin

The ligand, \( \beta \)-mangostin was isolated as fine yellow needle with melting point of 174 – 175°C (Lit. 175 – 176°C, [12]). \( \beta \)-mangostin was obtained via column chromatography and eluted with chloroform hexane mixture in 9:1 ratio and showed good agreement with Gopalakrishnan et al., [13]. Figure 6 shows the structure of \( \beta \)-mangostin with molecular formula of \( \text{C}_{25}\text{H}_{28}\text{O}_{6} \). Meanwhile, the IR spectrum showed the presence of hydroxyl group at 3399 cm\(^{-1}\) and chelated carbonyl group at 1647 cm\(^{-1}\). The absorption bands are situated at 1600, 1571, 1458 and 1278 cm\(^{-1}\) and are related to carbon vibration in benzene rings. The purity of isolated \( \beta \)-mangostin determined from HPLC analysis was 98%. Table 1 represents \(^1\text{H}-\text{NMR}, \(^{13}\text{C}-\text{NMR} \) and DEPT of \( \beta \)-mangostin. \(^1\text{H}-\text{NMR} \) and \(^{13}\text{C}-\text{NMR} \) result was compared with reported data by Sen et al. [14], Al-Massarani et al. [15] and Syam et al. [16] before identified as \( \beta \)-mangostin.

![Image of \( \beta \)-mangostin structure](image_url)
**Table 1: $^1$H-NMR, $^{13}$C-NMR and DEPT of $\beta$-mangostin in acetone-$d_6$**

<table>
<thead>
<tr>
<th>Carbon</th>
<th>$\delta_{C}$</th>
<th>$\delta_{H}$</th>
<th>DEPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>159.63</td>
<td>13.66 (1H, s, OH)</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>110.94</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>163.5</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>90.00</td>
<td>6.53 (1H, s)</td>
<td>CH</td>
</tr>
<tr>
<td>4a</td>
<td>155.31</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>5</td>
<td>101.83</td>
<td>6.87 (1H, s)</td>
<td>CH</td>
</tr>
<tr>
<td>6</td>
<td>156.69</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>7</td>
<td>143.70</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>8</td>
<td>137.25</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>8a</td>
<td>111.18</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>9</td>
<td>182.06</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>9a</td>
<td>103.27</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>10a</td>
<td>155.42</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>11</td>
<td>26.00</td>
<td>4.15 (2H, d, J = 6.6 Hz)</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>12</td>
<td>122.41</td>
<td>5.22 (2H, t, J = 7.3 Hz)</td>
<td>CH</td>
</tr>
<tr>
<td>13</td>
<td>130.66</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>14</td>
<td>25.05</td>
<td>1.67 (3H, s)</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>15</td>
<td>16.97</td>
<td>1.84 (3H, s)</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>16</td>
<td>21.02</td>
<td>3.34 (2H, d, J = 7.2 Hz)</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>17</td>
<td>123.78</td>
<td>5.29 (2H, t, J = 6.7 Hz)</td>
<td>CH</td>
</tr>
<tr>
<td>18</td>
<td>130.62</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>19</td>
<td>25.00</td>
<td>1.65 (3H, s)</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>20</td>
<td>17.40</td>
<td>1.79 (3H, s)</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>7-OMe</td>
<td>60.46</td>
<td>3.99 (3H, s)</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>3-OMe</td>
<td>55.67</td>
<td>3.81 (3H, s)</td>
<td>CH$_3$</td>
</tr>
</tbody>
</table>
Physical Properties of Metal Complexes

The metal complexes $[\text{CuL}_2(\text{H}_2\text{O})_2]$ and $[\text{FeL}_2(\text{CH}_3\text{O})_2]$ appeared as green and dark brown complex respectively. They are stable at room temperature. The percent yield of Cu(II) and iron(II) were 72% and 83% respectively. Elemental analysis suggested the ratio between metal to ligand was 1:2. Table 2 displays the actual and experimental values of carbon and hydrogen for both metal complexes.

Table 2: Elemental analysis of copper (II) and iron (II) complexes

<table>
<thead>
<tr>
<th>Metal complexes</th>
<th>Theoretical value</th>
<th>Experimental result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (%)</td>
<td>H (%)</td>
</tr>
<tr>
<td>$\text{CuL}_2(\text{H}_2\text{O})_2$</td>
<td>62.6</td>
<td>5.4</td>
</tr>
<tr>
<td>$\text{FeL}_2(\text{CH}_3\text{O})_2$</td>
<td>64.5</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Spectroscopic Study of Metal Complexes

IR spectra of $\beta$-mangostin showed the shifting of $\nu$(C=O) peak from 1647 cm$^{-1}$ to 1615 and 1610 cm$^{-1}$ for Cu(II) and Fe(II) respectively. The carbonyl frequency in the ligand is shifted to lower frequency. Thus, this showed that the electron density in the carbonyl was slightly decreased probably due to back bonding process. It was in a good agreement with Shen et al., [9] and Wang et al., [10]. Moreover, a broad band was observed at 3438 cm$^{-1}$ and 3429 cm$^{-1}$ for Cu (II) and Fe (II) complex respectively suggesting that the oxygen from hydroxyl also involved in coordination bond with the Cu$^{2+}$ and Fe$^{2+}$ ion.

Another significant difference between the ligand and its metal complex was observed from UV spectrum. The UV spectrum of copper(II) complex showed a broad band at 22000 cm$^{-1}$ (450 nm) which presumably corresponded to $d$-$d$ transition with octahedral arrangement as described by Yousef et al. [17]. While, Iron(II) complex has a broad band at 19157 cm$^{-1}$ (522 nm) [18] indicating an octahedral environment to the surrounding metal. The chelation of Cu(II)-$\beta$-mangostin and Fe(II)-$\beta$-mangostin complexes with bidentate ligand occurred with hydroxyl and carbonyl group at C$_9$ and C$_1$ respectively. The proposed structures of the complexes
are shown in Figure 7. The chelation formation in this study was similar to Farhan [6](2013) and Wang et al. [10].

![Figure 7: Proposed structure for (a) Cu(II) and (b) Fe(II) complexes](image)

**Antimicrobial Activity**

Table 3 illustrates the classification of antimicrobial properties adopted from Pessini et al. [19]. The evaluation of antibacterial against bacteria is given in Table 4. β-mangostin was inactive against five Gram-negative bacteria strains. However, both metal complexes exhibited moderate inhibition towards *Escherichia coli* and weak inhibition to other bacteria.

**Table 3: Classification of antimicrobial properties [19]**

<table>
<thead>
<tr>
<th>Antibacterial properties</th>
<th>Range (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong inhibition</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>Moderate inhibition</td>
<td>100 - 500</td>
</tr>
<tr>
<td>Weak inhibition</td>
<td>500 - 1000</td>
</tr>
<tr>
<td>Inactive inhibition</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>
Table 4: MICs (MBCs) of ligand and metal complexes against range of microorganisms

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC (MBC) ppm</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-mangostin</td>
<td>Cu-β-mangostin complex</td>
<td>Fe-β-mangostin complex</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1800</td>
<td>900</td>
<td>900</td>
</tr>
<tr>
<td>Protes vulgaris</td>
<td>1800</td>
<td>900</td>
<td>900</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>1800</td>
<td>900</td>
<td>900</td>
</tr>
<tr>
<td>Salmonella pneumoniae</td>
<td>1800</td>
<td>900</td>
<td>900</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1800</td>
<td>450</td>
<td>450</td>
</tr>
</tbody>
</table>

CONCLUSION

Two new metal complexes containing copper and iron were successfully synthesized with general formula of \([\text{CuL}_2(\text{H}_2\text{O})_2]\) and \([\text{FeL}_2(\text{CH}_3\text{O})_2]\). The elemental analysis and spectra data suggested the octahedral geometry for both Cu(II) and Fe(II) complexes. The IR spectroscopy showed that the oxygen of the carbonyl and hydroxyl of β-mangostin formed a coordination bond with Cu\(^{2+}\) and Fe\(^{2+}\) ion. The newly synthesized compounds, Cu(II) and Fe(II) complexes exhibited better activity towards antimicrobial than ligand itself, indicating that it has a good potential as bactericide.

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