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Value addition of *Coffea canephora robusta* husk as an agro-waste for synthesis of antimicrobial zinc oxide nanoparticles

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Abstract
Aqueous extract of *C. canephora robusta* husk was used to synthesize ZnO nanoparticles as an innovative and sustainable strategy to combat the threat of multi-drug-resistant pathogens. The monsooned variety of Robusta coffee is a geographical indication of the district of Wayanad husk thereby a major agro-waste product produced during the processing and poses a significant environmental threat. Various bioactive molecules present in the coffee husk were thereby an excellent option for the green synthesis of Zinc oxide nanoparticles. An SPR (surface plasmon resonance) band at 374 nm confirmed the presence of zinc oxide. The antibacterial potential was evaluated through micro broth dilution assay and a minimum inhibitory concentration (MIC) of 1000 µg/mL and a minimum bactericidal concentration of 2000 µg/mL against the multi-drug resistant (MDR) test strains was observed. Therefore, the incorporation of nanotechnology to valorize an agro-waste product to combat the threat of MDR- pathogens was found to be effective.

Keywords: Bioactive compounds, coffee husk, multi-drug resistant pathogens, valorisation, zinc oxide nanoparticles

1. Introduction
Worldwide, coffee is cultivated using two varieties of coffee species *Coffea canephora arabica* and *C. robusta* which account for 60 and 40% of coffee production, respectively. Coffee is the most important cash crop cultivated in the tropics since it is the most consumed and traded beverage commodity in India, which is also used for export. Owing to these facts around three-fold increase in the cultivation of coffee has been reported in the Indian subcontinent in the past few years. India is the seventh largest producer accounting for around 3% of global contribution. The monsooned variety of *C. robusta* is predominantly cultivated in the Wayanad district of Kerala and accounts for the major part of agro-waste generated.
This large-scale production imparts an environmental hazard in the form of agro-waste products such as spent beans and coffee husks during post-harvesting processing. Wherein, the coffee husks constitute around 0.5 tons of waste generated with each ton of coffee processed through dry and wet processing. Therefore, in recent times innovative strategies to mitigate the environmental impact through valorisation processes have been an interesting field of research. Another emerging threat to the public health domain is the emergence of multi-drug-resistant (MDR) pathogens and the significant health hazards imposed by them. This threat is signified by the fact that the WHO has included antimicrobial resistance (AMR) and its implications as one of the top ten threats to humanity. Furthermore, it has been estimated that the global death toll due to AMR could reach up to 10 million per year by 2050. The receding antibiotic pipeline and lack of novel sustainable strategies to combat this silent pandemic make the situation more difficult.
The utilization of bioactive compounds obtained from plant extracts for various purposes such as antimicrobial applications has been extensively explored in the context of combating AMR. Many phytochemicals such as caffeic acid, phenolic, and chlorogenic compounds are present in the coffee husk extracts thus making it an excellent candidate. Yet another area that has gained recent interest is the use of nanotechnology to devise a sustainable and novel solution to combat MDR pathogens. Zinc oxide has been widely accepted as a 'generally recognized as safe' (GRAS) material and has shown excellent antibacterial activity as a nanomaterial. Value addition to coffee husks with green synthesis methods using the prospect of nanotechnology and thereby incorporating bioactive molecules present in them to combat the ever-present

threat of multi-drug resistant pathogens could prove to be an innovative strategy to combat AMR.

2. Materials

The *C. robusta* husks were collected after dehulling from a coffee plantation in Muttil, Wayanad, Kerala (11º40'10.4" N; 76º07'20.1" E). The MDR strains of bacteria *Salmonella enterica* Typhimurium, *S. enteritidis*, enteroaggregative *Escherichia coli* (EAEC), and methicillin-resistant *Staphylococcus aureus* (MRSA) maintained in the Zoonoses laboratory of the Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Pookode as a part of the ICAR- NASF funded research project was used for the study.

2.1 Preparation of aqueous extract of *C. robusta*

The *C. robusta* husks were screened for any visible dirt and foreign objects were finely ground to form a fine powder using a mortar and pestle. This powdered coffee husk (10 g) was then transferred to 100 mL of nanopure water (Merck Millipore, USA). The mixture (100 mL) was then kept in a water bath maintained at 60 °C for one hour. Later, all the solid particulates were removed by centrifugation at 3000 rpm for 15 min. The supernatant extract thus obtained was stored at 4 °C until further use (Silva *et al*., 2020) [1].

2.2 Green synthesis of ZnO nanoparticles (NP) prepared using aqueous extract of coffee husk

The synthesis of ZnO NPs was achieved by mixing 0.10 M zinc acetate dihydrate [$Zn(CH3COO)_2(H2O)_2$] solution and coffee husk extract at a 6:1 ratio. The mixture was then continuously stirred at 210 rpm in a magnetic stirrer (Neaution Technologies Pvt. Ltd., India) for 15 min. This solution was transferred into a Teflon-lined sealed stainless-steel autoclave (Shilpent Enterprises Pvt. Ltd., India) and kept in a hot air oven at a temperature of 120 °C for 6 h. Later, after the reaction mixture had cooled down the ZnO NPs formed were washed thrice alternatively in isopropyl alcohol (Loba Chemie) and nanopure water to remove any impurities (Varsha *et al*., 2022) [2]. Finally, the ZnO NPs were air-dried, calcinated at 500 °C in a muffle furnace, and stored at 4 °C until further use (Dat *et al*., 2023) [3].

2.3 UV-Vis spectroscopy ZnO NPs prepared using aqueous extract of coffee husk

The synthesized ZnO NPs were characterized by UV- Vis spectroscopy, obtained ZnO NPs were dissolved in ultrapure water at a 1 mg/mL concentration and scanned within the range of 300 to 500 nm using a UV- Vis spectrophotometer, keeping nanopure water as the blank.

2.4 *In vitro* antimicrobial efficacy of ZnO NPs prepared using aqueous extract of coffee husk

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of ZnO NPs prepared using an aqueous extract of coffee husk against the MDR test strains of EAEC, *S. typhimurium*, *S. enteritidis* and MRSA, were determined by micro broth dilution technique (CLSI, 2020) [4].

Individual test cultures (1 x 10^7 CFU/ml; 100 µL) were co-incubated with serial fold dilution of ZnO NPs prepared using an aqueous extract of coffee husk (1000 to 0.244 µg/ mL; 100 µL) in a 96- well flat- bottom microtiter plate (Tarsons) with cation-adjusted Mueller Hinton broth (CA-MH; HiMedia). After the incubation at 37 °C for 18 to 24 h, the resazurin dye (0.015%; 20 µL) was added to all the wells and incubated at 37 °C for 20 min to determine the reduction dye. The lowest concentration of NPs with no visible bacterial growth was designated as MIC.

Later, to determine the MBC dilution of ZnO NPs prepared using an aqueous extract of coffee husk, 10 µL of the aliquots drawn from each well of the MIC plate indicating no visible growth were inoculated onto the eosin methylene blue (EMB) agar, xylose lysine deoxycholate (XLD) agar and Baird-Parker (BP) agar (HiMedia) supplemented with 100 µg of ampicillin (SRL) as all the four MDR test strains used in this study were ampicillin-resistant (Miles *et al*., 1938) [5]. The lowest concentration of the NPs which revealed 99.90 per cent killing of the test cultures in the selective agar media was determined to be their MBC (NCCLS, 1999) [6].

3. Results and Discussion

3.1 UV- Vis spectroscopy of ZnO NPs prepared using aqueous extract of coffee husk

A broad surface plasmon resonance (SPR) peak observed at 374 nm (Fig.1), suggested the formation of ZnO NPs using the aqueous extract of coffee husk by UV-Vis spectroscopy. The peak observed at 374 nm could be due to the reduction of Zn^{2+} Cations by the biomolecules present in the aqueous extract of coffee husk which validated the presence of zinc oxide NPs (Tsegaye *et al*., 2023) [7]. Furthermore, the presence of a peak at this point could be indicative of collective oscillations of the electrons present in the conduction band of the Zn^{2+} which again revalidates the presence of ZnO NPs (Khan *et al*., 2023) [8].

3.2 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ZnO NPs prepared using aqueous extract of coffee husk

The MIC values of ZnO NPs prepared using aqueous extract of coffee husk against the tested MDR pathogens observed in the assay were 2000 µg/mL, while the MBC values were determined to be 4000 µg/mL (Table 1). In this study, the MBC values obtained were twice greater than the MIC values. Zinc oxide NPs have been commonly found to have antibacterial efficacy against both Gram-positive and Gram-negative bacteria. The major mechanism by which the antibacterial activity is elicited is through the generation of reactive oxygen species such as superoxide ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), superoxide anion ($O^{-}{}_2^{\bullet}$), hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) (Abdelghany *et al*., 2023) [9]. Another proposed mechanism is the damage caused to the bacterial cell wall due to the electrostatic interactions caused by Zn^{2+} cations (Singh *et al*., 2023) [10]. Yet another mechanism is through the intake of Zn^{2+} into the bacterial cytoplasm which can also be detrimental to the survivability of the bacteria by causing osmotic imbalance and thereby inhibiting their growth (Packialakshmi *et al*., 2023) [11]. Furthermore, the bioactive molecules such as caffeic acid, chlorogenic acid, and phenolic compounds from the *C. robusta* husk extract have also been found to contribute towards the antimicrobial activity (Maimulyanti *et al*., 2023 [12]; Okhale *et al*., 2023) [13].

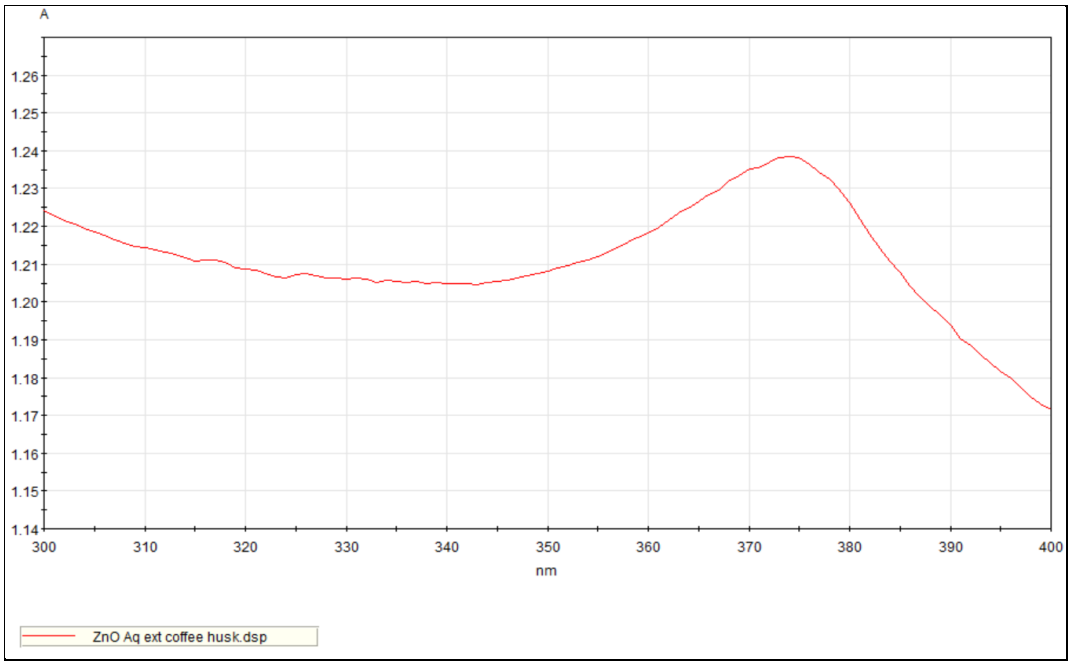

Fig 1: UV- Vis spectroscopy (300-400 nm)

Table 1: MIC and MBC values of ZnO NPs prepared using aqueous extract of coffee husk against MDR test strains

MDR- ISOLATES	MIC (µg/mL)	MBC (µg/mL)
EAEC	2000	4000
S. enteritidis	2000	4000
S. typhimurium	2000	4000
MRSA	2000	4000

4. Conclusion

The synthesis of ZnO NPs using the aqueous extract of *C. robusta* husk had antimicrobial potential. The use of plants and plant extracts has proven to be a safe and eco-friendly approach to nanoparticle synthesis. The value addition to an agro-waste product thereby enabling it to be a sustainable and safe alternative to combat the threat of multi-drug resistant pathogens through nanotechnology could be an interesting area of research.

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6. References

1. Silva MD, Honfoga JN, Medeiros LL, Madruga MS, Bezerra TK. Obtaining bioactive compounds from the coffee husk (*Coffea arabica* L.) using different extraction methods. Molecules. 2020;26(1):46.
2. Unni V, Abishad P, Ram PV, Niveditha P, Yasur J, John L, *et al*. Green synthesis, and characterization of zinc oxide nanoparticles using Piper longum catkin extract and its *in vitro* antimicrobial activity against multi-drug-resistant non-typhoidal Salmonella spp. Inorg. Nano-Met. Chem. 2022;17:1-9.
3. Dat NM, Nam NT, Hai ND, An H, Cong CQ, Dat NT, *et al*. An overall assessment of the pathway procedures, analytical approaches, and potentially essential applications of zinc oxide nanostructured materials. Inorg. Chem. Commun. 2023;12:111430.
4. CLSI [Clinical and Laboratory Standards Institute]. Performance Standards for Antimicrobial Susceptibility Testing. (30th Ed.). Wayne, USA; c2020. p. 214.
5. Miles AA, Misra SS, Irwin JO. The estimation of the bactericidal power of the blood. J Hyg. 1938;38:732-749.
6. NCCLS [National Committee for Clinical Laboratory Standards]. Methods for determining bactericidal activity of antimicrobial agents; approved guidelines. Wayne, USA. 1999;19:14.
7. Tsegaye G, Kiflie Z, Mekonnen TH, Jida M. Synthesis and characterization of coffee husk extract (CHE)-capped ZnO nanoparticles and their antimicrobial activity. Biomass Convers. Biorefin. 2023;26:1-3.
8. Khan AU, Malik N, Singh B, Ansari NH, Rehman M, Yadav A. Biosynthesis, and characterization of Zinc oxide nanoparticles (ZnONPs) obtained from the extract of waste of strawberry. J Umm Al-Qura University for Applied Sciences. 2023;20:1-8.
9. Abdelghany TM, Al-Rajhi AM, Yahya R, Bakri MM, Al Abboud MA, Yahya R, *et al*. Phytofabrication of zinc oxide nanoparticles with advanced characterization and its antioxidant, anticancer, and antimicrobial activity against pathogenic microorganisms. Biomass Convers. Biorefin. 2023;13(1):417-30.
10. Singh N, Singh R, Shah K, Pramanik BK. Green synthesis of zinc oxide nanoparticles using lychee peel and its application in anti-bacterial properties and CR dye removal from wastewater. Chemosphere. 2023;327:138497.
11. Packialakshmi JS, Kang J, Jayakumar A, Park S, Chang Y, Kim JT. Insights into the antibacterial and antiviral mechanisms of metal oxide nanoparticles used in food packaging. Food Packag. Shelf Life. 2023;40:101213.
12. Maimulyanti A. Green Extraction Technique to Separate Bioactive Compounds from Coffee Husk Waste Using Natural Deep Eutectic Solvent (NADES) Based on Choline Chloride-Proline. Available at SSRN 4267841.
13. Okhale SE, Okoli CP, Imoisi C, Aliyu A, Kazeem T, Ogwekpe V. Phytochemical and synergistic antimicrobial effects of Morinda lucida, *Anogeissus leiocarpus* and *Sarcocephalus latifolius* leaf extract. Life Sci. 2023, 20(4).