

Adsorptive Interaction of Natural Antifungals with Metal Hexacyanoferrate (II) Complexes and its Medical Applications

ABSTRACT

The study aimed to investigate the adsorptive interaction between metal hexacyanoferrates (II) and natural antifungals. Nickel, manganese and copper hexacyanoferrates (II) were synthesized and characterized by elemental and spectral analysis. The antifungal potentials of each metal hexacyanoferrate (II) at 10 mg/mL were combined with two natural antifungals: coconut (*Cocos nucifera*) and ochro (*Abelmoschus esculentus*) leaves. The ethanol and acetone extracts at 20 mg/mL and 10 mg/mL, respectively have been evaluated against fungus *Candida albicans*. The antifungal potential was executed by the disc diffusion method. The acetone and ethanol extract of *A. esculentus* at 10 mg/mL were found to possess the most effective antifungal potential. Each interaction pair was also characterized by spectral analysis and phytochemical analysis.

Keywords: Metal hexacyanoferrates (II), *Cocos nucifera*, *Abelmoschus esculentus*, Natural antifungals, *Candida albicans*, Disc diffusion, Green drugs.

1. INTRODUCTION

Metal hexacyanoferrates (II) serve three functions: as adsorbents, ion exchangers and photosensitizers [1]. Biosorption of heavy metals has been a recent research area where plants, bacteria and fungi facilitate the degradation of metal- cyanide complexes, including large complexes such as ferricyanides and ferrocyanides. The biodegradation occurs when the impermeable membrane of the metal-cyanide complexes is disrupted [2].

25 In other cases, the fungi do not tolerate the toxicity of the heavy metal and its complexes at
26 high concentrations. Some of the mechanisms by which metal hexacyanoferrates (II) function
27 to degrade the fungi instead are: inhibiting cell division, enzyme activity, disrupting cell
28 membrane and denaturing of protein among other actions [3, 4]. It can be assumed that either
29 the action of the fungi to degrade the metal-cyanide complex would result in its decrease in
30 growth or the fungi would be intolerable to the toxicity of the metal hexacyanoferrates (II).
31 The effects of interacting natural antifungals with transition metal ferrocyanides. The
32 transition (II) metal ferrocyanides included nickel, copper, manganese which interacted with
33 methanolic leaf extracts. The effects were observed using scanning electron microscope. The
34 interaction was tested in against *Rhizoctoniasolani*, a fungal disease which affects potatoes.
35 One of the leaves extracts in particular *Cassia alata* (Canicro bush) was least effective when
36 paired with nickel ferrocyanide. The effects of the leaves extracts paired with manganese
37 ferrocyanide was greater, while the leaf extracts paired with copper ferrocyanide was the
38 greatest [5].

39 The experiment was repeated with the same metal ferrocyanides and fungal disease. The
40 only difference being the methanolic leaf extracts. The results remained unchanged with
41 cadmium ferrocyanide being the most effective followed by manganese and nickel
42 ferrocyanides [6]. The antifungal activity of nickel and copper ferrocyanide were tested
43 against *Aspergillus niger* and *Candida albicans*. Neither of the ferrocyanides showed any
44 significant effectiveness against *C. albicans* [7]. Metal ferrocyanides: nickel, copper and
45 manganese against *Verticillum fungicola* and *Mycogone perniciosa*. *M. perniciosa* (wet
46 bubble disease) and *V. fungicola* (dry bubble disease) are fungal diseases which affect
47 mushrooms. All of the ferrocyanides exhibited antifungal potential against *M. perniciosa*.
48 The main findings revealed that nickel ferrocyanide was most effective in inhibiting the
49 activity of *M. perniciosa* [8]. The antifungal potential of nickel and copper ferrocyanides was

50 tested against the dry bubble disease (*V. fungicola*) of *Agaricus bisporus*. The results
51 concluded that both ferrocyanides has some amount of effectiveness against the fungal
52 disease [9].

53 The *C. nucifera* oil contains medium fatty acids such as caprylic acid and lauric acid. These
54 fatty acids are the antifungal agents [10]. The oil has been linked to fight against fungal
55 infections such as ringworm, Athlete's foot, toenail fungus [11] and dandruff [12]. *C.*
56 *nucifera* oil cream was created using varying amounts of *C. nucifera* oil (sourced from
57 coconut fruit). The creams were tested on skin inoculated with *Candida albicans*, *Aspergillus*
58 *niger*. After the cream was applied, five (5) minutes later the area was swabbed and then
59 incubated overnight. The coconut oil creams exhibited antifungal potential which was
60 boosted due to the presence of an emulsifying agent which allowed monolaurin to acquire an
61 increased penetration of the skin. Inhibition of the growth of *Aspergillus niger*, *Candida*
62 *albicans* began before the first hour was achieved and was maintained for 1-3 weeks [13].
63 This study is well thought out because of the objectives to formulate creams which not only
64 fight fungi with the use of *C. nucifera* oil but also consider its stability, that is, how it would
65 interact with the additional components, and with human skin which has been infected.
66 Further research and testing done on the successful batches of the formulation could lead to
67 an alternative treatment. In 2016 a research was published on the disease of early childhood
68 caries (ECC), which occurs when a child's teeth are decayed, missing or their surfaces are
69 filled. ECC is caused by microorganisms such as *C. albicans* which affects children who are
70 6 years 11 months or younger. The fungus was isolated in children affected with ECC, its
71 susceptibility was tested against 0.2 % chlorhexidine, *C. nucifera* oil obtained from the fruit,
72 probiotics and 2 % ketoconazole. All of the antifungal agents were found to inhibit the
73 growth of the fungus. However, chlorhexidine and coconut oil demonstrated the highest
74 antifungal activity which can be compared to ketoconazole [14].

75 *Abelmoschus esculentus*/*Hibiscus esculentus* contains flavones and triterpenoids which are
76 the antifungal agents that fight against *A. flavus*, *A. niger* and *C. albicans* [15]. The antifungal
77 potential of *A. esculentus* involved the synthesis of gold nanoparticles using seed aqueous
78 extract obtained from plant and tested on fungal diseases, *C. albicans* being one. The
79 antifungal potential of gold nanoparticles containing the seed aqueous extracts was evaluated
80 by the maximum zone of inhibition of each concentration which was compared to
81 amphotericin B (positive control). The antifungal potential of the nanoparticles was greater
82 than that of amphotericin B at higher volumes against the fungi. This advance research
83 reveals the pairing of the natural antifungal potential of seed aqueous extract of *A. esculentus*
84 and advancement in nanotechnology, which enhanced the antifungal ability of the plant's
85 seeds [16]. The entire *A. esculentus* plant was and tested against three fungi: *C. albicans*, *A.*
86 *niger* and *S. cerevacaе*. The crude methanolic extracts and ciprofloxin were applied to each
87 microorganism where the growth of *A. niger* was the most inhibited; followed by *C. albicans*
88 ciprofloxacin demonstrated greater inhibitions than the extracts where *C. albicans* was the
89 most susceptible [17].

90 A search of literature indicated that very few reports are available from our laboratory on
91 interaction of natural antifungals with metal hexacyanoferrates (II) and their medical
92 applications [18, 19]. In view of this, an attempt was made to study the phytochemical
93 analysis and the antifungal potential of some natural antifungals and metal hexacyanoferrates
94 (II). In addition the present work describes phytochemical analysis and antifungal potential of
95 natural antifungals: *C. nucifera* and *A. esculentus* leaves extract (ethanol and acetone) and
96 metal hexacyanoferrates (II) (CuFc , NiFc , MnFc) suspension.

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98

99 2. METHODOLOGY

100 2.1 Chemicals

101 Potassium ferrocyanide, Nickel (II) chloride, Manganese (II) chloride, Copper (II) chloride
102 were obtained from BDH, Poole, England. Nystatin, Picric acid, ferric chloride, metal
103 magnesium, acetic anhydride were obtained from Aldrich. All chemicals were of analytical
104 reagent grade and used without further purification. Solutions were prepared in
105 doubly distilled water.

106 *Microorganism: Candida albicans* and *Media:* Mueller Hinton, BD BBL was obtained from
107 Department of Biology, University of Guyana.

108 2.2 Synthesis of Metal Hexacyanoferrates (II)

109 The copper, manganese and nickel hexacyanoferrates (II) were synthesized by similar method
110 reported in literature by Kourim, et al. [20]. 500 m L, 0.1 M of the metal (II) chloride
111 solution was added to 167 m L, 0.1 M of potassium ferrocyanide solution with constant
112 stirring. The reaction mixture was heated on a water bath at 85 °C for 4 hours, occasionally
113 being stirred. Afterwards the mixture was kept at room temperature for 24 hours. The mixture
114 was filtered using Whatman filter paper and washed several times with distilled water. The
115 residue was dried in an oven at 60 °C to remove the moisture. The dried product was ground
116 with a mortar and pestle and sieved to 125 µm particle size.

117 2.3 Characterization of Metal Hexacyanoferrates (II)

118 The synthesized copper, manganese and nickel hexacyanoferrates (II) were found to have
119 dark brown, grey and light green colours, respectively. Elemental analysis: Atomic
120 absorption spectrophotometer was used to analyze the amount of iron, metal, carbon,
121 hydrogen and nitrogen. These values are given in Table 1. The spectral analysis of each metal
122 hexacyanoferrates (II) was done using Infrared Spectrophotometer. The spectral analysis will

be recovered in KBr disc using KBr pellets of **BUCK Scientific M530**. All of the samples were placed in the infrared spectrophotometer and read at 4000 cm^{-1} - 400 cm^{-1} . The infrared spectral data of the metal ferrocyanides are given in Table 2. The infrared spectral peaks around 3500 cm^{-1} and 1600 cm^{-1} characteristics of water molecules / OH groups and HOH bending, respectively. Two sharp bands around 2000 cm^{-1} and 580 cm^{-1} are characteristics of cyanide and Fe - C stretching, respectively. Another sharp band at 500 cm^{-1} probable shows the presence of metal-nitrogen band due to polymerization.

2.4 Preparation of Natural Antifungal Extracts

Extraction for all leaf samples, a modified procedure is reported by Mworio et al. [21]. The leaves were picked, washed with tap water and dried at room temperature to remove the excess water. The sample was ground using a Wiley Mill. A 50 g of the ground sample was soaked in 200 mL of acetone. The soaking sample via cold percolation was kept for 48 hours at room temperature. The acetone-sample extract was decanted and filtered every 48 hours using Whatman filter paper no. 41 ashless and fresh acetone was added. This was repeated three times. The acetone extracts were pooled and concentrated using rotary evaporator (40°C , 70-80 kPa), until a dry crude was obtained. The residue of the sample left after the acetone extraction was allowed to dry. The dried sample was further extracted with ethanol. The ethanol extract obtained was concentrated using rotary evaporator (50°C , 90 kPa).

2.5 Plate Preparation

Distilled water was added to Mueller Hinton agar; with frequent agitation the mixture was heated to a slow boil until the powder was dissolved. Mueller Hinton agar was autoclaved at 259°F at 20 psi for 20 minutes. After removal from the autoclave Mueller Hinton agar was allowed to cool to room temperature for comfortable handling. The cap was removed. The

mouth of the flask containing the Muller Hinton agar was placed over a flame for a few seconds. This was done to prevent any organism in the area from falling into the flask. The molten agar was poured slowly into the base of the sterile petri dish and covered with the lid under a laminar air flow cabinet. The petri dishes were left to solidify for approximately 30 minutes and UV sterilized for 20 minutes. The petri dishes were inverted and placed in transparent plastic bags tightly wrapped. The bags were then stored in the refrigerator overnight.

2.6 Disc Diffusion Assay for Microbial Sensitivity Testing

The dried crude extract was dissolved in their respective solvents to a stock solution of 20 mg/mL. The stock solution was used to make a 10 mg/mL solution. 12 ultraviolet sterilized paper discs of 6 mm (Whatman filter paper no. 1) in diameter were soaked in mini disposable petri dishes containing their respective extracts at the different concentrations for complete saturation. The agar plates were dried and ultraviolet sterilized in the laminar air flow cabinet. The *C. albicans* which was prepared prior was dissolved in 10 mL of distilled water in a test tube and its turbidity was compared to 0.5 M McFarland Standard (1.5×10^8 - approximate bacterial suspension/mL). Once identical turbidity is achieved, the mouth of the test tube is uncapped and flamed before a sterile cotton swab is submerged in the mixture.

The test tube is flamed and capped; the sterile cotton swab is streaked unto the agar plate in the different directions. The soaked paper discs containing their respective plant extracts at different concentrations with metal hexacyanoferrate (II) suspension were transferred to the agar plate (four discs on one plate).

A positive control (concentrated Nystatin suspension) and negative controls (solvent only) were also used. The plates were taped to prevent any contamination when being transferred

from the laminar air flow cabinet to the incubator. The plates were inverted and placed in an incubator at 37 °C for 48 hours. The diameter zone of inhibition of the leaves extracts of *C. nucifera* and *A. esculentus* with metal hexacyanoferrates (II) suspension against *C. albicans* are given in Tables 3 and 4, respectively.

2.7 Phytochemical Screening

The phytochemical screening followed the methodology of Harborne [22] and Kokate [23]. The presence of alkaloids, tannins, saponins, flavonoids, volatile oils, phenols and steroids was determined for each leaves extract of *C. nucifera* and *A. esculentus*. The results are given in Tables 5 and 6.

Alkaloids

2 mL of extract was measured in a test tube to which picric acid solution was added. An orange colour indicated the presence of alkaloids.

Tannins

A portion of the extract was diluted with water, 4 drops of 10 % ferric chloride solution was added. A blue colour indicated gallic tannins and green colour indicated catecholic tannins.

Saponins

About 0.5 mL of extract was measured in a test tube and was shaken with 2 mL of water. If foam persisted for 10 minutes it indicated saponins.

Flavonoids

4 mL of extract solution was treated with 1.5 mL of 50 % methanol solution. The solution was warmed and metal magnesium was added. To this solution, 6 drops of concentrated

194 hydrochloric acid was added and red colour indicated flavonoids and orange colour indicated
195 flavones.

196 **Volatile Oils**

197 2 m L of extract was shaken with 0.1 m L dilute NaOH and a small quantity of dilute HCl. A
198 white precipitate indicated volatile oils.

199 **Phenols**

200 1 m L of extract was treated with 4 drops of ferric chloride solution. A bluish black colour
201 indicated phenols.

202 **Steroids**

203 1 m L of extract was treated with 0.5 m L of acetic anhydride and 0.5 m L of chloroform.
204 Then concentrated solution of sulphuric acid was slowly added. A greenish bluish colour
205 indicated steroids.

206 **2.8 Characterization of Metal Hexacyanoferrates (II) and Plant Extract**

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208 The spectral analysis of plant extract with each metal hexacyanoferrate (II) was recovered
209 using Nujol Mull of **BUCK Scientific M530**. All of the samples were placed in the infrared
210 spectrophotometer and read at 4000 cm^{-1} - 400 cm^{-1} . A small drop of the plant extract with
211 metal hexacyanoferrate (II) was placed on one side of the KBr window followed by one drop
212 of Nujol Mull solution. The mixture was then covered and pressed with the other KBr
213 window and placed in the IR spectrophotometer. These values are given in Tables 7 and 8.

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2.9 Statistical Treatment

t- Test was used to compare the zones of inhibition of the plant extract only and plant extract with the addition of each metal hexacyanoferrate (II) at the same concentration. For each $p < 0.05$ - there is a significant difference, $p >$ there is no significant difference.

The inference “significant difference” implies that based on the zone of inhibition, the activities are not the same and thus the treatment is different. Therefore “no significant difference” would imply that the activities are the same, thus the treatments are the same.

Ultimately, it is to determine whether the addition of the metal hexacyanoferrate (II) is to be considered for its antifungal activity. Comparison of the zones of inhibition of the plant extract, with the addition of each metal hexacyanoferrate (II) at the different concentrations for those which are of opposite significant difference, will verify if concentration is a factor to be considered for its antifungal activity.

Comparisons were also conducted on the zones of inhibition of the plant extract with the addition of each metal (II) hexacyanoferrate, which demonstrated no significant difference in preliminary analysis and Nystatin suspension (positive control). Another preliminary analysis was conducted which compared the plant extract at the concentrations of the following to analyze if there was significant difference from Nystatin suspension.

3. RESULTS

It can be observed from Tables 3 that the acetone extract of *C. nucifera* at 20 mg/m L and 10 mg/m L with manganese and copper hexacyanoferrates (II) demonstrated effective antifungal potential. The ethanol extracts at both concentrations showed no antifungal activity.

On the other hand in Table 4, all of the acetone extracts of *A. esculentus* at 20 mg/m L and 10 mg/m L indicated showed effective antifungal potential when paired with all three metal hexacyanoferrates (II) (with the exception of combining manganese hexacyanoferrate (II)

with the plant extract at 10 mg/m L). The ethanol extracts at both concentrations demonstrated effective antifungal potential (with the exception of combining manganese hexacyanoferrate (II) with the plant extract).

The phenolic compounds such as phenols and flavonoids are antifungal agents. In Table 5, the phytochemical analysis of acetone and ethanol extracts of *C. nucifera* with copper, nickel and manganese hexacyanoferrate (II) suspensions. The results revealed that the acetone extract combined with copper and manganese hexacyanoferrate (II) contained phenols. Phenols were absent in the ethanol extract of *C. nucifera* with all three hexacyanoferrates (II).

Based on the data from Table 6, the phytochemical analysis of *A. esculentus* indicated that the acetone extracts when combined with copper, nickel and manganese hexacyanoferrates (II) contained phenols. While ethanol extracts when combined with nickel and manganese hexacyanoferrates (II) contained flavonoids and phenols respectively.

A preliminary test was conducted on the plant extract only which was used for comparison. Based on the peaks before and after the addition of each metal hexacyanoferrate (II) to the plant extract, the disruption of the antifungal potential can be determined. The infrared spectral data given in Table 7 showed that the antifungal potential was disrupted when the *C. nucifera*, ethanol extracts were combined with metal hexacyanoferrates (II). The exception was acetone extract with manganese and copper hexacyanoferrates (II).

The acetone and ethanol *A. esculentus* extracts combined with metal hexacyanoferrates (II) did not disrupt the antifungal potential when the infrared peaks were compared. The exception of the ethanol extract when combined with manganese hexacyanoferrate (II).

Interestingly the acetone extract when combined with manganese hexacyanoferrate (II) was partially disrupted based on the diminished sharpness of the peak. This is demonstrated in

Table 8

The most effective antifungal activities were demonstrated by 10 mg/m L ethanol extract of *A. esculentus* with nickel hexacyanoferrate (II) and 10 mg/m L acetone extract of *A. esculentus* with copper hexacyanoferrate (II). This is shown in Figures 1 and 2, respectively.

4. DISCUSSION

To investigate the interaction of metal hexacyanoferrate (II) and natural antifungals, the samples were tested individually. Recent research revealed that *C. albicans* has developed the ability to utilize the nitrogen present after the degradation of the cyanide bond to grow itself. This occurs due to the degradation of the metal-cyanide bond [24].

However, the acetone and ethanol extracts of *C. nucifera* and *A. esculentus* at the different concentrations exhibited antifungal potential. When the acetone extract of *C. nucifera* interacted with manganese and copper hexacyanoferrate (II) at both concentrations, antifungal potential was exhibited. While the acetone extract of *C. nucifera* with nickel hexacyanoferrate (II) and the ethanol extract with nickel, manganese and copper hexacyanoferrate (II) at both concentrations did not exhibit any inhibition [Table 3].

The interaction of the acetone and ethanol *A. esculentus* extract with nickel and copper hexacyanoferrate (II) at both concentrations, exhibited antifungal potential, while exhibiting antifungal potential was acetone extract of *A. esculentus* with manganese hexacyanoferrate (II) complex at 20 mg/m L. The interactions which showed antifungal potential indicated that the plant extract the metal hexacyanoferrate (II) had adsorbed the antifungal property from the natural antifungal. This is based on the observation that nickel and copper hexacyanoferrate (II) did not exhibit any antifungal potential at 10 mg/m L. The acetone extract of *A. esculentus* with manganese hexacyanoferrate (II) at 10 mg/ml and the ethanol

extract with manganese hexacyanoferrate (II) at both concentrations did not exhibit any inhibition [Table 4].

The addition of the metal hexacyanoferrate (II) inhibited the natural antifungal's potential or the growth of the fungus fueled by the source of nitrogen was exceeded by the antifungal potential of the plant extract. A trend was observed with the interaction between the ethanol plant extracts and manganese hexacyanoferrate (II) at both concentrations, where there was no inhibition of the growth of *C. albicans*. Ultimately, it was determined that the antifungal potentials of 10 mg/mL of ethanol extract of *A. esculentus* and acetone extract with nickel and copper hexacyanoferrate (II), respectively had no significant difference from that of Nystatin, a pharmaceutical treatment.

The presence of phenol has been observed in acetone extracts of *C. nucifera* with manganese and copper hexacyanoferrates (II). Phenols were also present in acetone and ethanol extracts of *A. esculentus* with copper hexacyanoferrate (II).

Whereas the ethanol extracts of *A. esculentus* with nickel hexacyanoferrate (II) has been observed to contain flavonoids, a small quantity of phenol was observed for the acetone extract of *A. esculentus* with manganese hexacyanoferrate (II), which is a possible explanation as to why this extract at 20 mg/mL exhibited antifungal potential [Tables 5 and 6].

The acetone extracts of *C. nucifera* with nickel and copper hexacyanoferrates (II) showed similar IR spectra; however, their antifungal potential was different. This explanation also applies to ethanol extracts of *A. esculentus* with manganese and nickel hexacyanoferrates (II). All of the ethanol extracts of *C. nucifera* showed the absence of the peaks at 3504 cm^{-1} and

3510 cm⁻¹. The acetone extract of *A. esculentus* with manganese hexacyanoferrate (II) did not show as being a strong peak when compared to the plant extract. This could be the reason for its antifungal potential being exhibited at 20 mg/m L [Tables 7 and 8].

5. CONCLUDING REMARKS

1. The acetone extracts of *C. nucifera* both concentrations revealed antifungal potential when combined with copper and nickel hexacyanoferrates (II).
2. All of the ethanol extracts of *C. nucifera* did not exhibit antifungal potential with any of the metal hexacyanoferrates (II).
3. The metal hexacyanoferrates (II)- extracts of *C. nucifera* interaction will demonstrate significant difference in the antifungal potential against *C. albicans* .
4. Ethanol and acetone extract of *A. esculentus* at both concentrations demonstrated antifungal potential when combined with copper and nickel hexacyanoferrates (II).
5. Acetone extracts of *A.esculentus* with manganese hexacyanoferrate (II) only showed antifungal potential at 20 mg/m L.
6. Some of the metal hexacyanoferrates (II)-extracts of *A. esculentus* interaction will demonstrate significant difference in the antifungal potential against *C. albicans* .
7. The metal hexacyanoferrate (II)-extracts of *A. esculentus* interaction will not demonstrate significant difference in the antifungal potential against *C. albicans*. This applies to 10 mg/m L of ethanol of *A. esculentus* with nickel hexacyanoferrate (II). Because there is no significant difference between the two latter interactions and Nystatin suspension, deduces that these possess the best antifungal potential.
8. The metal hexacyanoferrate (II)-extracts of *A. esculentus* interaction will not demonstrate significant difference in the antifungal potential against *C. albicans*. This applies to 10 mg/m L of acetone of *A. esculentus* with copper hexacyanoferrate (II).

Because there is no significant difference between the two latter interactions and Nystatin suspension, deduces that these possess the best antifungal potential.

9. It was observed that in some cases the metal hexacyanoferrates (II) did not inhibit the antifungal compounds: phenols and flavonoids.

10. The acetone extract of *C. nucifera* with copper and manganese hexacyanoferrates (II) contain phenols.

11. The acetone extract of *A. esculentus* combined with each of the metal hexacyanoferrates (II) contain phenols.

12. The ethanol extract of *A. esculentus* combined with copper hexacyanoferrate (II) contains phenol.

13. The ethanol extract of *A. esculentus* combined with nickel hexacyanoferrate (II) contains flavonoids.

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Table 1 . Element analysis of copper, manganese and nickel hexacyanoferrates (II)

Metal Hexacyanoferrates (II)*	Percentage (%) found				
	Metal	Iron	Carbon	Hydrogen	Nitrogen
CuFc	37.3	15.4	14.7	3.1	18.1
MnFc	19.4	19.5	16.3	2.8	18.6
NiFc	20.8	19.6	20.3	1.5	21.4

*CuFc, copper hexacyanoferrate (II); MnFc, manganese hexacyanoferrate (II); NiFc, nickel hexacyanoferrate (II)

Table 2 . Infrared spectral data of copper, manganese and nickel hexacyanoferrates (II)

Metal Hexacyanoferrates (II)*	Adsorption frequency (cm ⁻¹)				
	H ₂ O molecules/OH groups	HOH bending	$\nu\text{C}\equiv\text{N}$ stretching	$\nu\text{Fe-C}$	Metal-N ^a
CuFc	3733	1628	2069	600	450
MnFc	3698	1632	2066	600	510
NiFc	3690	1619	2071	590	430

*CuFc, copper hexacyanoferrate (II); MnFc, manganese hexacyanoferrate (II); NiFc, nickel hexacyanoferrate (II)

^ametal-nitrogen band due to polymerization

Table 3. Diameter zone of inhibition (mm) of extracts of *C. nucifera* leaves against *C. albicans*

Metal hexacyanoferrates (II) suspension* (10 mg/m L)	Acetone Extract (mg/m L)		Ethanol Extract (mg/m L)	
	20	10	20	10
NiFc	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MnFc	7.6 ± 3.8	5.6 ± 4.5	0.0 ± 0.0	0.0 ± 0.0
CuFc	8.7 ± 1.1	8.7 ± 1.2	0.0 ± 0.0	0.0 ± 0.0

*CuFc, copper hexacyanoferrate (II); MnFc, manganese hexacyanoferrate (II); NiFc, nickel hexacyanoferrate (II)

Table 4. Diameter zone of inhibition (mm) of extracts of *A. esculentus* leaves against *C. albicans*

Metal hexacyanoferrate (II) suspension* (10 mg/m L)	Acetone Extract (mg/m L)		Ethanol Extract (mg/m L)	
	20	10	20	10
Nickel	5.1 ± 4.5	6.0 ± 4.5	4.8 ± 4.2	10.1 ± 6.5
Manganese	8.8 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Copper	10.5 ± 1.2	7.9 ± 3.8	8.9 ± 5.1	6.7 ± 4.1

*CuFc, copper hexacyanoferrate (II); MnFc, manganese hexacyanoferrate (II); NiFc, nickel hexacyanoferrate (II)

Table 5 . Qualitative tests for the phytochemical analysis of acetone and ethanol extracts of *C. nucifera* nickel, copper and manganese hexacyanoferrates (II) suspension

Phytochemical	Inference					
	Acetone extract			Ethanol extract		
	NiFc	CuFc	MnFc	NiFc	CuFc	MnFc
Alkaloids	-	-	-	+	-	+
Tannins	-	-	-	-	-	-
Saponins	+	+	+	+	-	+
Flavonoids	-	-	-	-	-	-
Volatile Oils	-	-	-	-	-	+
Phenols	-	+	+	-	-	-

Steroids	-	-	-	-	-	-
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*CuFc, copper hexacyanoferrate (II); MnFc, manganese hexacyanoferrate (II); NiFc, nickel hexacyanoferrate (II). (+) – present, (-) - not present

Table 6 . Qualitative tests for the phytochemical analysis of acetone and ethanol extracts of *A. esculentus* nickel, copper and manganese hexacyanoferrates (II) suspension

Phytochemical	Inference					
	Acetone extract			Ethanol extract		
	NiFc	CuFc	MnFc	NiFc	CuFc	MnFc
Alkaloids	-	-	-	-	-	-
Tannins	-	-	-	-	-	+
Saponins	+	+	+	+	-	-
Flavonoids	-	-	-	+	-	-
Volatile Oils	+	-	+	-	-	-
Phenols	+	+	+	-	+	-
Steroids	-	-	+	+	+	+

*CuFc, copper hexacyanoferrate (II); MnFc, manganese hexacyanoferrate (II); NiFc, nickel hexacyanoferrate (II). (+) – present, (-) - not present

Table 7. Infrared spectral data of acetone and ethanol extracts of *C.nucifera* and suspension of metal hexacyanoferrates (II)

	Metal hexacyanoferrates (II) suspension*		
Plant Extract [adsorption frequency (cm ⁻¹)]	NiFc	MnFc	CuFc
Acetone [below 600]	Antifungal potential disrupted	Antifungal potential not disrupted	Antifungal potential not disrupted

Ethanol [3504, 3510]	Antifungal potential disrupted	Antifungal potential disrupted	Antifungal potential disrupted
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*CuFc, copper hexacyanoferrate (II); MnFc, manganese hexacyanoferrate (II); NiFc, nickel hexacyanoferrate (II)

Table 8. Infrared spectral data of acetone and ethanol extracts of *A. esculentus* and suspension of metal hexacyanoferrates (II)

	Metal hexacyanoferrates (II) suspension*		
Plant Extract [adsorption frequency (cm ⁻¹)]	NiFc	MnFc	CuFc
Acetone [2840]	Antifungal potential not disrupted	Antifungal potential partially disrupted	Antifungal potential not disrupted
Ethanol [below 600]	Antifungal potential not disrupted	Antifungal potential disrupted	Antifungal potential not disrupted

*CuFc, copper hexacyanoferrate (II); MnFc, manganese hexacyanoferrate (II); NiFc, nickel hexacyanoferrate (II)

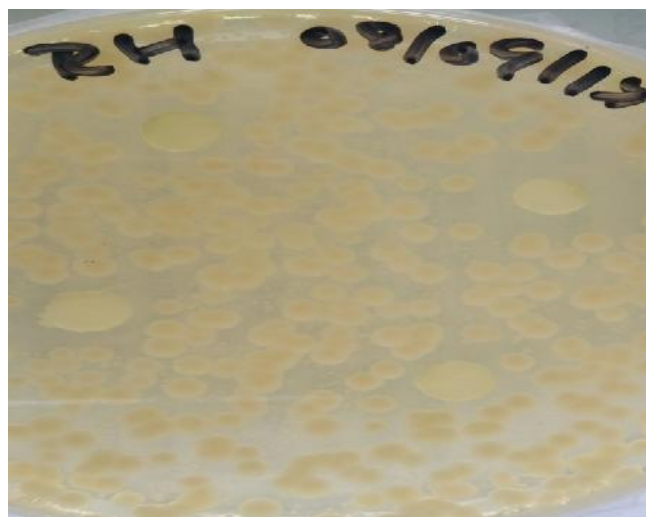
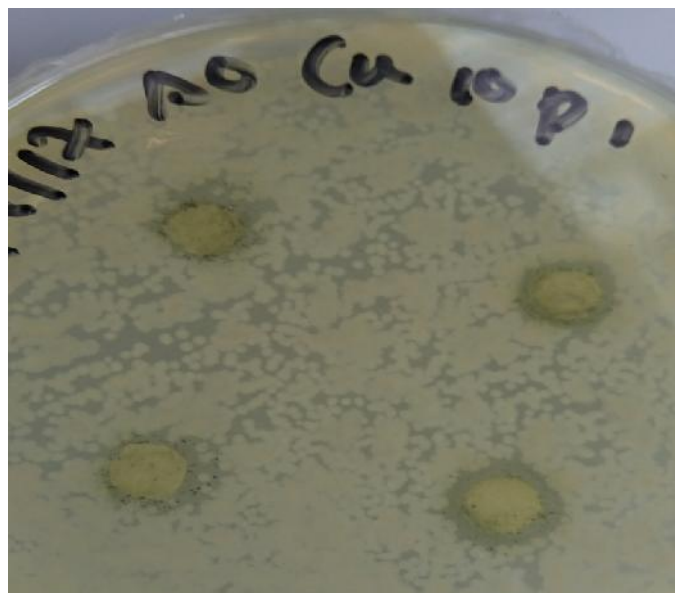


Figure 1. 10 mg/ml Ethanol extract of *A. esculentus* with nickel hexacyanoferrate (II)



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477 **Figure 2. 10 mg/ml Acetone extract of *A.esculentus* with copper hexacyanoferrate (II)**

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