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Antimicrobial activity of green synthesized tri-metallic oxide Ni/Cr/Cu nanoparticles

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Abstract

The tri-metallic oxide Ni/Cr/Cu nanoparticles (NPs) were synthesized using *Coriander sativum* extract as the reducing agent. The precursors namely $CuSO_4.5H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$ and Cr $(NO_3)_3{\cdot}9H_2O$ were used for the green synthesis. Further, the prepared NPs were characterized using Ultraviolet-Visible (UV-Vis) spectroscopy and X-ray diffraction (XRD) studies. Its antimicrobial property against two fungal and two bacterial species was determined by measuring the respective zone of inhibition (ZOI) in well diffusion method. A dose dependent inhibition was observed in all the four species of pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus flavus* and *Penicillium sp*. This antimicrobial property of tri-metallic oxide NPs may be utilized in the field of medical research, pharmaceutical industries and environmental sciences.

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Keywords: Tri-metallic oxide NPs, UV-Vis, XRD, antimicrobial activity, zone of inhibition.

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1. Introduction

The common methods to synthesize of multi metallic NPs includes laser ablation, ultrasonication, sol-gel, hydrothermal, co-precipitation and green synthesis [1,2]. The physical and chemical methods involve higher cost and are not eco-friendly. On the other hand, use of green synthesis methods offer several advantages such as cost-effectiveness, simplicity, and environmentally friendliness. The green sources used for synthesizing NPs are micro-organisms and plants [3-6]. The choice of these sources as bioreducing agents should be delicately considered in the green synthesis approach [7,8]. On comparing, the choice of plants is less expensive than micro-organisms.

Coriander sativum is an important medical plant belonging to Umbelliferae family comprising several bio-active compounds such as flavonoid, phenol, terpenoid, tannin and glycosides with high level of *in vitro* radical scavenging activity [9,10]. These bio-active compounds were believed to play a major role in the synthesis of metal oxide NPs.

Due to the distinctive properties of bimetallic NPs, it has attracted the attention of research community [11,12]. For various applications, the most suitable methods were to fabricate multi-metallic NPs followed by core-shell and/or alloy forma-

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tion. Extensive research has been established on traditional single metallic methods. Exploration tri-metallic nanostructures have been initiated recently [13] since an elaborate study with respect to its application is still at its infancy.

The greatest obstacle for scientists trying to eradicate dangerous pathogens such as bacteria, moulds, yeast and viruses has been growing resistance to antibiotics. Multi-metallic NPs have been heavily studied against these dangerous pathogens as feasible therapeutic and diagnostic methods [14,15]. An effective approach to overcome these issues is to synergistically engineer these NPs with various formulations, such as the creation of multi-metallic composites, to strengthen current vulnerabilities.

The antimicrobial resistance has long been of concern to researchers and the use of metal oxide NPs has increased the interest in nanomaterial researchers. Hence in the present study, a green method to synthesize tri-metallic oxide NPs comprising Nickel (Ni), Copper (Cu), and Chromium (Cr) using the *Coriander sativum* extract will be developed. Subsequently, its antimicrobial efficacy against two bacterial species and two fungal species will be assessed. This investigation of antimicrobial activity of tri-metallic oxide NPs could be encouraging for a pharmaceutical application.

2. Materials and Methods

2.1. Materials required

Chemicals including $CuSO_4{\cdot}5H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, $Cr\ (NO_3)_3{\cdot}9H_2O$ were purchased from SRL India. Double distilled water was used for the preparation of chemical reagents.. All antibacterial and antifungal media were purchased from Himedia. Laboratory grade solvents were used for our study. The bacterial and fungal species were obtained from MTCC.

2.2. Collection of plant

Coriander sativum leaves were used for our study. The collected leaves were washed with tap and double distilled water and finally dried. The leaves were crushed and grounded to fine powder. The extract was prepared using 5 g of fine powder in 100 mL distilled water for 15 min at 400W. It was filtered using Whatman no.1 filter paper and filtrate was used as a reducing agent for green synthesis of tri-metallic oxide NPs.

2.3. Green synthesis of tri-metallic oxide NPs

The method involves simultaneous reduction of precursor salts $CuSO_4{\cdot}5H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$ and $Cr(NO_3)_3{\cdot}9H_2O$ with the *C. sativum* extract [16]. Typically, 0.01M of salt solution comprising all three metals was mixed with the leaf extract in 250 mL Erlenmeyer flask. The temperature of the reaction mixture was maintained 40°C for 45 min in a water bath. After completion of the reaction, mixture was cooled to achieve room temperature. The mixture was centrifuged at 3000 rpm for 15 min and washed several time with distilled water. Finally the mixture was washed with ethanol to remove impurities. The sediment comprising the tri-metallic NPs were stored in vacuum oven at 45°C to maintain its stability and integrity.

2.4. Characterization of NPs

The synthesized tri-metallic oxide NPs were characterized using UV-Vis spectroscopy and X-ray diffraction (XRD). The composition of the Ni/Cr/Cu NPs were investigated using a Empyrean X-ray diffractometer, operated at 45 kV, 40 mA, with CuKα1 radiation (wavelength λ=1.5406 Å) and copper filter. The X-ray diffractogram was recorded in the 2θ range from 10° to 80° at scanning steps of 0.026°. An UV-Vis spectrum was measured between 190-800 nm using Jasco spectrophotometer to determine the characteristic peaks of the synthesized NPs.

2.5. Antimicrobial activity

The antimicrobial activity of tri-metallic NPs was evaluated against two bacterial and two fungal species by well diffusion method [17,18]. 1 mL of the selected microbial species was inoculated over the entire agar surface. A well with 5 mm diameter was bored aseptically, and 50μL of the NPs solution at desired concentration was introduced into the well. The inoculated plates were incubated under suitable conditions depending upon the test microorganism. The details of the medium, species and incubation conditions are provided below.

2.5.1. Antibacterial activity

Mueller Hinton Agar plates were prepared and inoculated with standardized bacterial strains comprising *E. coli* and *S. aureus* in individual plate. Five different wells were bored on the agar plates where, four different concentrations of the NPs were added in four separate wells and control (amoxicillin) was added in the other well. The plates were incubated (37℃/24 hours) and observed for zone of inhibition (ZOI).

2.5.2. Antifungal activity

Sabouraud Dextrose Agar plates were prepared and inoculated with standardized fungal strains of *A. flavus* and *Penicillium sp*. Five different wells were bore on the agar plates where, four different concentrations of the NPs were added in four individual wells and control (fluconazole) was added in the other well. The plates were then incubated at 25℃ for 72 hours and observed for zone of inhibition (ZOI).

2.6. Statistical analysis

All results of antimicrobial activity are expressed only numerically as mean ± standard deviation (SD) without any analysis.

3. Results and discussions

3.1. Synthesis and characterization of tri-metallic NPs

Initially, the extract and the precursor solution looked tanned brown colour. After the reduction reaction, the solution turned dark brown in colour. After centrifugation and drying, a dark brown powder was collected. The UV-Vis absorption of the *C. sativum* extract was compared with the collected tri-metallic oxide NPs. The tri-metallic oxide NPs showed three characteristic peaks at 261 nm, 426 nm and 564 nm (Figure 1) that correspond to tri-metallic oxide Ni/Cr/Cu NPs reported earlier

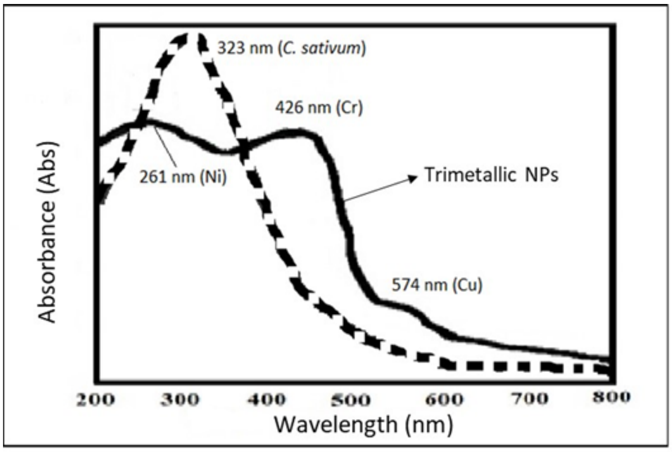

Figure 1. UV-Visible spectrum of tri-metallic oxide Ni/Cr/Cu NPs

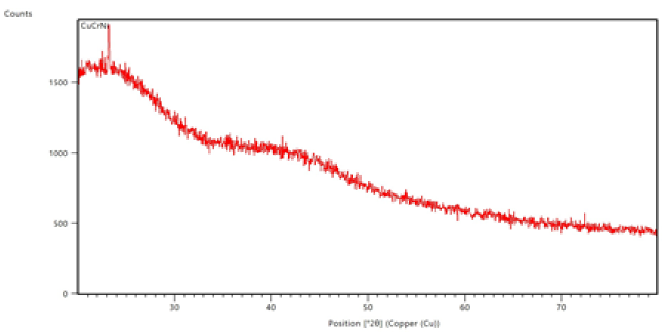

Figure 2. Powder XRD of tri-metallic oxide Ni/Cr/Cu NPs

[16]. Similar results were obtained in monometallic NPs synthesized using individual metallic precursors [19–21].

The Ni, Cu, and Cr NPs tend to show characteristic XRD peaks at 40-50° and 50-60° [19–21] individually. A combination of peaks was observed in XRD (Figure 2) and new peak at 20° characteristic to that of graphene nanomaterials was also observed. This may be due to the reduction reaction of phytochemical that is present in the leaf extract of *C. sativum*. Using Scherrer's equation, the crystallite size of the tri-metallic oxide NPs was calculated as 17.72 nm from the XRD. In the present study, the cumulative peaks of all the three metal were exhibited in both UV-Vis and XRD. This shows that tri-metallic oxide NPs comprising Ni, Cr and Cu were synthesized.

3.2. Antibacterial activity

The antibacterial activity of the tri-metallic NPs was determined against *E. coli* and *Staph. aureus* by measuring the ZOI after incubation (37°C/ 24 h) (Table 1). The observed results are presented in figure 3. It can be seen that the tri-metallic NPs exhibited dose-dependent antibacterial activity. That is, the zone of inhibition increased proportionally with the concentration of NPs. Among the tested species, the tri-metallic NPs showed better antibacterial activity against *E. coli* than *Staph. aureus*. There are reports that discussed the antibacterial activity of these metals. In a study by O. Dlugosz et. al., copper showed improved antibacterial activity against *E. coli* and *Staph. aureus* with respect to increasing size. But its effect reduced when it is combined with silver. On the other hand, it exhibited inhibition against broader spectrum of microbes. In another study, the bimetallic NPs made of gold and silver showed that the size and the antimicrobial property are inversely proportional to each other [15]. There is only one study which discussed similar antibacterial property of similar tri-metallic NPs wherein all the three metals showed synergistic antimicrobial effect [16]. Hence as per the earlier studies and the present results, it can be understood that the antibacterial property of the metallic NPs was due to the size and the charge of the active surface area.

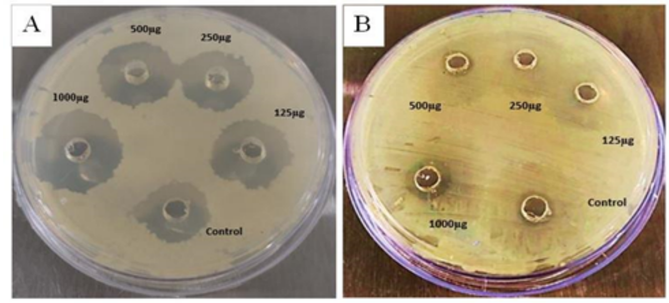

Figure 3. Agar plates showing ZOI against (a) *E. coli* and (b) *Staph aureus*. Control - Amoxicillin

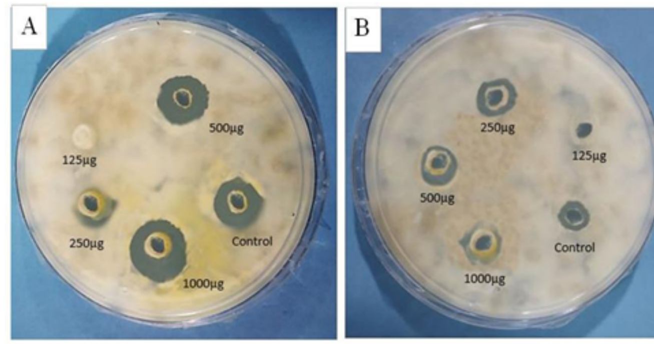

Figure 4. Agar plates showing ZOI against (a) *A. flavus* and (b) *Penicillium sp*. Control - Fluconazole

3.3. Antifungal activity

The antifungal activity of the tri-metallic NPs was determined against *A. flavus* and *Penicillium sp.* by a similar method to that of antibacterial activity. The measured ZOI and its respective plates are provided in Table 1 and figure 4. It was observed that the organism showed resistance towards the tri-metallic NPs at 125 μg/mL concentration. But as the nanoparticle's concentration increased, the diameter of the zone of inhibition also increased. Thus, the dose and inhibition were directly proportional to each other. In earlier studies, the bimetallic NPs showed good activity towards fungal species *Candida sp.* and *B. cinerea* [22,23] due to their external charge and size. The tri-metallic NPs showed better antifungal activity against *Penicillium sp.* than *A. flavus* that can be attributed towards even

Table 1. Zone of inhibition (mm) measured for each microbes across the concentration gradient. All the values are given as mean value.

S No	NPs Concentration (μg/mL)	*E. coli* (mm)	*Staph aureus* (mm)	*A. flavus*(mm)	*Penicillium sp.* (mm)
1	125	19	13	Nil	Nil
2	250	17	15	13	12
3	500	21	17	18	14
4	1000	27	24	23	15
5	Control	16	18	18	16

size distribution and cumulative charge of the NPs.

4. Conclusion

Tri-metallic NPs comprising Ni, Cr and Cu were synthesized using the leaf extract of *Coriander sativum*. Characteristic peaks were observed in both UV-Vis spectroscopy and XRD diffractogram. The main objective of this research is to investigate the recognition and targeting capability of the tri-metallic oxide NPs and use it to deliver the antimicrobial drugs for resistant species. As per the results, these NPs showed good antimicrobial property against *E. coli*, *Staph. aureus, Penicillium sp.* and *A. flavus.* Further investigation is required to determine other parameters like minimum inhibitory concentration, critical dose and lethal dose.

References

[1] M. Parashar, V. K. Shukla & R. Singh, "Metal oxides nanoparticles via sol–gel method: a review on synthesis, characterization and applications", Journal of Materials Science: Materials in Electronics **31** (2020) 3729.

[2] W. M. Rangel, R. A. A. Boca Santa & H. G. Riella, "A facile method for synthesis of nanostructured copper (II) oxide by coprecipitation" J Mater Res Technol. **9** (2020) 994.

[3] S. P. Patil, "Ficus carica assisted green synthesis of metal nanoparticles: A mini review", Biotechnology Reports **28** (2020) 00569.

[4] I. Fatimah, "Synthesis of metal and metal oxide nanoparticles using plant extract: a review", J Eksakta. **17** (2017) 66.

[5] Y. Kato, E. Yoshimora & M. Suzuki, "Synthesis of Gold Nanoparticles by extracellular components of Lactobacillus casei", Chemistry select **4** (2019) 7331.

[6] Y. Kato & M. Suzuki "Synthesis of metal nanoparticles by microorganisms", Crystals, 10 (2020) 589.

[7] A. Muthuvinothini & S. Stella, "Green synthesis of metal oxide nanoparticles and their catalytic activity for the reduction of aldehydes", Process Biochem. **77** (2019) 48.

[8] R. Chokkareddy & G. G. Redhi, "Green synthesis of metal nanoparticles and its reaction mechanisms" In: The Macabresque: Human Violation and Hate in Genocide Mass Atrocity and Enemy-Making (2018) 113.

[9] N. Pathak, S. B. Kasture & M. M. Bhatt, "Phytochemical screening of Coriander Sativum linn", Int J Pharm Sci Rev Res., 9 (2011) 027.

[10] S. S. Jangra, V. K. Madan & S. I. Dusyant, "Comparative analysis of phytochemical profile and antioxidant activity of coriander (Coriandrum sativum L.)", Asian J Chem. **30** (2018) 508.

[11] T. Mazhar, V. Shrivastava & R. S. Tomar, "Green synthesis of bimetallic nanoparticles and its applications: A review" Journal of Pharmaceutical Sciences and Research **9** (2017) 102.

[12] O. Długosz, M. Sochocka, M. Ochnik & M. Banach, "Metal and bimetallic nanoparticles: Flow synthesis, bioactivity and toxicity", J Colloid Interface Sci. **586** (2021) 807.

[13] M. Nasrollahzadeh, M. Sajjadi, S. Iravani & R. S. Varma, "Trimetallic nanoparticles: Greener synthesis and their applications", Nanomaterials **10** (2020) 1784.

[14] N. Arora, K. Thangavelu & G. N. Karanikolos, "Bimetallic Nanoparticles for Antimicrobial Applications", Frontiers in Chemistry **8** (2020) 00412.

[15] B. Syed, N. Karthik, P. Bhat, N. Bisht, A. Prasad & S. Satish, "Phytobiologic bimetallic nanoparticles bearing antibacterial activity against human pathogens", J King Saud Univ - Sci. **31** (2019) 798.

[16] Z. Vaseghi, O. Tavakoli, A. Nematollahzadeh, "Rapid biosynthesis of novel Cu/Cr/Ni trimetallic oxide nanoparticles with antimicrobial activity", J Environ Chem Eng. **6** (2018) 1898.

[17] Ahmad I, Mehmood Z, Mohammad F, "Screening of some Indian medicinal plants for their antimicrobial properties", J Ethnopharmacol. **62** (1998) 183.

[18] Irshad S, Mahmood M, Perveen F, "In-Vitro Anti-Bacterial Activities of Three Medicinal Plants Using Agar Well Diffusion Method", Res J Biol. **2** (2012).

[19] Elango G, Roopan SM, Dhamodaran KI, Elumalai K, Al-Dhabi NA, Arasu MV, "Spectroscopic investigation of biosynthesized nickel nanoparticles and its larvicidal, pesticidal activities", J Photochem Photobiol B Biol. **162** (2016) 162.

[20] Ramyadevi J, Jeyasubramanian K, Marikani A, Rajakumar G, Rahuman AA, Santhoshkumar T, "Copper nanoparticles synthesized by polyol process used to control hematophagous parasites", Parasitol Res. **109** (2011) 1403.

[21] Jaswal VS, Arora AK, Kinger M, Gupta VD, Singh J, "Synthesis and characterization of chromium oxide nanoparticles", Orient J Chem. **30** (2014) 559.

[22] Al-Dhabaan FA, Shoala T, M Ali AA, Alaa M, Abd-Elsalam K, Abd-K, "Chemically-Produced Copper, Zinc Nanoparticles and Chitosan-Bimetallic Nanocomposites and Their Antifungal Activity against Three Phytopathogenic Fungi", Int J Agric Technol. **13** (2017) 753.

[23] J. A. Gutiérrez, S. Caballero, L. A. Díaz, M. A. Guerrero, J. Ruiz & C. C. Ortiz CC, "High Antifungal Activity against Candida Species of Monometallic and Bimetallic Nanoparticles Synthesized in Nanoreactors", ACS Biomater Sci Eng. **4** (2018) 647.