Studies on the Interaction of Natural Antifungals with Metal Ferrocyanides and Their Medicinal Applications

Abstract: Manganese, Silver and Titanium ferrocyanides were synthesized and characterized by elemental and spectral studies. The stabilities of these metal ferrocyanides were investigated in the presence of acids, bases, organic solvents, tap and sea water at room and boiling temperature. The natural antifungal plants studied were azadirachta indica (Neem), ocimum sanctum (tulsi), cassia obtusifolia (money bush), cassia alata (canicro bush), tagetes patula (marigold). The natural antifungal plant extract with metal ferrocyanides complexes were found to be have more antifungal property in comparison to metal ferrocyanides and natural antifungals alone. Antifungal activity of natural antifungals, metal hexacyanoferrate(II) compound and natural antifungal metal ferrocyanide complexes were tested by well known cultured fungus (Aspergillus Niger). The titanium ferrocyanide with neem extract and manganese ferrocyanide with money bush extract complexes were found to have maximum and minimum antifungal property, respectively.
[Nature and Science. 2009;7(3):1-7]. (ISSN: 1545-0740).

Commented [A1]: The abstract you provided can serve as a basis for a chapter in a book, but some modifications could improve it to better fit a chapter format. Here are a few suggestions:

- Add an introductory sentence explaining why the study of natural antifungals and metal ferrocyanides is important. This will help situate the reader in the topic.
- Clarify the study's objectives early on so that the reader immediately understands what to expect from the chapter.
- Since this is a chapter, it might be useful to include some details about the methodology used for synthesis and antifungal testing.
- Conclude with a sentence about the implications of your findings for future research or practical applications, providing a broader perspective.

Keywords: metal ferrocyanides, natural antifungals, aspergillus niger, medical value, skin infection.

1. INTRODUCTION

Antifungal extracts are to be obtained from various local plants in Guyana. These plants include azadirachta indica, cassia alata, cassia obtusifolia, oscimum sanctum and togetes patula. Studies on these plants have shown that they possess antifungal compounds mainly in their leaves, bark and fruits. The extract may show various activities depending on the method of extraction – such methods include wet, dry and steam distillation. In addition they are also used for various medicinal purposes such as laxative and antibacterial among others. Azadirachta indica is believed to be an answer to many incurable diseases. A mixture of the extracts from the leaves, bark, fruit and seeds is used efficiently to treat skin diseases[1]. Cassia alata leaves and flowers are used as remedy for asthma, bronchitis, diabetes, ulcers, scabies and skin diseases such as puritis eczema, etc.[2,3]. Cassia obtusifolia leaves extracts are known to be used to treat feet rashes, lotta, scabies, ringworm, and other skin infections. The oil extract of Ocimum sanctum consists of eugenol, eugenol methyl ether and carvacrol which are main contributors to the medicinal value of Tulsi. It helps to eradicate ringworm and other skin diseases when applied to such skin infections[4]. The whole herb of tagetes patula is used in coughs and dysentery, taken internally in the form of a decotion. Extracts of Marigold can be used as fungicides. In addition to antifungal properties plants extract also have various other medicinal uses[5-10]. To analyze the nature of metal ferrocyanides and the extracts, the best suited fungal spore is Aspergillus niger. A. niger is omnivorous and one of the most common easily identifiable species of the genus. A. niger may also be a common laboratory contaminant [11].

Primitive earth atmosphere was anoxygenic and reducing potential of atmosphere was not high enough hence metals like iron, chromium, molybdenum, manganese and tin etc. were in the form of their lower oxidation states. Considering the fact that cyanide was formed in all simulated

experiments of primitive earth conditions, cyanide could have combined with a large number of metal ions present in primeval sea. Consequently, several insoluble metal ferrocyanides of general formula $M_2[Fe(CN)_6] \cdot x\ H_2O$, where M = Fe, Cr, Mo, Zn, etc. could have been formed. It is well established that metal ferrocyanides acts as adsorbents[12], ion-exchangers[13] and photosensitizers[14].

Literature survey indicates that no report is available on medical value of natural antifungal – metal ferrocyanide complex. In view of this attempt were made to study medical application of these complexes. In addition present work describes synthesis, characterization and medical application of manganese, silver, titanium ferrocyanides – natural antifungal complexes.

2. EXPERIMENTAL SECTION

2.1 Chemicals

All chemicals used were of AnalaR grade and used as such without any further purification. Potassium ferrocyanide, manganese chloride, silver nitrate, titanium tetrachloride were obtained from BDH, Poole, England. Solutions were prepared in doubly distilled water.

2.2 Synthesis of metal ferrocyanides

Manganese and silver ferrocyanides were prepared by Kourim's method[15]. Whereas titanium ferrocyanide was prepared according to the procedure reported by Bastian et al. [16].

The manganese and silver ferrocyanides were prepared by adding potassium ferrocyanide (167 ml; 0.1 M) slowly to metal chloride/nitrate (500 ml; 0.1 M) with constant stirring. Reaction mixture was heated on water bath for 2-3 h and cured for 24 h. The precipitate was washed with distilled water and dried at 60 C. The dried product was ground and sieved to 125 µm BSS mesh size. In case of silver ferrocyanide all reactions were performed in the dark. Silver ferrocyanide was kept in the dark bottle.

The best condition for the preparation of titanium ferrocyanide involves variation in the mole ratio of titanium to hexacyanoferrate(II), which vary between 10 to 1 and 1 to 10, respectively. For this experiment we will use a 0.5 M solution of titanium tetrachloride in 2.0 M aqueous hydrochloric acid and 0.34 M solution of hexacyanoiron(II) acid. The solution of hexacyanoiron(II) acid is won by pouring a solution of potassium hexacyanoferrate(II) over a Dower-50-exchanger and then poured into the 2.0 M $HCl/TiCl_4$ solution. The filling material from the exchanger is centrifused out after 24 h and dried over phosphorous pentaoxide and potassium hydroxide in a vacuum desiccators. The dried product was washed with water free from chloride ions and then dried again in the vacuum desiccators. The dried product was ground and sieved to 125 µm BSS mesh size.

2.3 Characterization of metal ferrocyanides

Manganese, silver ferrocyanides are found to have light blue colour, while titanium ferrocyanide have forest green colour. All are amorphous solid and shows no X-ray pattern. The metal ferrocyanides were characterized on the basis of elemental and spectral studies.

The percentage composition of metals were determined by IL – 751 atomic absorption spectrophotometer. Carbon, hydrogen and nitrogen analysis were carried out by CEST – 118, CHN analyzer. Percentage composition of all three metal ferrocyanides are given in Table 1.

Infrared spectra of the metal ferrocyanides were recovered in KBr disc on Beckman IR – 20 spectrophotometer. All three metal ferrocyanides show a broad peak at 3800 cm^{-1} is characteristics of water molecules and OH groups. Also a peak at around 1600 cm^{-1} is due to H-O-H bending. A sharp band at 2000 $cm^{-1}$and a broad peak at 600 cm^{-1}were observed in all three metal ferrocyanides are characteristics of cyanide and Fe – C stretching, respectively. A band around 500 cm^{-1} is observed in all three metal ferrocyanides may be due to polymerization of metal – nitrogen bond.

2.4 *Stability of metal ferrocyanides*:

Commented [A2]: Scientific Comments:
1. **Plant Names in Italics**:
   - Ensure that all plant names are italicized (e.g., *Azadirachta indica*, *Cassia alata*, *Cassia obtusifolia*, *Ocimum sanctum*, and *Tagetes patula*).
2. **Extraction Methods**:
   - When mentioning extraction methods, specify how each method (wet, dry, steam distillation) may influence the yield and efficacy of the antifungal compounds. A brief explanation of each method's advantages would add depth.
3. **Chemical Composition**:
   - For *Ocimum sanctum*, consider elaborating on the specific roles of eugenol, eugenol methyl ether, and carvacrol in the antifungal activity. Mentioning their ...

Commented [A3]: Your introduction provides a good overview of the antifungal properties of local plants in Guyana and sets the stage for the discussion on metal ferrocyanides. To enhance clarity and engagement, it is recommended to structure the text more clearly by breaking up long paragraphs and using headings to organize sections. Ensure that all genus and species names are in italics. Add transition sentences to facilitate reading and clarify certain ...

Commented [A4]: Your text is generally clear, but here are some points to consider for improvement in writing and format:
Suggested Corrections:
- Use a consistent verb form. Instead of "were used and used as such," consider "were used as received without any further purification."
- To improve readability, you could list the chemicals. ...

Commented [A5]: Your text is mostly clear, but there are some areas where improvements can be made in terms of writing, clarity, and formatting. Here are some suggestions:
Punctuation and Formatting:
- Use a semicolon or a comma instead of "and" in "500 ml; 0.1 M" to maintain consistency.
- Add a comma after "the reaction mixture" in "Reaction mixture was heated..." ...

Commented [A6]: Scientific Comments:
1. **Method References**:
   - Ensure that the references to Kourim's method and Bastian et al. are correctly cited with full bibliographic details in your references section for clarity and proper attribution.
2. **Chemical Formulas**:
   - When introducing chemical compounds, consider ...

Commented [A7]: Here are some comments and corrections for your section on the characterization of metal ferrocyanides:
Suggested Corrections:
1. **Subject-Verb Agreement**:
   - Change "titanium ferrocyanide have" to "titanium ferrocyanide has" for correct subject-verb agreement.
2. **Punctuation and Clarity**: ...

Commented [A8]: Scientific Comments:
1. **Color Description**:
   - Consider specifying that the color perception can vary depending on the concentration and lighting conditions. You might also relate the color to the electronic structure of the complexes.
2. **Amorphous Nature**:
   - You mention that the metal ferrocyanides show no X ...

All three metal ferrocyanides were found to be stable in acids (HCl, H_2SO_4, HNO_3, CH_3COOH) bases (NaOH, KOH, NH_4OH) in concentration range 0.5 – 2.0 M at room and boiling temperature. Metal ferrocyanides unaffected by salt (LiCl, NaCl, KCl, NH_4Cl, RbCl, CsCl, $BaCl_2$ and $CaCl_2$) solutions at room temperature in concentration range of 0.5 – 2.0 M.

Metal ferrocyanides are also found to be stable in tap and atlantic ocean water at room and boiling temperature. The change in colour of metal ferrocyanides of various conditions are may be due to loss of water molecules from the compound.

2.5 Preparation of natural antifungal extracts

The extraction from cassia obtusifolia was done by wet method. The leaves of the plant were picked and soaked by covering with 95% ethanol solvent for 24 h. The ethanol – extract was filtered using glass wool. The filtrate was vaporized using a rotovapourizer at 45 C until all the ethanol is removed. The final extract was considered as the stock solutions from which further dilutions would be made for analysis cassia alata, cassia obtusifolia, ocimum sanctum and azadirachta indica extraction were done using dry method. The green leaves were dried for three days at 45 C, then grounded using an electric mill. The powdered leaves were then be soaked by covering with 95% ethanol solvent for 24 h. The soaking was repeated three times. The ethanol – extracts were then filtered by gravitation filtration using whatman filter paper. The ethanol was then be removed using rotovaporizer until the extract solidifies. The antifungal activity of plant extracts, metal ferrocyanides and plant extract – metal ferrocyanide complexes, were tested on a known cultured fungus, Aspergillus niger.

2.6 Test on antifungal activity

2.6.1 Testing the antifungal activity of metal ferrocyanides only Metal ferrocyanide (10 mg) was placed in a sterilized petri dish containing media.

The fungal spores were then sprayed on the entire bottom of the dish using an aspirator. The similar method was repeated using different metal ferrocyanides.

2.6.2 Testing the antifungal activity of extract only

The antifungal plant extract (10 mg) was placed by means of washing with 20 ml ethanol in a sterilized petri dish containing media. The fungal spores were sprayed on the entire bottom of petri dish using an aspirator. The same method was repeated using different plants extract.

2.6.3 Testing the antifungal activity of antifungal plant extract – metal ferrocyanide complexes

Metal ferrocyanide (10 mg) and antifungal plant extract (10 mg) were placed in sterilized petri dish containing media. The fungal spores were sprayed on the entire bottom of the petri dish using an aspirator. This method was repeated using different extract and metal ferrocyanide complexes.

2.6.4 Testing the antifungal activity of control (ethanol only) Ethanol (20 ml) was placed in sterilized petri dish containing media. The fungal spores were then sprayed on the entire bottom of the dish using an aspirator. The essay was left to stand in sealed container in an incubator at 28 C for 168 h.

3. RESULTS AND DISCUSSION

3.1 Antifungal activity of metal ferrocyanides only

Antifungal activities of manganese, silver and titanium ferrocyanides was studied. Titanium ferrocyanide and manganese ferrocyanide were found to have maximum and minimum antifungal property respectively. The following order of antifungal activity was observed in metal ferrocyanides:

Titanium ferrocyanide > silver ferrocyanide > manganese ferrocyanide

The observation of bioassay test of metal ferrocyanides with fungal spores are given in Table 3.

3.2 Antifungal activity of extract only

Antifungal activity of azadirachta indica, cassia alata, cassia obtusifolia, ocimum sanctum and targetes were studied. Azadirachta indica and cassia obtusifolia were found to have maximum and minimum antifungal property respectively. The following order of antifungal property was

Commented [A9]: Suggested Corrections:
1. **Clarity and Punctuation**:
   - Change "stable in acids (HCl, H2SO4, HNO3, CH3COOH), bases (NaOH, KOH, NH4OH) in concentration range" to "stable in acids (HCl, H_2SO_4, HNO_3, CH_3COOH) and bases (NaOH, KOH, NH_4OH) within a concentration range" for better clarity and grammatical correctness.
2. **Consistency in Temperature Descriptions**:
   - Use "at room temperature and boiling temperature" for consistency.
3. **List Formatting**:
   - Consider using a bullet list for the salts to improve ...

Commented [A10]: Scientific Comments:
1. **Clarity on Stability**:
   - Clearly define what you mean by "stability." Does this refer to chemical integrity, structural integrity, or both? It may be helpful to specify how stability was assessed (e.g., by observing physical changes, chemical reactivity, etc.).
2. **Concentration Ranges**:
   - The concentration range for acids and bases (0.5 – 2.0 M) should be justified. Explain why these specific ...

Commented [A11]: Suggested Corrections:
1. **Clarity and Punctuation**:
   - Change "The extraction from cassia obtusifolia was done by wet method" to "The extraction from *Cassia obtusifolia* was performed using a wet method" for improved clarity and proper italicization of the species name.
2. **Verb Consistency**:
   - Change "were picked and soaked by covering with 95% ethanol solvent" to "were picked and soaked in ...

Commented [A12]: Scientific Comments:
1. **Extraction Method Details**:
   - Specify why the wet extraction method was chosen for *Cassia obtusifolia*. Discuss any advantages this method offers in terms of yield or the preservation of bioactive compounds.
2. **Ethanol Concentration Justification**:
   - Explain why 95% ethanol was used as the solvent. Is there evidence in the literature that supports the efficacy of this concentration for extracting antifungal ...

Commented [A13]: Suggested Corrections:
1. **Clarity and Punctuation**:
   - In "metal ferrocyanides only," consider changing it to "Testing the antifungal activity of metal ferrocyanides only" for better readability.
   - Change "was placed in a sterilized petri dish containing media" to "was placed in a sterilized Petri dish containing the media" for clarity and consistency in capitalization.
2. **Consistency in Terminology**: ...

Commented [A14]: Scientific Comments:
1. **Methodology Clarity**:
   - Ensure that the method of preparing the media is described elsewhere in the document, as it's critical for reproducibility. Specify the type of media used (e.g., potato dextrose agar, Sabouraud dextrose agar).
2. **Concentration Details**:
   - You mention "10 mg" for both the metal ferrocyanides and the plant extracts. It might be helpful to include the rationale behind this concentration. Are there previous ...

observed in plant extracts.

Azadirchta indica > tagetes patula > oscimum sanctum > cassia alata > cassia obtusifolia

The observations of bio assay test of natural antifungal extract only with cultured fungal spore are given in Table 4.

3.3 *Antifungal activity of metal ferrocyanides and metal antifungal complexes*

The following order of antifungal activity was observed in natural antifungal with metal ferrocyanide complexes.

(i) Manganese ferrocyanide
Azadirachta indica > tagetes patula > ocimum sanctum > cassia alata > cassia obtusifolia

(ii) Silver ferrocyanide
Azadirachta indica > tagetes patula > ocimum sanctum > cassia alata > cassia obtusifolia

(iii) Titanium ferrocyanide
Azadirachta indica > tagetes patula > ocimum sanctum > cassia alata > cassia obtusifolia

Titanium ferrocyanide – azadirachta indica and manganese ferrocyanide – cassia obtusifolia complexes were found to have maximum and minimum antifungal properties, respectively. The observation of bioassay test of metal ferrocyanide – natural antifungal complexes with cultured fungal spore are given in Table 5.

3.4 Antifungal activity of control (ethanol only)

It was observed that fungal spores were able to grow in the control. The growth of fungal spore was unaffected by ethanol.

Table 1. Elemental analysis of manganese, silver and titanium ferrocyanides

Metal Ferrocyanides*	Percentage found				
	Metal	Iron	Carbon	Hydrogen	Nitrogen
MnFc	26.90	13.12	16.30	2.80	18.60
AgFc	40.22	8.75	11.47	2.75	13.77
TiFc	25.35	11.95	15.62	3.17	18.25

*MnFc = Manganese ferrocyanide; AgFc = Silver ferrocyanide;

TiFc = Titanium ferrocyanide

Table 2. Infrared spectral data of manganese, silver and titanium ferrocyanides

Metal Ferrocyanides	Absorption frequencies (cm^{-1})				
	H_2O molecules/ OH groups	HOH bending	τ C ≡ N	τ Fe – C	Metal – N*
MnFc	3800	1600	2000	610	500
AgFc	3800	1600	2010	600	490
TiFc	3800	1615	2020	600	500

* metal – nitrogen band due to polymerization

Table 3. Observation of bioassay test of metal ferrocyanides only with cultured fungal spores

Manganese ferrocyanide	Silver ferrocyanide	Titanium ferrocyanide
Evidence of few fungal spores growth was seen	Few spores of fungus were seen in area where silver ferrocyanide was not present.	No evidence of fungal spores growth seen.

Bio assay: 10 mg metal ferrocyanide per petri dish

Room temperature: 30 ± 1 C

Time: 168 h

Cultured fungus: Aspergillus niger

Order of antifungal activity: TiFc > AgFc > MnFc

Table 4. Observations of bioassay of natural antifungal extract only with cultured fungal spore

Azadirachta indica	Cassia alata	Cassia obtusifolia	Ocimum sanctum	Tagetes patula
Evidence of small amount of spores was seen. It had least evidence of fungal spores.	Evidence of more fungal spores was seen but less than cassia obtusifolia extract	Evidences of wide spread fungal spores was seen in comparison to al other plant extract.	Evidences of fungal spores was seen but more than tagetes patula plant extract.	Evidences of small amount of spores was seen but more than azadirachta indica plant extract.

Bio assay: 10 mg plant extract per petri dish

Room temperature: 30 ± 1 C

Time: 168 h

Cultured fungus: Aspergillus niger

Order of antifungal activity: Azadirachta indica > targets patula > oscimum sanctum > cassia alata > cassia obtusifolia

Table 5. Observations of bioassay test of metal ferrocyanides and natural anti fungal extract with cultured fungal spore

MFc	Azadirachta indica	Cassia alata	Cassia obtusifolia	Ocimum sanctum	Tagetes patula
MnFc	Little sign of fungal growth seen	Some fungal spores seen growing but more than O. Sanctum	Clumps of fungus were seen in the petri dish maximum growth	Few fungal spores seen growing more than T. Patula	Evidence of small amount of fungal growth but more than A. Indica.
AgFc	Very little sign of fungal growth seen	More spores were seen growing than O. Sanctum	Some spores were seen growing in clumps.	Evidences of small amount of fungal growth but more than T. Patula	Evidences of small amount of fungal growth but more than A. Indica
TiFc	No evidence of fungal growth maximum inhibition	Evidences of small amount of fungal growth but more than O. Sanctum	Evidences of small amount of fungal growth but more than C. Alata	Few fungal spores seen growing but more than T. Patula	Little fungal spores seen growing

Bio assay: 10 mg metal ferrocyanide plus 10 mg antifungal plant extract per petri dish

Room temperature: 30 ± 1 C

Time: 168 h

Cultured fungus: Aspergillus niger

CONCLUDING REMARKS

The following conclusions can be drawn from the present studies

(a) Antifungal activity of secondary metabolities are enhanced through interaction with metal ferrocyanides.
(b) Titanium and manganese ferrocyanides were found to have maximum and minimum antifungal property, respectively.
(c) Azadirachta indica and cassia obtusifolia were found to have maximum and minimum antifungal properties, respectively.
(d) Azadirachta indica extract – titanium ferrocyanide complex and cassia obtusifolia extract – manganese ferrocyanide complex were found to have maximum and minimum antifungal property respectively.
(e) It may be also concluded from present studies that titanium ferrocyanide – azadiracha indica extract complex may be used as effective medicine for skin infection.

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3. S. Palanichamy, Wound Healing Activity of Cassia Alata, *Fitoterapia* 62 (2) (1991) 336-338.

Commented [A15]: General Suggestions

1.**Structure and Flow**: Ensure that each subsection flows logically into the next. Use transitional phrases to connect ideas.
2.**Clarity of Terms**: Define any specific terms or abbreviations at first mention, especially if they are not common in all fields (e.g., "bioassay").
3.**Quantitative Data**: Where possible, include specific numerical values or percentages to quantify the antifungal activity rather than just stating "maximum" or "minimum."
4.**Table Titles**: Ensure that each table title clearly indicates what data is being presented. For example, "Table 1: Elemental Analysis of Ferrocyanides" could also specify which elements are analyzed.
5.**Consistency**: Ensure consistency in naming conventions (e.g., plant names in italics, consistent use of full names versus abbreviations).

Specific Suggestions

3.1 Antifungal Activity of Metal Ferrocyanides Only
- **Rephrase for Clarity**: Instead of saying "maximum and minimum antifungal property," you could say "Titanium ferrocyanide demonstrated the highest antifungal activity, while manganese ferrocyanide showed the least."

3.2 Antifungal Activity of Extracts Only
- **Provide Context**: Briefly explain why the antifungal activity of each extract is significant, perhaps linking it to traditional uses or existing literature.

3.3 Antifungal Activity of Metal Ferrocyanides and Antifungal Complexes
- **Clarify Complexes**: Specify the nature of the complexes formed between the metal ferrocyanides and plant extracts. This could involve a brief mention of the interaction mechanism, if known.

3.4 Antifungal Activity of Control (Ethanol Only) ...

Commented [A16]: **Rephrase for Clarity**: Instead of "The following conclusions can be drawn from the present studies," you might say, "This study leads to the following conclusions:"

Conclusion (a): Consider specifying the type of secondary metabolites involved. For example:
- "(a) The antifungal activity of secondary metabolites is enhanced through interactions with metal ferrocyanides."

Conclusion (b): Add context on the implications of antifungal properties:
- "(b) Titanium ferrocyanide exhibited the highest antifungal activity, while manganese ferrocyanide demonstrated the least effectiveness."

Conclusion (c): Clarify the significance of the findings:
- "(c) Among the plant extracts, *Azadirachta indica* showed the strongest antifungal properties, whereas *Cassia obtusifolia* displayed the weakest."

Conclusion (d): Ensure consistent naming for the complexes:
- "(d) The complex formed between *Azadirachta indica* extract and titanium ferrocyanide exhibited the highest antifungal activity, while the complex with *Cassia obtusifolia* extract and manganese ferrocyanide showed the lowest."

Conclusion (e): Strengthen the implication:
- "(e) These findings suggest that the titanium ferrocyanide–*Azadirachta indica* extract complex may serve as an effective treatment for skin infections, warranting further investigation into its medicinal potential."

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1/24/2009

Commented [A17]: General Suggestions
1.**Consistent Formatting**: Ensure consistency in how authors, titles, and publication details are formatted.
2.**Standardization**: Use a consistent citation style throughout (e.g., APA, MLA, Chicago).
3.**Detail Completeness**: Make sure all necessary details (like volume, issue number, and page ranges) are included.