New symbiotic antifungal strategies based on medicinal plants and metal complexes

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Abstract
The combination of previously manufactured metal complexes with extracts from medicinal herbs such as Withania somnifera, Azadirachta indica, Ocimum sanctum and Tinospora cordifolia L., renowned for their intrinsic antimicrobial activities, produced a synergistic effect. Comparative investigation demonstrated that medicinal plant extracts mixed with metal complexes had higher antimycotic action than individual metal complexes or plant extracts alone.

Notably, cadmium ferrocyanide combined with Tinospora cordifolia extract and copper ferrocyanide combined with Withania somnifera extract exhibited the highest antimycotic activities against Phytopthora infestans, the causal agent of late blight in potatoes. These findings show that synergistic interactions between metal complexes and medicinal plant extracts improve antimicrobial activity, underlining their potential as long-term disease management options.

Keywords: Synergistic effects, medicinal plants, transition metal ferrocyanides, *Phytopthora infestans*.

Introduction
Medicinal plants are recognised to play an important role for mankind. For thousands of years, plant elements such as roots, bark, seeds and leaves have been used for medicinal purposes. In recent years, there has been a significant interest in assessing several plant extracts for their antibacterial activities against microorganisms that cause tooth caries and periradicular pathology[12,24]. The rise in microbial resistance to currently available antimicrobial treatments is mostly to blame for the growing clinical warning sign of microbial infections which can have fatal effects. Because of this, methods for determining an antibiotic's susceptibility and developing novel antibiotics are commonly used and are still being developed[17].

A common Ayurvedic herb is *Withania somnifera* (L.) Dunal, a Solanaceae medicinal plant also referred to as winter cherry, Indian ginseng, or ashwagandha. It is a well-known medicinal herb with antifungal properties against various fungus species[18]. It is widely utilized throughout the world because of its low toxicity and high medical efficacy. Roots and less frequently leaves and fruits, have been utilized as phytomedicines in decoctions, infusions, ointments, powders and syrups. Currently, only a few studies have been conducted to investigate the anticancer properties of *W. somnifera* root extracts, with comparably less study on aqueous extracts[4].

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The objectives of this work were to assess *W. somnifera* leaf extract antimicrobial properties *in vitro* in order to address a perceived research gap (Fig. 1). It is now grown as a crop to meet the enormous demand for biomass and to maintain a long-term dominance in the pharmaceutical industry[14,25]. Perhaps the most effective traditional medicinal herb is *Azadirachta indica*, also known as neem, a member of the Meliaceae family. To treat a variety of human illnesses, all parts of the tree have been utilized as home remedies[23]. In India, the tree is still acknowledged as a "village dispensary". This plant has gained international attention due to its many medicinal uses. The majority of its parts including fruits, seeds, leaves, bark and roots, contain compounds that have been found to be germicidal, interferon, febrifuge, antileukotriene, anti-peptic ulcer and antimicrobial, among others (Fig. 2)[27].

Since ancient times, Ayurvedic medicine *Ocimum sanctum*, also known as Tulsi, a tried-and-true medicinal herb has been used and it belongs to the *Lamiaceae* family. Its numerous therapeutic characteristics have contributed significantly to modern research. Various plant parts have immaculate, inflammation reducer, anaesthetic, antiulcer, cytostatic, abortifacient and anticancer properties. This medicinal plant is a source of tremendous economic significance around the world. Nature has given upon us a vast botanical wealth and a wide variety of plant varieties flourish across the country[1]. Alternative antimicrobial medicines derived from medicinal plants are required to treat infectious diseases. Conventional synthetic chemicals have caused ecological concerns because of their high cost, negative impact on the ecosystem and potential for pathogen resistance.

Keeping these factors in mind, attempts have been made to manage the fungus using natural extracts. Apart from its therapeutic use, *Ocimum spp*. (Fig. 3) produces essential oils that are valuable in perfumery. Essential oil contains a novel terpene, whereas seeds contain a lot of mucilage. Nature has produced medical ingredients for thousands of years and an incredible number of modern drugs are derived from natural

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resources. Folk medicines are valuable source of potentially beneficial novel chemicals for the evolution of annihilator drugs. Tulsi is extensively recognized to have antioxidant, anticancer, antidiabetic, antibacterial, antifungal, hepatoprotective, analgesic, antifertility, anti-inflammatory, antiulcer and antihypertensive properties[13].

Menispermaceae member *Tinospora cordifolia*, popularly referred to as "Guduchi," possesses several medicinal qualities such as antihyperglycaemic, inflammation reducer, antiperistalsis, anti-rheumatic, abortifacient, antiallergenic and de-stress effects[7,21]. Because of its alkaloids, flavonoids and carbs content, this plant can effectively replace clinical isolates that have demonstrated *in vitro* antibacterial action. The increasing prevalence of drug resistance in healthcare facilities across the globe may be addressed by the use of plant-based therapeutic alternatives. This medicinal plant's leaves stem and roots are among its constituent parts that possess therapeutic and medicinal qualities (Fig. 4)[11].

Tests have shown that the plant extracts are efficient against a number of bacterial agents such as *Proteus vulgaris, Pseudomonas aeruginosa, Enterobacter aerogenes, Salmonella typhi, Shigella flexneri, Salmonella paratyphi and Salmonella typhimurium*. One important Indian remedy that might be used to counteract the growing antimicrobial resistance of today is *Tinospora cordifolia*[10,26]. Positively charged ions are produced which play a crucial role in stabilizing or modifying the quaternary or tertiary structure of electron-rich biomolecules like DNA and proteins. The current investigation will concentrate on metal complex-based antibacterial medications[2]. Metal coompound have arisen as excellent alternatives to organic molecules due to their unique steric and electronic properties, which result in various modes of action like electron transfer and redox processes[6,9]. The fundamental goal of this study is to look at the antibacterial properties of transitional metal complexes in combination with secondary metabolites found in medicinal plants against bacterial and fungal infections. The study's specific goal is to assess the antibacterial synergy between transitional metal complexes and medicinal plant secondary metabolites.

Examine the efficacy, safety and environmental impact of these combinations as alternative treatments for microbial infections, as well as the mechanisms underlying their antimicrobial properties, to better understand their mode of action against microbial pathogens.

Material and Methods

Collection of fungal cultures: *Phytophthora infestans*, a fungal strain that causes late blight disease in potatoes, was acquired from the Central Potato Research Institute (CPRI), Modipuram, Meerut, India, in February 2024. The pathogen was cultivated on potato dextrose agar (PDA) medium for two days at 25-30°C.

Figures 1-4: Leaves of *Withania somnifera*, *Azadirachta indica*, *Ocimum sanctum* and *Tinospora cordifolia*

Collection of medicinal plants: In the month of November and December 2023, samples of leaves of *Withania somnifera*, *Azadirachta indica*, *Ocimum sanctum* and *Tinospora cordifolia* were collected from the Botanical Garden of Banasthali Vidyapith, Banasthali, Rajasthan, India, which is located at 1010ft altitude and has a latitude range of 26°24'13''N-26°24'11''N. The longitude is 75°52'21''E-75°52'36''E. The region receives an average annual rainfall of 79.02 millimeters and temperatures range from 23.31-45.58 ºC. The plant species were dried in the shade for 15-20 days at 30-35 ºC. Each plant species' shade-dried leaves were pulverized in a mixer and stored. For every gram of plant material, four to five milliliters of methanol were used to extract the dry leaf powder. These extracts were all mixed together and concentrated at 40°C using flash evaporation.

Synthesis of transition metal ferocyanides: The Kourim technique[16] is employed to synthesized these metal complexes. Using this procedure, Ferrocyanides of manganese, cobalt, nickel, copper, zinc and cadmium were synthesized and identified using magnetic susceptibility testing (Sherwood Scientific), IR spectra (KBR disc on Bio-red FTIR spectrophotometer) and X-ray diffraction analysis (SCXRD) results had already been published[8].

Antifungal activity of plant extracts with metal ferrocyanide complexes: The antifungal activity was tested using the paper disc method[22]. The latest investigations used medicinal plant extracts combined with metal complexes. In a sterile petri dish filled with potato dextrose agar (PDA) media, a selected medicinal plant extract (5 mg) and transition metal ferrocyanide (5 mg) were added and with the help of aspirator, fungal spores were sprayed across the whole bottom of the petri dish. The process is repeated using a new extract and ferrocyanide metal. The Vincent formula[28] is used to determine growth percentage inhibition (%) as (Cg-Tg)/Cg100. Cg represents growth under control in millimeters, while Tg represents growth during therapy in millimetres. Each experiment was performed three times and the average value was utilized to interpret the results (Tables 1 and 2). Each experiment was performed in triplicate and the average value was used to interpret the results.

Synergistic effect of medicinal plant extracts with metal complexes: Take a sterile petri dish and add 5 mg of plant extract and 5 mg of metal ferrocyanide. Fungus spores were liberally spread on the bottom of the petri plate using an aspirator. A similar method was frequently used when combining other plant extracts and metal complexes.

Statistical analysis: The statistical analysis was carried out using SPSS Statistics IBM base 22.0. The study used descriptive statistics, paired t-tests and Shapiro-Wilk tests to determine normality. The data was understood and described using descriptive statistics, as well as by Shapiro-Wilk test to corroborate the parametric methods' assumptions.

Results and Discussion

Antimycotic activity of plant extracts: The examination of fungicidal activity of *O. sanctum*, *W. somnifera*, *T. cordifolia* and *A. indica* revealed that *W. somnifera* and *T. cordifolia* extracts had the highest antimycotic properties respectively. Thus, the antimycotic properties of the plant extracts under evaluation are listed in the following order: *W. somnifera* > *T. cordifolia* > *A. indica* > *O. sanctum.*

Table 1
Antifungal activity of metal complexes

Metal complexes	*Phytopthora infestans*	
	Inhibition zone (mm)	Inhibition (%)
Mn (II)	5	28
Co (II)	6	33
Ni (II)	3	9
Cu (II)	8	40
Zn(II)	3	13
Cd(II)	13	80

Table 2
Synergistic effect of medicinal plant extracts with metal complexes

Metal complexes	*O. sanctum*	*W. somnifera*	*T. cordifolia*	*A. indica*
	Growth Inhibition zone (mm)			
Mn (II)	6	12	11	9
Co (II)	9	14	12	10
Ni (II)	5	8	9	7
Cu (II)	10	17	14	11
Zn (II)	5	10	9	6
Cd (II)	13	18	19	15

Antifungal activity of metal complexes: Investigated fungicidal activity was reported in table 1 for Mn-II, Co(II), Ni(II), Cu(II), Zn(II) and Cd(II). Cd(II) and Ni(II) have the highest and lowest fungicidal properties respectively. Table 1 clearly shows that Cd (II) had the highest growth inhibition of 88% against *P. infestans*, Cu (II) had a considerable growth inhibition of 40% and Mn-II and Co (II) had antmycotic potential of 28-33% against the same fungal pathogen. On the other hand, Ni (II) and Zn(II) were found to be less effective with growth inhibition, which is only up to 9-13% against fungal pathogen. Thus the antifungal activity of metal ferrocyanides was recorded in following order: Cd(II)>Cu(II) > Co(II)> Mn(II)> Zn(II) >Ni(II).

Synergistic effect of medicinal plant extracts with metal complexes: The data provided in table 2 indicated that the combinations of Cd(II) metal complex with plant extract of *W. somnifera* were recorded to have maximal fungicidal potential up to 13-19 mm growth inhibition against fungus taken for the current experiment. Co(II) and Cu(II) showed considerable antifungal capability up to 9-17 mm when combined with all of the plant extracts studied, Mn(II) showed better fungal growth inhibition (6-12mm) with all of the plant extracts. In contrast, the pairings of Ni(II) and Zn(II) with all of the plant extracts under study demonstrated the lowest antifungal activity in the 5-10 mm range. Thus, the following sequence of antimycotic action was observed in the combination of transition metal complexes with medicinal plant extracts under study (Fig. 5):

1) Mn(II): *W. somnifera* > *T. cordifolia* > *A. indica* > *O. sanctum*
2) Co(II): *W. somnifera* > *T. cordifolia* > *A. indica* > *O. sanctum*
3) Ni(II): *W. somnifera* > *T. cordifolia* > *A. indica* > *O. sanctum*
4) Cu(II): *W. somnifera* > *T. cordifolia* > *A. indica* > *O. sanctum*
5) Zn(II): *W. somnifera* > *T. cordifolia* > *A. indica* > *O. sanctum*
6) Cd(II): *W. somnifera* > *T. cordifolia* > *A. indica* > *O. sanctum*

When only metal ferrocyanides are considered, Cd(II) and Ni(II) have the strongest and lowest antimycotic capabilities respectively. However, secondary metabolites antimycotic activity is increased when they interact with metal ferrocynides. Furthermore, it was discovered that *W. somnifera* extract with cadmium ferrocyanide and O. sanctum extract with nickel ferrocyanide had the highest and lowest levels of fungicidal activity respectively.

Thus, the current study's findings suggest that a combination of Cd(II) and *W. somnifera* extract could be used as an effective antimycotic agent for potato late blight. The findings demonstrated that these transition ferrocyanides are utilized to treat a wide range of pathogenic illnesses and that Cu(II), Co(II) and Cd(II) might be used to investigate the likely reason of the synergistic action of bioactive chemical compounds discovered in medicinal plants.

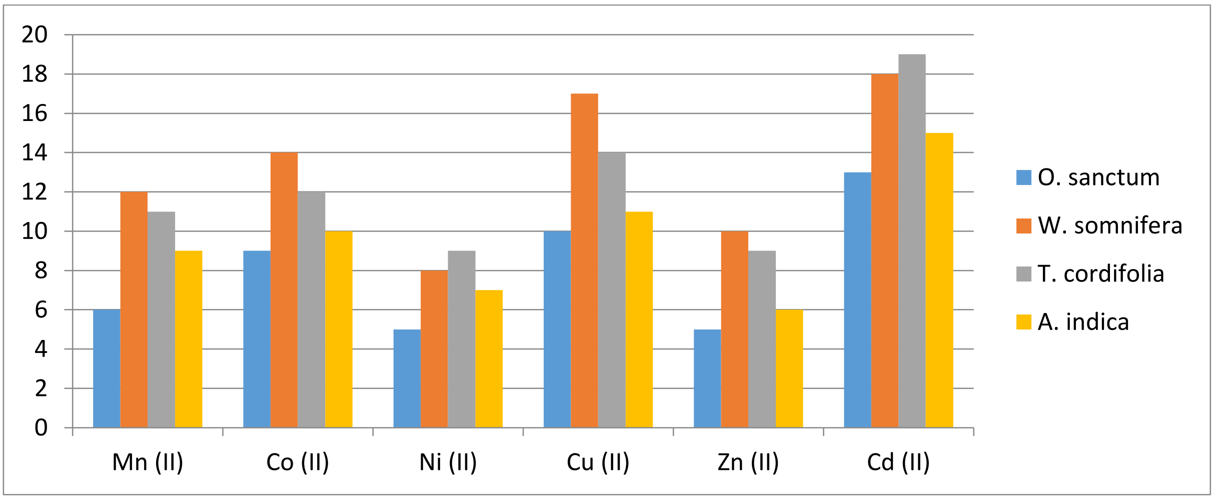

Fig. 5: Graphical representation of synergistic effect of medicinal plant extracts with metal complexes

Table 3
Statistical analysis of Synergistic effect of medicinal plant extracts with metal complexes

Name of the Plant	**Growth Inhibition against *Phytopthora infestans***		
	Mean	**Standard deviation**	**Paired t-test value (p-value)**
	7.83	3.87	Before Compare
O. sanctum	8.17	3.66	5.31 (< 0.001)
W. somnifera	13.68	4.02	16.02 (< 0.000)
T. cordifolia	11.74	3.71	7.660 (< 0.000)
A. indica	9.51	3.35	8.000 (< 0.000)

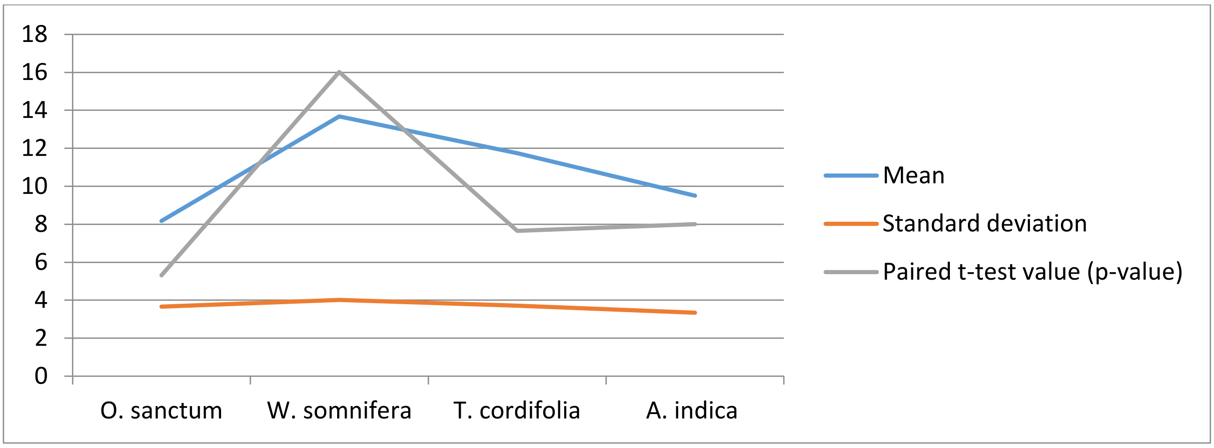

Fig. 6: Statistical analysis of Synergistic effect of medicinal plant extracts with metal complexes

These findings suggest that these transition metal complexes can be applied as fungicides on their own or in conjunction with other plant-botanical fungicides to create environmentally benign fungicides that can protect our environment and health from soil contamination.

Statistical analysis: A paired t-test was used to determine the significance of the mean difference between metal ferrocyanides and medicinal herbs. Value was considered as in table 3. The paired t-test results show a significant difference in fungal growth between *P. infestans*, *O. sanctum* (paired t-test value-5.31 (< 0.001) *W. somnifera* (paired t-test value-16.02 (< 0.000), *T. cordifolia* (paired t-test value-16.02 (< 0.000) and *A. indica*. paired t-test value-7.660 (< 0.000). Overall, we have found the highly significant p-value in all four medicinal plants extract and antimycotic action was seen in the combination of transition metal complexes with the help of statistical analysis (Figure 6).

Conclusion

Although combination medicine therapy is not a new idea, the future of infectious disease control and environmental safety may depend on creative and practical applications of phytochemicals to create safe and efficient combination therapies. On the other hand, synergism can be viewed as a type of combination therapy where the presence of one element greatly enhances the activity of the other. Comprehending synergism and its diverse manifestations may need a thorough understanding of molecular mechanics, statistical techniques and modern, improved technology. In this study, we attempted to summarize the most potent and widely utilized bioactive compounds for the management of infectious diseases along with any known mechanisms of action.

The persistent and repeated use of synthetic pesticides against plant diseases degrades the environment day after day. Scientists are currently focused on finding a long-term and environmentally benign solution to the aforementioned issue. The findings of this study also point to the possibility of using metal complexes as fungicides on their own or in combination with various secondary metabolites of plants, resulting in the development of environmentally friendly, safer-to-use fungicides capable of protecting our environment from various types of pollution in the future.

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