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Harnessing Plant-Based Nanoparticles for Combatting Multidrug-Resistant Bacteria

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Abstract

The emergence of multidrug-resistant organisms poses a significant challenge to public health worldwide. In response, plant-based nanoparticles have garnered attention for their potential as novel antimicrobial agents. These nanoparticles are derived from various plant sources and exhibit inherent antimicrobial properties due to their phytochemical composition. Nanoparticles synthesized from spinach (*Spinacia oleracea*) and orange (*Citrus sinensis*) extracts, loaded with salts of Cu and Zn, were evaluated for their antimicrobial activity against multidrug-resistant bacteria. Antimicrobial efficacy was assessed through agar well diffusion assays against clinically relevant multidrug-resistant bacterial strains of *Pseudomonas aeruginosa* MTCC 74, *Staphylococcus aureus* MTCC 902, *Klebsiella pneumoniae* MTCC 661. The nanoparticles exhibited potent antimicrobial effects, significantly inhibiting the growth of tested bacteria even when compared to commercially available antibiotics. These findings highlight the potential of plant-based nanoparticles loaded with Cu and Zn salts as effective agents against multidrug-resistant bacteria, offering a promising avenue for future antimicrobial therapies.

Keywords: Nanoparticles, Spinacia oleracea, Citrus sinensis, Multi-drug resistant bacteria, Phytochemicals

Introduction

Nanotechnology is transforming medicine by offering groundbreaking tools and techniques to diagnose, treat, and prevent diseases at the molecular level. Operating on a scale of nanometers (one billionth of a meter), nanotechnology enables precise manipulation of materials to create structures with unique properties. In medicine, this translates to innovations like nanoparticles designed to deliver drugs directly to diseased cells, enhancing treatment efficacy while minimizing side effects.

Over the last few decades, the application of nanotechnology, particularly the use of nanoparticles for drug delivery, has generated significant impact in medicine (Xu et al 2008. Farokhzad OC *et.al.*, 2015). As conventional antibiotics struggle against resistant bacteria, nanotechnology offers innovative solutions by leveraging nanoparticles to enhance antimicrobial efficacy. One of the perks of nanoantibiotics can be the simultaneous mechanisms of action against microbes which would require

high and vigorous gene mutations in the same bacterial cell for antibacterial resistance to develop; therefore, it is difficult for bacterial cells to become resistant to NPs (Wang *et.al.* 2017).This non-traditional method also provides a very less opportunity for pathogens to develop resistance because of availability of different groups and different targets of nanoparticles. Nanoantibiotics represent a cutting-edge approach in combating drug-resistant organisms, which pose a significant global health challenge

These nanoantibiotics can be engineered to overcome the mechanisms that bacteria develop to resist traditional treatments (Hetta et al., 2023). Nanoparticles, typically ranging from 1 to 100 nanometers in size, can be functionalized with antimicrobial agents like antibiotics or antimicrobial peptides. Their small size allows them to penetrate bacterial cell walls more effectively, delivering a concentrated dose of antimicrobial agents directly to the target.

Moreover, nanoantibiotics can be designed to release their antimicrobial payload in a controlled manner, optimizing effectiveness and reducing the likelihood of resistance development. They can also be tailored to specifically target pathogens while sparing beneficial bacteria, minimizing collateral damage to the host microbiome.

In addition to direct antimicrobial action, nanoparticles can synergize with existing antibiotics, restoring or enhancing their efficacy against resistant strains. Furthermore, nanoantibiotics have the potential to overcome biofilm formation, a common defense mechanism of bacteria that complicates treatment.

Overall, nanoantibiotics represent a promising frontier in the fight against drug-resistant organisms, offering targeted and potent solutions to combat infections that are increasingly difficult to treat with traditional antibiotics alone.

Nanoparticles such as silver nanoparticles exhibit strong antibacterial properties against multidrug-resistant pathogens. They can disrupt bacterial cell membranes, inhibit enzyme activity, and interfere with bacterial DNA replication, effectively combating resistant strains (Sondi et al., 2004). Nanoparticles, particularly those functionalized with antibiotics, can bypass or inhibit these efflux pumps, allowing the antibiotics to accumulate inside bacterial cells and exert their antimicrobial effects more effectively (Rai et al., 2014, Jacob rt al., 2012, Jaidev et al., 2010).Nanoantibiotics can act synergistically with conventional antibiotics, restoring or enhancing their activity against resistant bacteria. For instance, nanoparticles can disrupt bacterial biofilms, which are often resistant to antibiotics, thus making the bacteria more susceptible to treatment (Hajipour et al., 2012).

Nano-antibiotics incorporating natural vegetable extracts represent a novel approach to leveraging both traditional medicine and nanotechnology for combating drug-resistant organisms. Nanoantibiotics using Garlic (Allium sativum) extract, particularly allicin, has known antimicrobial properties against a wide range of bacteria, including drug-resistant strains. These nanoantibiotics have shown effectiveness against antibiotic-resistant bacteria such as MRSA (Methicillin-resistant *Staphylococcus aureus*) (Ankri and Mirelman, 1999). Nanoparticles have been formulated with tea tree oil to improve its solubility and bioavailability, enhancing its antimicrobial efficacy against drug-resistant bacteria as *Staphylococcus aureus* and *Escherichia coli* (Carson et al., 2006). Silver nanoparticles functionalized with turmeric extract exhibit enhanced antimicrobial activity against resistant bacteria.These nanoantibiotics have shown promise in treating infections caused by antibiotic-resistant bacteria due to their dual action of silver nanoparticles and curcumin (Prasad et al., 2014, Gulel et al., 2024). Nanoantibiotics with Cinnamon (Cinnamomum verum) extract have demonstrated effectiveness against antibiotic-resistant bacteria such as *Pseudomonas aeruginosa* and *Salmonella typhimurium* (Rao et al., 2016).

Spinach (Spinacia oleracea) and oranges (Citrus sinensis) both possess natural bioactive compounds such as flavanoids, phenolics etc., that confer antibacterial properties, making them potentially valuable in the development of antimicrobial agents. Both of the extracts inhibit the growth of *Escherichia coli* (E. coli) *and Staphylococcus aureus*, both of which can be pathogenic and resistant to antibiotics in certain forms. Incorporating extracts from these sources into nanoantibiotics could enhance their effectiveness against drug-resistant bacteria by leveraging their natural antibacterial mechanisms in a targeted and controlled manner.

In this study we have assessed the effect nanoparticles formulated with Cu and Zn salts functionalized with extracts of spinach and orange against bacterial strains of *Pseudomonas aeruginosa* MTCC 74, *Staphylococcus aureus* MTCC 902, *Klebsiella pneumoniae* MTCC 661, multi drug resistance organisms obtained from clinical isolates.

Materials and methods:

The leaves of spinach (*Spinacia oleracea)* and peels of orange *(Citrus X sinensis*) was collected. The extract was prepared by sun drying. The plant part is separated from the unwanted parts. Washed with tap water followed by distilled water, thereafter washed by 70% ethanol and then paper dried by tissue. The samples were then thoroughly sundried weighed 30gm on the weighing balance and ground to a fine powder. The powder was then dissolved in various solvents such as hexane, petroleum ether, distilled water, acetone and benzene, placed for 24 hrs on a rotary shaker, the aqueous ectracts were the filtered and store in the refridgerator. These prepared extracts were then screened against bacterial strains of *Pseudomonas aeruginosa* MTCC 74, *Staphylococcus aureus* MTCC 902, *Klebsiella pneumoniae* MTCC 661.

Phytochemical analysis: The confirmatory qualitative phytochemical screening of plant extracts was performed to identify the main classes of compounds (tannins, flavonoids, alkaloids, steroids, and terpenoids) present in the extracts following standard protocols (Lawal et al., 2019, Solanki et al., 2019, Wilde et al., 2013)

Test for Terpenoids:

Chloroform and concentrated H_2SO_4 was added to the sample The observation of reddish brownish colour indicates the presence of terpenoids.

Test for Flavonoids:

1ml of extract was dissolved in the diluted NaOH &HCl were added. A yellow solution that turns colourless indicates the presence of flavonoids.

Test for Phlobatannins:

1ml of extract was dissolved in the distilled water and filtered the filtrate was boiled with 2% HCl solution. A red precipitate indicates positive result of the test.

Test for Tannins:

The extract was boiled and filtered. To the extract 1% lead acetate solution was added, the formation of a yellowish precipitate confirms the presence of tannins.

Test for Leucoanthocyanin:

The test is considered positive when isoamyl alcohol reacts with the sample extracts and forms a red precipitate..

Test for Coumarin:

3ml of 10% of NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates the

spresence of coumarin.

Test for Steroids:

1ml of extract was dissolved in 10ml chloroform and equal volume of concentrated H_2SO_4 was added on the sides of tubes. The upper layer turns red & H_2SO_4 layer is yellow with green fluorescence. This indicates the presence of steroids.

Test for Fatty acids:

1ml of extract was mixed with 10ml of ether, the extract was allowed to evaporate on the filter paper. The appearance of the transparency of the filter paper indicates presence of fatty acids.

Salt preparation:

0.5 M salts of copper sulphate, zinc sulphate and lead sulphate was prepared. These salts were prepared at different concentrations (80% - 20%).

Preparation of nano particles:

Centrifuge method: The NPs were prepared by incubating overnight a mixture of the prepared salt and extracts. This was centrifuged thrice and the pellet was collected and dissolved in ethanol.

Magnetic stirrer method: Various concentration of the Cu and Zn salts was prepared by mixing the desired concentration of the salt with the extract. This was incubated overnight, then subjected to heat (60°C) on a magnetic stirrer for 1hr.The rotation of the machine was calibrated time to time to avoid the formation of foam.

Antibiotic Sensitivity Test:

Anti-bacterial test was performed by agar well diffusion method in Mueller Hinton Agar. Wells were punctured and nano-particles prepared were added to the wells along with the 50µl control (extract) and salt solution. Incubated overnight at 37°C and the Zone of Inhibition (ZOI) was measured against the test organisms *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae.*

Minimum Inhibitory concentration:

In the blank set of test tubes, 0.2 ml of antimicrobial extract was added in the first test tube and then serially diluted in 5 tubes. Thus, making the concentration of the extract to decline in the order 10^{-1}, 10^{-2},10^{-3}, 10^{-4},10^{-5}. After the dilution, 20µl of pathogen was added in test tubes. The test tubes were incubated at 24hrs in the shaker incubator. OD was recorded at 620nm. The stoichiometric calculations were used to determine the MIC of the extracts.

Biofilm degradation:

The biofilm of *Pseudomonas aeruginosa* was prepared and then subjected to degradation by keeping it in contact with 10mg of the nano drug i.e. Cu 80% and 50% Cu drug in the tubes to visualize the degradation. The tubes were incubated for 5 days.

Endospore staining and degradation:

Endospores for *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia* were produced and subjected to degradation with the nanoparticles. The spore in the microcentrifuge tube is allowed to interact with the nano drug of 10mg overnight at room temperature. The smear is prepared the next day and stained with malachite green followed by safranin and visualized under microscope.

Comparative analysis:

The nano drug prepared were compared with market drugs *i.e* Norfloxacin, Tetracycline, and Ciprofloxacin

RESULTS:

The samples of *Spinacia oleracea* and *Citrus X sinensis* were collected from the local markets in Chhattisgarh (Figure 1). The samples were sundried and extracted in different solvents (acetone, ether, DW, Benzene, methanol). The solvents alone were tested as a control against *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Klebsiella pneumoniae* multi drug resistance organisms obtained from clinical isolates. None of the solvents showed any zone of inhibition against the test organisms. This indicates that the solvents alone had no inhibitory effect against the tested organisms (Table 2).

The extracts were assessed for the presence of phytochemicals. Both extracts showed the presence of trepenoids and tannins, however orange were positive for flavanoids and coumarin as well (Table 1).

Table 1: Phytochemical analysis of the extracts.

TESTS	SAMPLES	
	Spinach	**Orange**
Terpenoids	+ve	+ve
Flavanoids	-ve	+ve
Phlobatannins	-ve	-ve
Tannins	+ve	+ve
Leucoanthocyanins	-ve	-ve
Coumarin	-ve	+ve
Steroids	+ve	+ve
Fatty acids	-ve	+ve

These extracts were used for the preparation of nanoparticles (NP) for which salts of Cu and Zn was prepared in concentration of 60% and 80%. The salt solutions were combined with the extracts in desired concentrations and incubated overnight. The salt-extract solution was then subjected to the conventional centrifuge method and magnetic stirrer method for preparation nanoparticles. The prepared nanopracticles were evaluated for their antibacterial activity against the test organisms (Table 3)

Fig 1:*Spinacia oleracea* and *Citrus X sinensis*

The combination of phytochemicals from spinach and orange extracts with metal salts (Cu and Zn) appears to enhance the antimicrobial properties of the nanoparticles, possibly through synergistic interactions at certain concentrations. The highest anti-bacterial activity was observed with NP of spinach *i.e.*, 60% Cu-spinach, 80% Cu-spinach, 80% Cu-spinach showing ZOI of 10mm, 20mm and

21mm against *P. aeruginosa, S. aureus* and *K. pneumonia*e respectively followed by 80% Zn-spinach with ZOI of 18.4mm against *K. pneumoniae* and 80% Cu-orange with ZOI of 21mm against *P. aeruginosa* (Table 3). Cu-spinach NPs we which showed antibacterial activity against all the three test pathogen was considered and evaluated as the best candidate nano antibiotic for further analyses.

The Cu-spinach NP were then evaluated in detail firstly by varying the percentage of the Cu-salt used for preparing the NPs. Cu-spinach NPs was prepared with 70%, 60% 50%, 40%,30% and 20 % of cu-salt. The antibacterial activity of these NP's was tested against all the three test isolates. Cu-Spinach (50%, 20%) showed the best antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* followed by Cu-spinach (80%, 60%, 40%) against *Pseudomonas aeruginosa* and *Klebsiella pnemoniae* (Table 4, Fig 2). The Minimum Inhibitory Concentration (MIC) of Cu was determined at 80%, 50% and 20% and was estimated to be 10mg/ml. The Cu-Spinach NPs prepared with 10mg/ml 80%, 50% and 20% Cu salt showed the best antibacterial activity that was estimated by turbidometric analysis.

Table 2: Antibiogram of the different solvents

SR.no	**Pathogen**	**Sample**	**ZOI**				**Result**
			DW	**Acetone**	**Petroleum ether**	**Benzene**	
1.	***S. aureus***	**Spinach**	No zone	No zone	No zone	No zone	-ve
2.	***P. aeruginosa***	**Orange**	No zone	No zone	No zone	No zone	-ve

Table 3: Antibiogram of Spinach and Orange NPs made with the 80% and 60% Cu and Zn salts, against all three test isolates.

S. no	Pathogen	sample	NP (Sample)	SALT (Control)	ZOI	
					NP	SALT
1.	*Pseudomonas aeruginosa*	Spinach	80%Cu	80%Cu	No zone	No zone
			60% Cu	60% Cu	10mm	5mm
			80%Zn	80% Zn	No zone	No zone
			60%Zn	60%Zn	No zone	No zone
		Orange	80%Cu	80%Cu	21mm	13mm
			60% Cu	60% Cu	No zone	No zone
			80%Zn	80% Zn	No zone	No zone
			60%Zn	60%Zn	No zone	No zone
2.	*Staphylococcus aureus*	Spinach	80%Cu	80%Cu	20mm	12.8mm
			60%Cu	60%Cu	No zone	No zone
			80%Zn	80%Zn	10mm	10.6mm
			60%Zn	60%Zn	No zone	No zone
		Orange	80%Cu	80%Cu	14.6mm	17.7mm
			60%Cu	60%Cu	20.3mm	20.8mm
			80%Zn	80%Zn	No zone	No zone
			60%Zn	60%Zn	No zone	No zone

3.	*Klebsiella pnemoniae*	Spinach	80%Cu	80%Cu	21mm	14mm
			60% Cu	60% Cu	No zone	No zone
			80% Zn	80% Zn	18.4mm	12mm
			60%Zn	60%Zn	No zone	No zone
		Orange	80%Cu	80%Cu	No zone	24.0mm
			60% Cu	60% Cu	No zone	No zone
			80% Zn	80% Zn	10 mm	11mm
			60%Zn	60%Zn	No zone	No zone

Table 4: Antibiogram of Cu-Spinach NPs with varying percentage s of Cu salts against all three test isolates.

Pathogen	NP (Sample)	SALT (Sample)	ZOI	
			NP	SALT
Pseudomonas aeruginosa	Spinach	80% Cu	23mm	24.5mm
		70% Cu	22mm	25mm
		60% Cu	24.5mm	23mm
		50% Cu	23mm	24.5mm
		40% Cu	26.5mm	25mm
		30% Cu	19.5mm	24mm
		20% Cu	26mm	23mm
Staphylococcus aureus	Spinach	70% Cu	21.8mm	22.5mm
		60% Cu	34.5mm	34.5mm
		50% Cu	37mm	33mm
		40% Cu	38.5mm	38.5mm
		30% Cu	31.5mm	32mm
		20% Cu	32.5 mm	30.5 mm
Klebsiella pnemoniae	Spinach	80% Cu	34mm	31.5mm
		70% Cu	18.6mm	30.5mm
		60% Cu	31mm	30.5mm
		50% Cu	29.5mm	30mm
		40% Cu	31.5mm	30mm
		30% Cu	19.5mm	24mm
		20% Cu	20mm	23.5

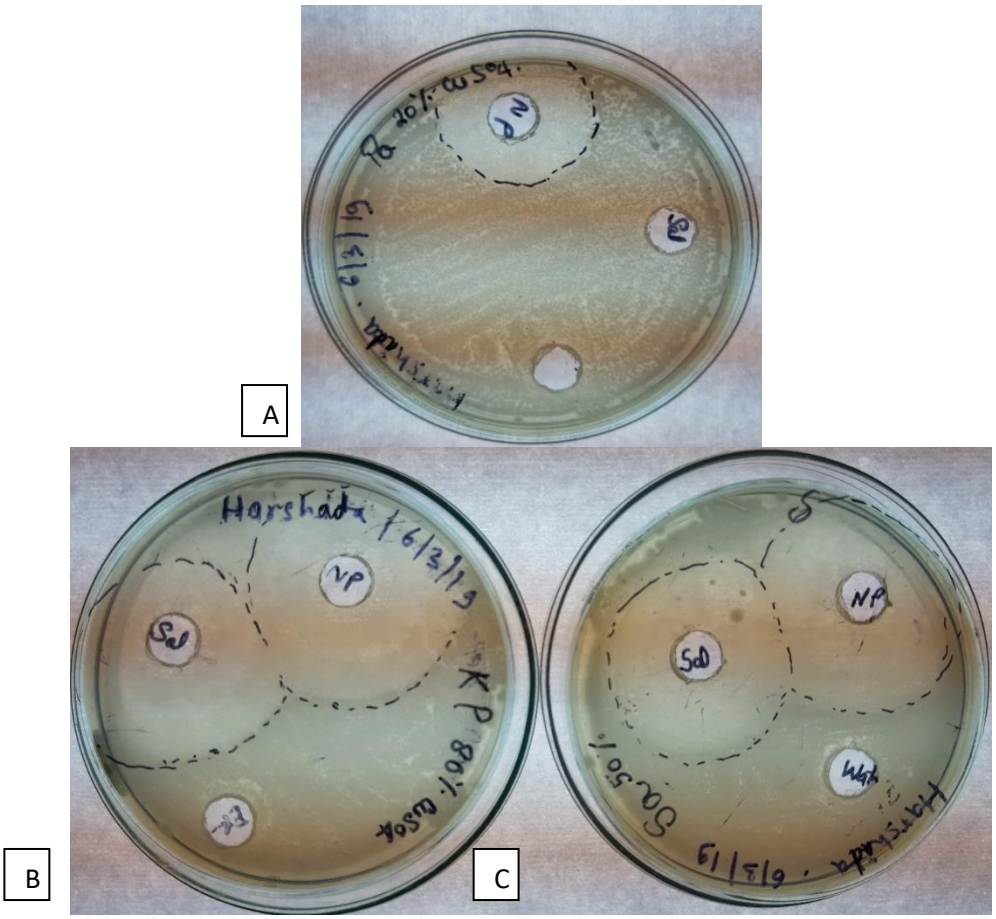

Figure 2: Zone of Inhibition of the best concentrations of the Cu-spinach NPs. A) ZOI of 26mm observed with spinach NP (20%Cu) against *Pseudomonas aeroginosa*, B) ZOI of 34mm observed with spinach NP(80%Cu) against *Klebsiella pnemoniae* C) ZOI of 37mm observed with spinach NP(50%Cu) against *Staphylococcus aureus*.

The Cu-spinach NPs prepared with10mg/ml Cu salt, was tested for their capability to degrade biofilms that were formed by *Pseudomonas aeroginosa.* Partial degradation of the biofilm was observed with 50% and 80% Cu-spinach NPs.

Finally the antibacterial activities of Cu-spinach NPs were compared with antibiotics such as Norfloxacin, Tetracycline and Ciprofloxacin that are commercially available in the market. Cu-spinach 50% and 80% showed a better antibacterial activity with ZOI of 31.5mm and 30mm against *Staphylococcus aureus* and *Klebsiella pnemoniae* compared to the commercial antibiotics tested (Fig.3)

Table 5: Comparison of the Cu-Spinach NP with commercially available antibiotics.

S.no	Pathogen	Nanoparticle	Norfloxacin	Tetracycline	Ciprofloxacin
1.	*Pseudomonas aeruginosa*	20% Cu (15mm)	17mm	26.5mm	16mm
2.	*Staphylococcus aureus*	50% Cu (31.5mm)	20mm	30mm	18.5mm
3.	*Klebsiella pnemoniae*	80% Cu (30mm)	28.5mm	27.5mm	15mm

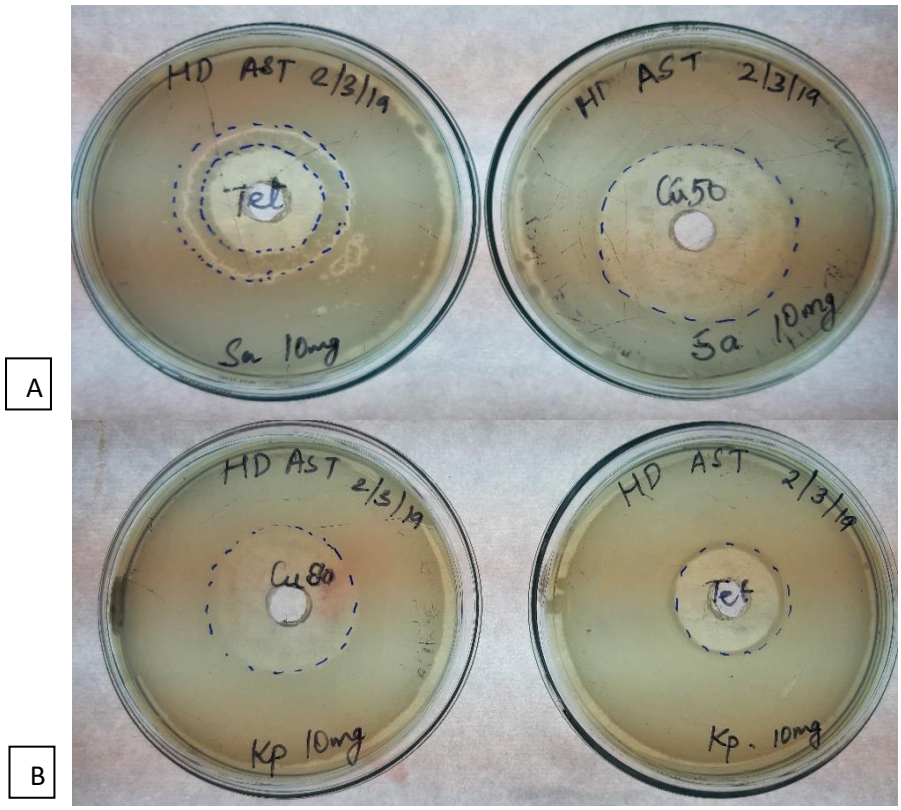

Figure 3: Comparative study of Cu-Spinach NPs against Tetracycline: A) ZOI of Cu-Spinach 50% (10mg) and Tetracycline (10mg) against *Staphylococcus aureus* B) ZOI of Cu-Spinach 80% (10mg) and tetracycline (10mg) against *Klebsiella pnemoniae.* The NP show better antibacterial activity against the test organisms.

Conclusion

The nanoparticles prepared from spinach and orange extracts with metal salts (Cu and Zn) exhibited potent antimicrobial activity against a panel of drug-resistant bacterial strains. This efficacy was comparable to or better than conventional antibiotics such as Norfloxacin, Tetracycline and Ciprofloxacin in inhibiting bacterial growth of multi resistant bacteria of clinical relevance. The combination of phytochemicals from spinach and orange extracts with metal salts (Cu and Zn) appears to enhance the antimicrobial properties of the nanoparticles at certain concentrations. In conclusion, nanoparticles synthesized from spinach and orange extracts, enriched with salts of Cu and Zn, have demonstrated significant potential as nanoantibiotics against drug-resistant organisms. Further research is warranted to optimize nanoparticle synthesis methods, enhance stability and shelf life, explore in vivo efficacy, and investigate potential applications in clinical settings.

References:

1. Xiaoyang Xu, William Ho, Xueqing Zhang, Nicolas Bertrand, Omid Farokhzad, Cancer nanomedicine: from targeted delivery to combination therapy, Trends in Molecular Medicine 2015, 21(4), 223-232.
2. Farokhzad, OC. Nanotechnology for drug delivery: the perfect partnership. Expert Opin Drug Deliv 2008, 5: 927-929.

3. Wang LL, Hu C, Shao LQ, The antimicrobial activity of nanoparticles: present situation and prospects for the future 2017, (12):1227-1249.
4. Sondi, I. and Salopek-Sondi, B. Silver Nanoparticles as Antimicrobial Agent: A Case Study on E. coli as a Model for Gram-Negative Bacteria. Journal of Colloid and Interface Science, 2004, 275, 177-182.
5. Rai Mahendra, Kon Kateryna, Ingle Avinash, & Duran Nelson, Galdiero Stefania & Galdiero Massimiliano, Broad-spectrum bioactivities of silver nanoparticles: the emerging trends and future prospects, Appl Microbiol Biotechnol 2014, 98:1951–1961.
6. Jacob SJP, Finu JS, Narayanan A. Synthesis of silver nanoparticles using Piper longum leaf extracts and its cytotoxic activity against Hep-2 cell line. Colloids Surf B: Biointerfaces 2012. 91:212–214
7. Jaidev LR, Narasimha G. Fungal mediated biosynthesis of silver nanoparticles, characterization and antimicrobial activity. Colloids Surf B: Biointerfaces 2010, 81:430–433
8. Hajipour Mohammad J, Fromm Katharina M, Ashkarran Ali Akbar, Aberasturi Dorleta Jimenez de, Larramendi Idoia Ruiz de, Rojo Teofilo, Serpooshan Vahid, Parak Wolfgang J, Mahmoudi Morteza, Antibacterial properties of nanoparticles, Trends Biotechnol 2012 Oct;30(10):499-511
9. Ankri, S. and Mirelman, D. Antimicrobial Properties of Allicin from Garlic. Microbes and Infection, 1999, 1, 125-129.
10. Carson C F, Hammer K A, Riley T V, Melaleuca alternifolia (Tea Tree) oil: a review of antimicrobial and other medicinal properties, Clin Microbiol Rev, 2006 Jan;19(1):50-62
11. Prasad Sahdeo , Gupta Subash, Tyagi Amit K, Aggarwal Bharat B, Curcumin, a component of golden spice: from bedside to bench and back, Biotechnol Adv 2014 Nov 1;32(6):1053-64.
12. Gulel GT, Kanat S, Kucukgoz E.Antibacterial effect of curcumin on *Salmonella* Typhimurium: *In vitro* and food model studies.Vet Med (Praha). Apr 2024, 26;69(4):115-122.
13. Rao Amanda, Steels Elizabeth , Inder Warrick J , Abraham Suzanne, Vitetta Luis, Testofen, a specialised Trigonella foenum-graecum seed extract reduces age-related symptoms of androgen decrease, increases testosterone levels and improves sexual function in healthy aging males in a double-blind randomised clinical study, Aging Male 2016 Jun;19(2):134-42.
14. Hetta Helal F., Ramadan, Alhanouf I. Al-Harbi Yasmin N., Ahmed Esraa A., Battah Basem, Abd Ellah Noura H., Zanetti Stefania, and Donadu Matthew Gavino Nanotechnology as a Promising Approach to Combat Multidrug Resistant Bacteria: A Comprehensive Review and Future Perspectives, Biomedicines. 2023 Feb; 11(2): 413.
15. Lawal AM, Lawan MM, Apampa SA. Phytochemical analysis and thin layer chromatography profiling of crude extracts from GuieraSenegalensis (Leaves). *J Biotechnol Biomed Sci*. 2019;3(3):7–12.
16. Solanki SL, Modi CM, Patel HB, Patel UD. Phytochemical screening and thin-layer chromatography of six medicinal plants from the surroundings of Junagadh, Gujarat, India. *J Pharmacogn Phytochem*. 2019;8(4):3122–3126.
17. Wilde TJJD, Ben IO, Woode E, Abotsi WKM. Preliminary phytochemical screening and in vitro antioxidant properties of Trichilia monadelpha (Thonn.) JJ De Wilde (Meliaceae). *J Med Biomed Sci*. 2013;2:6–15.