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Synergistic effects of some medicinal plants and transition metal ferrocyanides on some selected fungus

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Abstract
Transition ferrocyanides were synthesized and characterized by IR spectra, magnetic susceptibility and XRD studies. The medicinal plants which contain natural antimicrobial properties such as Phyllanthus emblica, Psidium guajava, Jatropha gossypifolia, Mangifera indica were showed synergistic effect with transition metal ferrocyanides. These plant extracts with metal ferrocyanides complexes were found to be having more antifungal property in comparison to metal ferrocyanides and plants extract individual. Antifungal activities of medicinal plants and metal hexacyanoferrate (II) compounds were tested against Rhizoctonia solani causing black scurf in potato. Cadmium ferrocyanide with Phyllanthus emblica extract and nickel ferrocyanide with Mangifera indica extract complexes were found to have maximum and minimum antifungal property, respectively.

Keywords: Medicinal plants, transition metal ferrocyanides, synergistic effects, *Rhizoctonia solani*.

Introduction
Phytochemicals are bioactive compounds found in vegetables, fruits, cereal grains, and plantbased beverages such as tea and wine. Phytochemical consumption is associated with a decrease in risk of several types of chronic diseases due to in part to their antioxidant and free radical scavenging effects. Because it is hypothesized that the beneficial health effects observed from phytochemicals are related to the synergistic mixture of phytochemicals and other nutrients found in whole foods and its components, consumption of variety of plant-based foods is encouraged (Chopra, 1956) [4]. Researchers are exploring the use of phytochemicals to product economically important crops against various pest and pathogens. Potato is world's fourth economically important food crop after wheat, rice and maize because of its greater yield potential and high nutritive value. Its constituents nearly half of the worlds annul output of all root and tuber crops. A large percentage of potential production is reportedly destroyed by pests and pathogens. Annual yield loss of potato crop quality is due to *Rhizoctonia solani* infection can be 15-20 % (Rauf, 1999 and Beagle-Ristaino *et al*., 1985) [1, 3].
Rhizoctonia solani is a fungus that attacks tubers, underground stems and stolons of potato plants. Although it probably occurs wherever potatoes are grown, it causes economically significant damage only in cool, wet soils (Frank, 1986) [2].
Rhizoctonia is a soil borne fungus with more or less continuous vegetative growth of brown threadlike branching mycelium in warm, moist soil conditions. These fungal strands grow between the soil particles and in dead non-living plant material to promote its decay and breakdown of organic matter in temperate production areas, losses from *R. solani* are sporadic and occur only when weather is cold and wet in the weeks following planting. In northern areas, where growers often must plant in cold soils, black scurf caused by *R. solani* is a more consistent problem. Poor stands, stunted plants, reduced tuber number and size, and misshapen tubers are characteristic of the black scurf disease (Frank, 1986) [2].
The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the near east, but it is doubtless an art as old as mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as holly back, these plants are still widely used in ethno medicine around the world (Khare, 2007) [8].
Phyllanthus emblica L. (syn. *Emblica officinalis*) is commonly known as Indian gooseberry. All parts of this plant are used for medicinal purposes, especially the fruit, which has been used in Ayurveda as a potent Rasayana (rejuvenator) (Neeraj *et al*., 2017) [5]. *P. emblica* contains phytochemicals including fixed oils, phosphatides, essential oils, tannins, minerals, vitamins, amino acids, fatty acids, glycosides, etc. Various pharmaceutical potential of *P. emblica* has been reported previously including antimicrobial, antioxidant, anti-inflammatory, analgesic and antipyretic, adaptogenic, hepatoprotective, antitumor and antiulcerogenic

activities either in combined formulation or *P. emblica* alone. The various other Ayurvedic potentials of *P. emblica* are yet to be proven scientifically in order to explore its broad spectrum of therapeutic effects (Chopra, 1956) [4].

Psidium guajava leaf extracts against two gram-negative bacteria (Escherichia coli and Salmonella enteritidis) and two gram-positive bacteria (Staphylococcus aureus and Bacillus cereus) which are some of food borne and spoilage bacteria. (Abad, *et al*. 2007) [6] According to the findings of the antibacterial assay, the methanol and ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, whereas the gram-negative bacteria were resistant to all the solvent extracts. On the basis of the present finding, guava leaf-extract might be a good candidate in the search for a natural antimicrobial agent. This study provides scientific understanding to further determine the antimicrobial values and investigate other pharmacological properties. (Fostel *et al*., 2000 and Abdelrahim *et al*., 2002) [7, 9]

Jatropha gossypifolia Linn. belongs to the family Eurphorbiaceae and the order, "Geraniale". A number of other species of *Jatropha like Jatropha curcas*, *Jatropha multifida*, *Jatropha podagrica* are also known. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Laxane *et al*., 2013) [10]. The aim of this study is to investigate the antimicrobial activity of *J. gossypifolia* leaves extracts as well as to identify the active constituents (Faokunla Opeyemi *et al*., 2017) [11].

Modern drugs are associated with several side effects like nausea and headaches. Man has resorted to plants for treatment due to high prices of synthetic drugs. Plants are regarded as cheaper and safe alternative source of drugs. However, cases of overdose or self poisoning through use of medicinal plants have been increasing. Investigations of the antibacterial activity of stem-bark extracts of *Mangifera indica* on Staphylococcus aureus was carried out with a view to screen for phyto-chemical compounds and determine susceptibility of the bacterium.

A large variety of chemical compounds have been reported in *Mangifera indica*. Among these, polyphenols (flavonoids, xanthones, and phenolic acids) are the most abundant compound types in *M. indica*. Various studies have been conducted with the extracts of roots, leaves, bark, fruit peel and flesh, and kernel of *M. indica* to investigate antibacterial properties. Among these parts, mango kernel and leaves are the most studied parts for antibacterial effects. A study carried out with four *M. indica* varieties grown in Kenya (Apple, Ngowe, Sabine, and Kent) found that the methanol extracts of kernels of Apple and Sabine varieties exert strong inhibitory effects against *Escherichia coli*. A number of studies have also been carried out to evaluate antibacterial effects of different extracts of *M. indica* leaves worldwide (Ajila, Bhat *et al*., 2007) [12].

Synergy is best defined as the interaction of two agents such that the combined effect is greater than the sum of expected individual effect. This is perhaps an antonym of antagonism meaning the interaction of two or more agents such that the combined effect is less than the sum of the expected individual effect. There are few reports are available on synergistic effect of antimicrobial activity of phytochemicals with synthetic chemicals in literature (Shahnaz *et al*., 2009) [13].

Transition metals have an important place within medicinal biochemistry. Research has shown significant progress in utilization of transition metal complexes as drugs to treat several human diseases like carcinomas, lymphomas, infection control, anti-inflammatory, diabetes, and neurological disorders (Warra *et al*., 2011) [15]. This activity of transition metals has started the development of metal based drugs with promising pharmacological application and may offer unique therapeutic opportunities. To provide an update on recent advances in the medicinal use of transition metals, a Medline search was undertaken to identify the recent relevant literature (Tao *et al*., 2010) [14].

There are few reports on antifungal and antibacterial activity of transitional metal complexes in literature. These complexes may possess antimicrobial activity against pathogenic fungi and bacteria being used as a test organism in the present study. Transition metals such as zinc, copper, cobalt, manganese, iron have been reported to be essential for crops (Arora *et al*., 2012) [16]. Under primitive earth conditions, cyanide could have combined with a large number of metal ions present in primeval sea they remain in soil in small quantity and known as micronutrient. If the deficiency of these elements is detected in soil these are recommended to be added to soil with fertilizer or in form of top dressing. These metals act as micronutrient in trace quantity and hence application of metal complexes in combination with other ecofriendly chemicals/botanicals may be screened for antimicrobial potential.

Material and Methods

Collection of fungal cultures: Fungal pathogen *Rhizoctonia solani* employed in the present investigations have been collected from Central Potato Research Institute Campus, Modipuram, Meerut, India. The pathogen was grown on potato dextrose agar (PDA) medium and incubated at 28 ± 1 ºC. The medium was incubated at 28 ± 1 ºC for 2 days for the *Rhizoctonia solani*.

Collection of plants: The samples of leaves of *Phyllanthus emblica*, *Psidium guajava*, *Jatropha gossypifolia* and *Mangifera indica* were collected from Harbal Garden, Patanjali Yogpeeth, Haridwar India. Plant material was dried in shade at 35 ºC for 15-20 days. The shade dried leaves of each plant spp. were grinded in mixer and stored in airtight containers after grinding. Dry powder of leaves was extracted four to five times with 5 mL methanol solvent/g of plant material. All these extracts were combined and concentrated by flash evaporation at 40 ºC.

Synthesis of transition metal ferocyanides: Manganese, cobalt, nickel, copper, zinc and cadmium ferrocyanides were prepared following the Kourim's procedure (Kourim *et al*., 1964) [17]. A solution of potassium ferrocyanide (167 mL, 0.1 M) was added to solution of desired metal salt (500 mL, 0.1 M) with constant stirring at room temperature. A slight excess of metal salt solution markedly improves the coagulation of the precipitate. The reaction mixture was heated on a water bath at 80 ºC for 3-4 h and allowed to stand at ambient temperature for 24 h. The precipitate was filtered under vacuum and washed thoroughly with double distilled water. It was dried in an oven at 60 ºC. The dried product was ground and sieved to 100 mesh sizes. The coloured powers of metal complexes were stable in air. These were characterized on the basis of IR spectra (KBR disc on Bio-red FTIR spectrophotometer) and magnetic susceptibility measurement (Sherwood Scientific) (Tables-1-8). The molecular formula of synthesized metal complexes established on the basis of data obtained from elemental analysis are Mn2[Fe(CN)6]·3H2O, Co2[Fe(CN)6]·2H2O, Ni2[Fe(CN)6]·5H2O, Cu2[Fe(CN)6]·7H2O, Zn2[Fe(CN)6]·3H2O and Cd2[Fe(CN)6], respectively.

Table 1: Infrared spectral data of metal ferrocyanide complexes

Complexes	**Adsorption Frequencies (cm^{-1})**				
	ν_{HOH}	$\nu_{C\equiv N}$	**HOH bending**	ν_{Fe-C}	$\nu_{Metal-N}$
$Mn_2[Fe(CN)_6].3H_2O$	3743	2065	1641	594	451
$Co_2[Fe(CN)_6].2H_2O$	3830	2087	1643	592	459
$Ni_2[Fe(CN)_6].5H_2O$	3423	2090	1645	592	462
$Cu_2[Fe(CN)_6].7H_2O$	3614	2104	1614	594	491
$Zn_2[Fe(CN)_6].3H_2O$	3618	2098	1516	599	497
$Cd_2[Fe(CN)_6]$	3448	2063	1637	592	445

Table 2: Magnetic moments and molar conductivity of metal ferrocyanide complexes.

Metal hexacyanoferrate (II)	**μ_{calc} (B.M.)**	**μ_{eff} (B.M.)**	**Molar conductance (μS)**
$Mn_2[Fe(CN)_6].3H_2O$	5.92	6.21	24.2
$Co_2[Fe(CN)_6].2H_2O$	3.87	4.36	9.81
$Ni_2[Fe(CN)_6].5H_2O$	2.83	2.99	6.61
$Cu_2[Fe(CN)_6].7H_2O$	1.73	2.45	6.72
$Zn_2[Fe(CN)_6].3H_2O$	0.00	0.81	2.70
$Cd_2[Fe(CN)_6]$	0.00	0.90	7.44

Table 3: Major X-Ray absorption peaks in the XRD spectra of manganese ferrocyanide

2 θ	**d-Spacing(Å) observed**	**Relative Intensity (%)**	**d-Spacing[Å] reported in PCPDF database**
17.6155	5.0348	56.48	5.8087
24.9795	3.56478	100.00	3.5570
29.6726	3.0117	7.07	3.0334
39.1584	2.3005	6.69	2.3081
40.0277	2.2525	9.91	2.5152
43.4091	2.0846	5.28	2.9043

Table 4: Major X-Ray absorption peaks in the XRD spectra of cobalt ferrocyanide

2 θ	**d-Spacing(Å) observed**	**Relative Intensity (%)**	**d-Spacing[Å] reported in PCPDF database**
17.7134	5.0072	60.23	5.0300
25.0657	3.5527	100.00	3.5600
35.8547	2.5045	64.74	2.5300
43.7255	2.0702	8.97	2.0800
44.9538	2.0165	13.02	2.2800

Table 5: Major X-Ray absorption peaks in the XRD spectra of nickel ferrocyanide

2 θ	**d-Spacing(Å) observed**	**Relative Intensity (%)**	**d-Spacing[Å] reported in PCPDF database**
17.7146	5.0069	60.93	5.0500
25.0107	3.5604	100.00	3.5700
35.7078	2.5145	53.31	2.5700
40.1426	2.2463	10.26	2.2600
44.0851	2.0542	15.62	2.0600
51.3617	1.7789	10.64	1.7840
54.7539	1.6765	4.39	1.6830
57.9877	1.5891	11.22	1.5230

Table 6: Major X-Ray absorption peaks in the XRD spectra of copper ferrocyanide

2 θ	**d-Spacing(Å) observed**	**Relative Intensity (%)**	**d-Spacing[Å] reported in PCPDF database**
25.1752	3.5375	79.69	3.5000
29.7271	3.0054	7.41	3.0200
36.0522	2.4913	36.50	2.5000
40.3144	2.2372	14.79	2.2300
44.3532	2.0424	12.34	2.0400

Table 7: Major X-Ray absorption peaks in the XRD spectra of zinc ferrocyanide

2 θ	d-Spacing(Å) observed	Relative Intensity (%)	d-Spacing[Å] reported in PCPDF database
16.3677	5.4157	100.00	5.4000
19.7227	4.5014	46.65	4.5100
21.7924	4.0783	90.70	4.0800
28.6684	3.1139	22.18	3.1100
29.7535	3.0027	9.27	3.0000
35.6073	2.5141	10.35	2.5400
37.7830	2.3810	7.67	2.3700
38.8405	2.3186	7.21	2.3200
40.9696	2.2029	11.16	2.2000
47.8545	1.9008	5.80	1.9500

Table 8: Major X-Ray absorption peaks in the XRD spectra of cadmium ferrocyanides

2 θ	d-Spacing(Å) observed	Relative Intensity (%)	d-Spacing[Å] reported in PCPDF database
19.5467	4.5415	3.45	4.1100
28.7088	3.1096	19.86	3.1600
31.7556	2.8178	3.52	2.8300
35.3196	2.5412	39.74	2.4900
39.6586	2.2726	19.20	2.2300
42.7467	2.1153	9.50	2.1100
49.1373	1.8541	1.50	1.8180
50.7909	1.7976	7.34	1.7470
57.3244	1.6073	10.31	1.6670
59.3363	1.5575	2.77	1.5760
61.4229	1.5095	1.43	1.5350
66.3195	1.4094	2.20	1.4740

Testing the antifungal activity of plant extracts-metal ferrocyanide complexes: Paper disc method (Perez *et al*., 1990) [18] was used for initial screening of antifungal potential of metal complexes and plant extracts chosen for present investigations. This method was based on diffusion capacity of test chemical(s) through agar medium. Metal ferrocyanide (5 mg) and antifungal plant extract (5 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.

Results and Discussion

Antifungal activity of plant extracts only

Antifungal activity of *Phyllanthus emblica*, *Psidium guajava*, *Jatropha gossypifolia*, and *Mangifera indicia* were studied. *Phyllanthus emblica extract* and *Mangifera indica* were found to have maximum and minimum antifungal property, respectively. The following order of antifungal lproperty was *Phyllanthus emblica* > *Jatropha gossypifolia* > *Psidium guajava* > *Mangifera indica*

Antifungal activity of metal ferrocyanides only: Antifungal activities of manganese, cobalt, nickel, copper, zinc and cadmium ferrocyanides were studied. Cadmium and nickel ferrocyanides were found to have maximum and minimum antifungal property, respectively. The following order of antifungal activity was observed in metal ferrocyanides. Cadmium ferrocyanide > copper ferrocyanide > cobalt ferrocyanide > manganese ferrocyanide > nickel ferrocyanide

Synergistic effect of medicinal plant extracts with metal ferrocyanides: Plant extract (5 mg) and metal ferrocyanide (5 mg) and 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. The following order of antifungal activity was observed**,** in natural antifungal with metal ferrocyanide complexes.

- Cadmium ferrocyanide: *Phyllanthus emblica* > *Jatropha gossypifolia* > *Psidium guajava* > *Mangifera indica*
- Copper ferrocyanides: *Phyllanthus emblica* > *Jatropha gossypifolia* > *Psidium guajava* > *Mangifera indica*
- Cobalt ferrocyanide: *Phyllanthus emblica* > *Jatropha gossypifolia* > *Psidium guajava* > *Mangifera indica*
- Manganese ferrocyanides: *Phyllanthus emblica* > *Jatropha gossypifolia* > *Psidium guajava* > *Mangifera indica*
- Nickel ferrocyanides: *Phyllanthus emblica* > *Jatropha gossypifolia* > *Psidium guajava* > *Mangifera indica*

The following conclusions were observed that antifungal activities of secondary metabolites are enhanced through interaction with metal ferrocynides. Cadmium and nickel ferrocyanides were found to have maximum and minimum anti fungal property, respectively. *Phyllanthus emblica* and *Mangifera indica* were found to have maximum and minimum antifungal properties, respecticely. *Phyllanthus emblica* extract-cadmium ferrocyanide complex and *Mangifera indica extract* with nickel ferrocyanide complex were found to have maximum and minimum properties, respectively. It may be also concluded from present studies that cadmium ferrocyanid *Phyllanthus emblica* extract complex may be used as effective antifungal drug for black scurf disease of potato.

The result lends credence to the folkloric use of these transition ferrocyanides in treating microbial infection and shows that ferrocyanides of cobalt, cadmium and copper could be investigate the probable reason of synergistic effect of bioactive fractions of medicinal plants. Synergistic effect of methanol extracts of medicinal plants with metal ferrocyanides against *R. solani* may be due to Porous surface structure and adsorption capacity of metal ferrocyanides. These metal ferrocyanides may adsorb active components of medicinal plants on its active sites thus the concentration of active components increases at the surface of metal ferrocyanides at active sites for adsorption as well as inside the pores present at their surface. The results suggest that these transition metal complexes may be used as fungicides alone or in combination with other plant botanicals fungicidal substances resulting in the development of ecofriendly fungicides for future that can help in protecting our environment for soil pollution and our health too.

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