Microbial and antioxidant efficacy of bimetallic copper-zinc nanoparticle using leaf extract of *Lantana camara*

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Abstract

Nanotechnology is becoming increasingly popular and it is currently a well-established subject of interdisciplinary science. Nanoparticles can be manufactured using a variety of processes, but they have a few disadvantages including toxicity. To circumvent the limits of traditional synthesis processes, the attention has switched to a new radius that includes biological entities such as plants, known as green synthesis. Nanotechnology is one of the most important areas of research for the development of environmentally friendly biological processes for nanoparticle synthesis. Copper-Zinc nanoparticles (Cu-Zn NPs) were synthesised at ambient temperature with leaf extract of Lantana camara acting as the reducing agent. Aqueous methanolic extracts were used to perform qualitative screening of phytochemical components which revealed the presence of glycosides, alkaloids, tannins, terpenes, flavonoids, saponins and carbohydrates. Cu, Zn and Cu-Zn NPs were designed with the aim of scavenging free radicals and functioning as antibacterial static agents. Biosynthesized NPs were found to be more effective DPPH radical scavengers than aqueous leaf extract.

Furthermore, the biosynthesized nanoparticles effectively reduced the growth of therapeutically relevant pathogenic Gram-positive bacteria (Staphylococcus aureus, Bacillus cereus, Candida albicans and Bacillus subtilis) as well as Gram-negative bacteria (E.coli and Pseudomonas aeruginosa). According to the study, biosynthesized nanoparticles have a great potential for use in the creation of medications used to treat a variety of ailments, as well as being a prospective contender for numerous medicinal applications.

Keywords: Phytoconstituents, Therapeutically, Biosynthesized.

Introduction

Lantana camara, commonly known as red sage, primarily a native to subtropical and tropical regions, can be found in South India, America and Africa. Numerous chemical components, including flavonoids, steroids, glycosides, triterpenes and essential oils, are abundant in leaves.[18,30] The plant is used to cure skin conditions like dermatitis, itching, scabies, leprosy and chickenpox since it has several therapeutic uses. In the current work, Cu-Zn nanoparticles for antibacterial potential were synthesized using leaf extract. Nanomaterials are nano scale particles that exhibit chemical stability due to their huge surface area to volume ratio, nonlinear optical performance, catalytic reactivity and thermal conductivity.[18]

Researchers focused on metallic nanoparticles because of their unique characteristics such as electrical, catalytic, optical and magnetic properties, which have been shown to be highly dependent on their size and form. The effect of this unique attribute of NPs, which differs from their bulk materials, has been noticed in a variety of disciplines including chemical[40], biomedical[14], agricultural[28] and engineering[24] research.

Nanoparticles are predicted to serve as the foundation for many of this century's technical and biological advancements with specific advantages in physical, chemical and biological aspects. Thus, plant-mediated synthesis, also known as green synthesis, has emerged as the most effective alternative to chemical synthesis. Plants contain the most bioactive organic compounds such as polyphenols, flavonoids, alkaloids, terpenes, tannins, steroids and saponins. These phytochemicals are non-nutritive in nature and are produced by plants as part of their defence mechanism to withstand stress[39]. Copper[7], zinc[8], gold[1,21] and silver are some of the metals utilised in nanotechnology to synthesise nanoparticles.[3,26,42] Zinc is the most deficient micronutrient in soils around the world. In India, 40-42% of cultivated fields are zinc deficient, resulting in a significant drop in output.

As a result, agricultural plants require zinc as a supplement. If crop plants are given Zn in their nano-formulation, their nutrient utilisation efficiency will increase. To be economically viable, nanoparticle production must be both low-cost and environmentally friendly. Cu NPs are highly interacting nanoparticles with a variety of biological properties including antioxidant capacity, antifungal and antibacterial activity, cytotoxicity and drug delivery against malignant and tumour cells. Bacteria, fungi, actinomycetes, algae and plants all produce Cu NPs, either extracellularly or intracellularly[36].

Most of the literature technique for producing Cu NPs, includes sonochemical synthesis, surfactant-based template methods, biosynthesis techniques, hydrothermal ultrasound irradiation, electron beam lithography, copper acetate decomposition, sol-gel, microwave-assisted protocols and solid-state reactions.[16,22,33,41] Furthermore, it has been demonstrated that the morphological characteristics and

toxic behaviour of Cu NPs are influenced by the process of their synthesis.[27] Plants produce a diverse spectrum of secondary metabolites, among them, antioxidants react with free radical and prevent damage before it starts by neutralizing them[30]. Because of this, the current study used the DPPH test and *Lantana camara* produced nano-Cu-Zn to examine the plant's capacity to scavenge free radicals. For the aforementioned reasons, the current study evaluated the antioxidant properties and antibacterial activity of *Lantana camara* leaf extract tested against pathogenic bacteria which included *Staphylococcus aureus*, *E. coli*, *Bacillus cereus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* in order to synthesise Cu-Zn NPs.

Material and Methods

Collecting plants and extractions: Weed was procured by the road side of the National Highway (NH-44) and identified at the Botany Department of Kurukshetra University Kurukshetra, Haryana. The harvested leaves of the plant *Lantana camara* were washed with distilled water and then air/oven dried for five days. After mixing 20g of the sample with 200 ml of deionised water and stirring it for an hour at 60°C, a combination was developed. The leaves extract was filtered using filter paper and filtrate was kept at 4°C for later use.

Synthesis of Cu-Zn bimetallic NPs: The biosynthesis of bimetallic nanoparticles involved the addition 6 gram of zinc nitrate (25mM) and 5 gram of copper sulphate pentahydrate (25mM), dissolved in 90 ml of de-ionized water. This mixture was then incubated at ambient temperature in water bath till solution became uniform. 90 ml of this dissolved copper zinc solution was added drop wise into 10 ml of plant extract and incubated in a water bath at 70°C for one hour. The pH level of the solution was corrected to a value of 8 by introducing a 1molar solution of NaOH. The following NPs were rinsed thrice with ethanol and dried at 90°C incubated in an oven. In the final step, the synthesized NP powder went through calcinations process at 400°C for 4hr.[20]

Phytochemical Screening: 10g of oven-dried leaf powder was placed in a 100ml methanolic solution. The solution was keeping for 72 hours at room temperature. Whatmann no.1 filter paper was used to filter the extracts and the supernatant was then collected. Using a water bath, the solvent was evaporated and the final volume was measured. Phytochemical screening of *L. camara* leaf extract was performed to determine the presence of phytoconstituents. The existence of several phytochemical elements such as amino acid, protein, carbohydrate, alkaloids, saponins, flavonoids, phenolic, tannins, cardiac glycosides, steroids and terpenoids was qualitatively tested using established methods.[10,12]

Collection of microbe: The human pathogenic microorganisms were acquired from the Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology (IMTECH) in Chandigarh. These include *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *E. coli* and *Candida albicans*. Muller Hilton's broth was prepared to preserve cultures. Broth test tubes were refrigerated at 4ºC for future study.

Determination of antimicrobial activity of leaf extract in various organic solvents: *L. camara* leaves solvent extracts were constituted by weighing 10g of leaf powder dissolving in 200ml of different solvents in a sterile test tube and then dry on hot plate, yielding a stock solution concentration of 100mg/100ml extract. The stock solution was kept at room temperature until used[11.]The antibacterial activity of the leaf extracts was assessed using the agar well diffusion method. The impact of different solvent extracts on pathogenic bacterial strains was evaluated. 1mg of each solvent extracts were dissolved in 1ml of DMSO (dimethyl sulfoxide) and approximately 150μl of this solution was loaded on the well. All cultured Petri plates were kept for 24h in incubator at 37°C and the bacterial inhibition zone were recorded in mm.

Determination of Minimum Inhibitory concentration: The minimum inhibitory concentration (MIC) is the lowest concentration required to prevent bacterial growth after 24 hours of incubation. MIC assay was used to analyse antimicrobial activity using micro dilution method. Dilutions of synthesised NPs were generated at various concentrations (mg/ml). To dilute the broth, use a final inoculum of 10^5cfu/ml and apply 1-2µl to the broth media. The minimum inhibitory concentration (MIC) values were established using two-fold serial dilutions.[26]

Determination of Antimicrobial Activity of different nanoparticle and its concentration: The antimicrobial activities of the nanoparticles were assessed using the agar well assay method. The experiment comprised of both Gram-negative and Gram-positive bacterial strains. Microbial inoculum was aseptically disseminated uniformly on the surface of pre-solidified Mueller-Hinton Agar (MHA) plates using a sterile glass spreader. Different concentrations of nanoparticles were dissolved in 1ml of DMSO and approximately 150μl of this solution was impregnated in the well. The well was impregnated with 150μl of copper, zinc and copper-zinc solution. Chloramphenicol was used as a control. An incubation of 37°C was given to cultured plates in an incubator for 24 hours and the microbial inhibition zone was measured and quantified in mm.[13,34]

Determination of Antibacterial Activity of Bandages: The sterile bandages (autoclaved) were cut into 1×2 cm size and impregnated with Cu-Zn solution. Bandages saturated in nanoparticles were dried in the vial at 60°C. The microbial activity of these bandages was tested on Muller Hinton agar medium at 37°C for 24 hours. The inhibition of bacterial growth zones in bandages containing nanoparticle was compared to non-impregnated bandages. The bandages were dipped in 150μl and dried in the vials, resulting in larger than predicted zones.

Antioxidant activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) method: 2,2-diphenyl-1-picrylhydrazyl (DPPH) technique exhibits antioxidant action. The approach was performed in accordance with minor modifications.[4] The capacity of Cu, Zn and Cu-Zn to decrease DPPH (2, 2-diphenyl-picrylhydrazyl) stable free radical, was tested. The antioxidant capabilities of the samples were compared to the standard ascorbic acid. The control solution was DPPH (all reagents except the sample) and the blank solution was methanol. NPs at varied concentrations (10µg, 30µg, 60µg, 90µg, 120µg, 150µg, 180µg and 210µg/ml) were combined with 1ml of freshly made DPPH (1mM in methanol) solution and thoroughly vortexed. The mixture of the solution was left undisturbed in the dark for 30 minutes at 37°C. The samples absorbance was measured at 517nm and DPPH was clearly detectable in the absence of any other standard. The scavenging activity was calculated in percentage using the formula:

$$\% \text{ of antioxidant activity} = [(Ac-As) \div Ac] \times 100$$

where Ac is the absorbance of control and As is the absorption of NPs/vitamin C.

Results

Phytochemical Analysis: The qualitative analysis of the phytochemical components revealed presence of glycosides, tannins, alkaloids, terpenes, flavonoids, saponins and carbohydrate performed using aqueous methanolic extracts as shown in table 1. These phytochemical studies focus on the unique separation of natural chemical elements from plant extracts, used in clinical applications. Methanol found to be the best extractant was opted for phytochemical analysis of phyto components to reveal alkaloid and flavonoids to be potent stabilizing and reducing agent to reduce salt zinc nitrate and copper sulphate to non-ionic or oxide forms in the process of synthesis of Cu-Zn bimetallic nanoparticles.

Rashid et al[36] and Ezebo et al[11] conducted a qualitative phytochemical examination of ethanolic and methanolic extracts of *Lantana camara* leaf, which revealed the presence of saponins, flavonoids, tannins, alkaloids and terpenoids. Similar to present study. Palei et al[33] results showed that alkaloids, terpenoids, phenolic, coumarine, tannin, phlobetanin, saponins and flavonoids were present in leaf extracts consistent with our findings.

Antimicrobial activity: Antimicrobial activities are inherent part of any medicinal plant exploited for drug discovery and the secondary metabolites along with phytochemicals or bioactive components that offer certain medicinal properties of plant to act against various ailments. Leaves extracted from the plant (*L. camara*) have to be effective antimicrobial agent to act against both bacterial and fungal pathogens. *Lantana camara* plant belonging to family *Verbenaceae* has been used as potent agent against standard pathogens which include *C. albicans*, *S. aureus*, *B. subtilis*, *B. cereus*, *E. coli* and *P. aeruginosa.* Leaf extract of the plant was utilized to synthesize bimetallic nanoparticles which include Cu and Zn nanoparticle. The standard drug used as control was chloramphenicol.

The results obtained using both original plant extract from leaves also serve as control and for further validation, we opted Cu-Zn solution (salt solution) also as control to reveal effect of synthesized bimetallic nanoparticle in comparison to opted control solutions. The plates prepared using MHA were kept for 3 days in laminar air flow chamber to avoid any contamination in plates owing to sterilization method and handling. The plates were punctured using tips after spread of respective bacteria. The extracts prepared of control, standard drug, Cu-Zn solution and Cu-Zn nanoparticles were poured at a dose of 150µl (as final capacity) into the punctured hole. The plates were incubated in incubator shaker at 37°C for overnight and then zone of incubation were measured using standard scale as minimum or maximum against all pathogens selected individually.

The source being vast including air, water, soils serve as minerals zones for *Bacillus* bacterial isolation. The present research resulted in potent zones against *B. subtilis* bacteria. The control (chloramphenicol) resulted in a zone of 21mm which was lesser as compared to *E. coli*. However, monometallic Cu and Zn nanoparticles produced 14mm and 12mm zone respectively.

Table 1
Phytochemical screening of aqueous leaf extract of *Lantana camara*

S.N.	Name of Phytochemicals	Presence(+)/ Absence(-)
1.	Amino acids	-
2.	Proteins	-
3.	Carbohydrates	+
4.	Alkaloids	+
5.	Cardiac glycosides	+
6.	Flavonoids	+
7.	Saponins	+
8.	Tannins	+
9.	Terpenoids	+
10.	Phenol	+

The salt solution also taken as an extra control resulted in 17mm zone only. The bimetallic nanoparticles synthesized salt solution and leaf extract of plant resulted in potent zone of 28mm against *B. subtilis*. Against *E. coli* bacteria, the poured sample resulted in different inhibition zones, a zone of of 27mm against chloramphenicol, while 33mm (maximum) zone was observed using Cu-Zn (bimetallic) nanoparticles. The control sample produced a bigger inhibition zone of 27mm against *E. coli* in comparison to salt solution of Cu-Zn bimetallic which resulted in a zone of 23mm.

Highest zone of 34mm inhibition was observed using bimetallic nanoparticle against *Bacillus cereus*. The inhibition zone was highest among all other zones obtained against other bacteria ranging from a minimum of 19mm against *P. aeruginosa* to 33mm against *E. coli*. Monometallic Cu nanoparticles produced a zone of 14mm which was the smallest of all. In comparison, Monometallic Zn nanoparticle resulted in almost similar zone of 12mm. The salt solution taken gave a zone of 15mm which is higher than even monometallic nanoparticle. The control also gave a significant zone of 31mm higher of all zones against different bacteria.

A zone of 19mm was obtained using bimetallic nanoparticle which was minimum of all zones obtained using bimetallic nanoparticles. So it is least effective against *P. aeruginosa*. In comparison, a similar zone of inhibition of 18mm was obtained using salt solution of Cu-Zn. The chloramphenicol taken as control however resulted in a zone of 28mm which was effective. Monometallic Cu and Zn nanoparticle resulted each 14mm zones which were lesser effective than plant extract and Cu-Zn salt solution.

S. aureus is a multidrug resistant pathogen and most of the reports published revealed the bacteria to become resistant to a large no. of antibiotics consumed by affected patients irrespective of specificity concentration and doctor prescription. The control chloramphenicol resulted in significant zone of 27mm against *S. aureus* while monometallic Copper and Zn nanoparticle resulted in 13mm and 12mm zones respectively. In comparison to monometallic nanoparticles, the bimetallic nanoparticles can serve as target drugs to combat ailments which are caused by *S. aureus*. But the toxicity and concentration of nanoparticles have to verify before testing on human beings.

The bimetallic and monometallic nanoparticles are quite effective against all bacterial pathogens irrespective of their being Gram negative and positive bacteria. So the sample prepared was tried against *C. albicans* to test their efficacy other than bacterial pathogens. A zone of 26mm almost equivalent to that obtained against *E. coli* and *S. aureus* was observed against *C. albicans* while in comparison, the monometallic Cu, Zn nanoparticles were observed to get restricted 12mm and 13mm respectively. The solution prepared by mixing Cu-Zn salt resulted in a zone of 19mm and in contrast, the Cu-Zn bimetallic nanoparticle synthesized resulted in zone of 30mm which again revealed the effectiveness of bimetallic nanoparticle against *C. albicans* caused ailments.

A zone size of 2mm is considered as significant and our nanoparticle resulted in higher zones as in figures 1, 3 and table 2. The results of the study suggested that the synthesized NPs showed potential antibacterial effects against difference between Gram positive and negative *Candida albicans, S. aureus, Bacillus cereus, Bacillus subtilis* and *E. coli and Pseudomonas aeruginosa*.

The Gram-negative bacteria have an outer membrane composed of negatively charged lipopolysaccharides molecules. The synthesized nanomaterial passes through the cell wall of the bacteria and damages from the interior. The physical interaction between bacterial cells and synthesised NPs disrupted the cell wall structure, resulting in breakdown and ultimately bacterial death. The nanoparticles penetrating the thick peptidoglycan layer of bacteria (Gram positive) while the Gram negative lack such thick layer to have drastic effect on their thin peptidoglycan layer. Cu-Zn nanoparticles had the significant microbial activity against all the tested microbes when compared to any other nanoparticle solvent.

The Cu-Zn nanoparticles exhibited the highest activity against *Bacillus cereus* and *E. coli* resulting in zones of 34mm and 33mm at 150μl volume of extract.

Table 2
Inhibition zone diameters (in mm) of control Cu, Zn, Cu-Zn solution, Cu-Zn solution with plant extract nanoparticles from *Lantana camara* tested against six pathogens.

Bacteria pathogens taken			Volume of nanoparticles solution(100/150µl)					
		Control	Cu	Zn	Cu-Znnps, 100 µl	Cu-Znnps 150µl	Cu-Zn solution without extract 100µl	150µl
1.	*E. coli*	27mm	13mm	11mm	16mm	23mm	20mm	33mm
2.	*Candida albicans*	26mm	12mm	13mm	22mm	19mm	24mm	30mm
3.	*Staphylococcus aureus*	27mm	13mm	12mm	14mm	20mm	16mm	29mm
4.	*Bacillus cereus*	31mm	11mm	11mm	21mm	15mm	22mm	34mm
5.	*Bacillus subtilis*	21mm	14mm	12mm	26mm	17mm	28mm	28mm
6.	*Pseudomonas aeruginosa*	28mm	14mm	14mm	14mm	18mm	14mm	19mm

S. aureus and *Bacillus subtilis* were also more vulnerable to Cu-Zn mixed nanoparticles with 29mm and 28mm zone of inhibition at 150 µl volume of extract. In contrast, other nanoparticles like Cu, Zn individually were less effective to all bacteria. If we compare the result with control, the Cu-Zn mixed nanoparticle showed highest antibacterial activity. Atacan et al[2] and Kyene et al[25] using ZnO NPs from *Cassia sieberiana* demonstrated a considerable zone of inhibition for *S. typhi* (20.0mm) and *S. aureus* (24.0mm), but less for *E. coli* (13.7mm) and *C. albicans* (11.7mm).

Kasthuri et al[18] and Hasanin et al[13] reported that nanocomposite displayed antibacterial activity against *Bacillus subtilis* (23mm), *Escherichia coli* (19mm), *Staphylococcus aureus* (16mm) and *Candida albicans* (16mm), with MICs of 7.81, 31.25 and 62.5µg ml^{-1} respectively. Tiwari et al[40] reported zone of Inhibition of diameter values 35.2 mm ± 0.9, 23.6 mm ± 0.1 and 13.5 mm ± 0.1 and the Zn NPs were highly effective against *S. Aureus* using leaf extract *Calendula officinalis.*[39]

Antibacterial activity of organic solvent: *L. camara* possessed numerous secondary metabolites which were evaluated using different solvent extracts against bacterial strains. Our findings showed that all solvent extracts of L. camara were efficient against both Gram positive and Gram negative bacterial strains examined, while their efficiency varied. The methanolic extract obtained resulted in a range of zone of inhibition with a minimum of 13mm against *B. cereus* and a maximum 21 against *E. coli* which resulted in a zone of 18mm against *S. aureus, C. albicans* and *P. aeruginosa*. A similar zone of 14mm was observed against *B. subtilis*. Acetone extracts resulted in zones of 19mm, 19mm, 22mm, 11mm, 12mm and 18mm against *E. coli, C. albicans, S. aureus, B. cereus, B. subtilis and P. aeruginosa* respectively.

Butanol extracts resulted in zones of 23mm, 20mm, 17mm, 11mm, 15mm and 15mm against *E. coli, C. albicans, S. aureus, B. cereus, B. subtilis and P. aeruginosa* respectively. Acetonitrophenol extracts resulted in zones of 12mm, 13mm, 18mm, 13mm, 12mm and 16mm against *E. coli, C. albicans, S. aureus, B. cereus, B. subtilis and P.aeruginosa* respectively in fig. 2 and table 3. In contrast, chloroform extract was less effective to all bacteria. *L. camara* is used in many regions of the world to treat a variety of human diseases.

Our reports support the findings of Ezebo et al[11] and Davydova et al[9] who revealed that ethanolic extract showed highest inhibition against *V. cholerae* (17.14+ 0.06) and the lowest inhibition was observed on *E. coli* (12.08+ 0.22). The methanol extract showed highest inhibition of *V. cholerae* (13.19+ 0.06) and lowest inhibition of *M. luteus* (9.22+ 0.09). Borge et al[6] and Natrayan et al[31] studied antimicrobial activity of different extracts obtained against *E. coli*, no significant differences were observed between methanol (16.7 mm), ethanol (16.7 mm), acetone (14.7 mm) and hexane (12.0 mm) ($p > 0.05$).

Antibacterial activity of Bandages: Cu-Zn bimetallic nanoparticles prepared using plant extract of *Lantana camara* were tested for their antibacterial activity by dipping the cotton bandages in bimetallic solution 150µl in a closed Erlenmeyer flask to avoid evaporation of nanoparticle solution as in fig. 4 and table 4. The bandages were dried inside the flasks and then tested against standard pathogens. The dose was kept same (60µl) for all bacterial pathogens to avoid dose dependent effects. The bandages were kept folded on bacterial culture plates and zones were measured around bandages as minimum and maximum. A range of minimum zone of 27mm which it in its own, is a significant zone of inhibition against *C. albicans* to 33mm.

A maximum was observed against *P. aeruginosa*. As *P. aeruginosa* causes a number of skin diseases, bandages coated in bimetallic nanoparticles have the potential to revolutionize the health/medical sector. Bandages showed a 28mm zone against *E. coli* Gram negative bacteria, while a similar zone of 27mm was observed against *C. albicans*. A bigger size zone of 29mm was obtained against *S. aureus* and *B. subtilis* (both Gram positive bacteria).

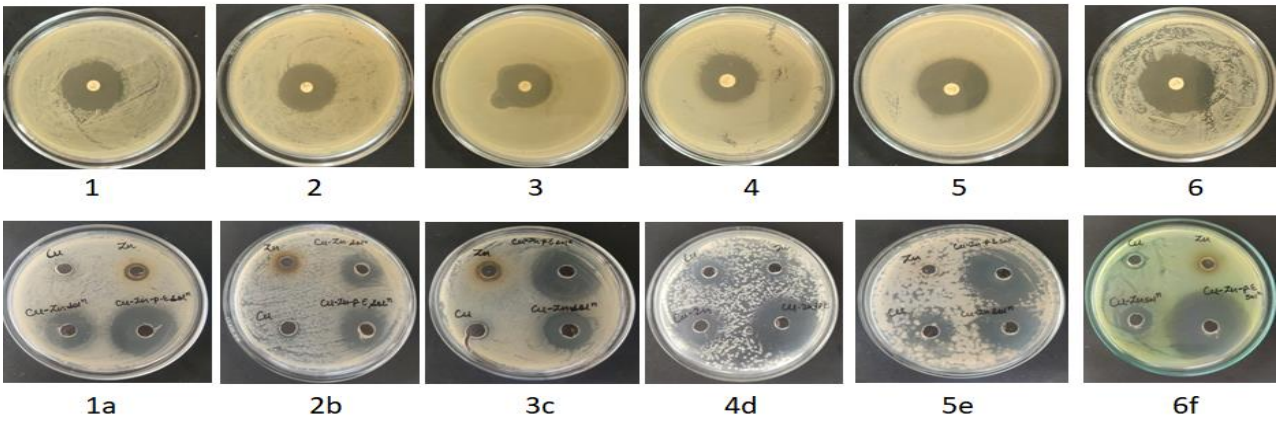

Figure 1: Zones of inhibition using Cu, Zn, Cu-Zn solution, Cu-Zn nanoparticles from *Lantana camara* leaf extracts tested against six pathogens. (1-6 control), (1a-6f Nps solution) 1, a) *B. cereus* 2, b) *S. aureus* 3, c) *C. albicans* 4, d) *B. subtilis* 5, e) *E. coli* 6, f) *P. aeruginosa* respectively.

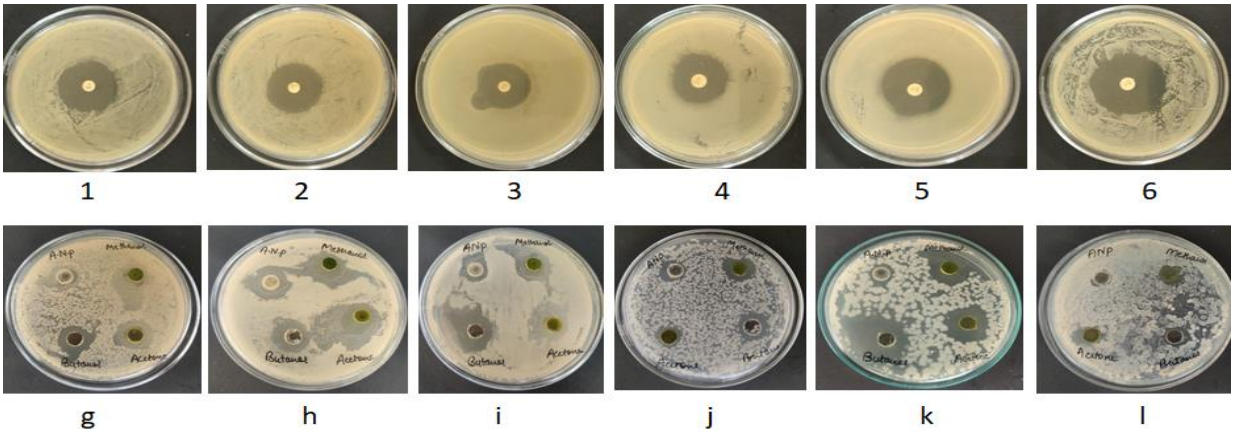

Figure 2: Zones of inhibition using leaf extract (*Lantana camara*) in different organic solvent tested against six pathogens (1-6 control), (g-l solvents). 1,g) *B. cereus* 2,h) *S. aureus* 3,i) *C. albicans* 4,j) *B. subtilis* 5,k) *E. coli* 6,l) *P. aeruginosa*

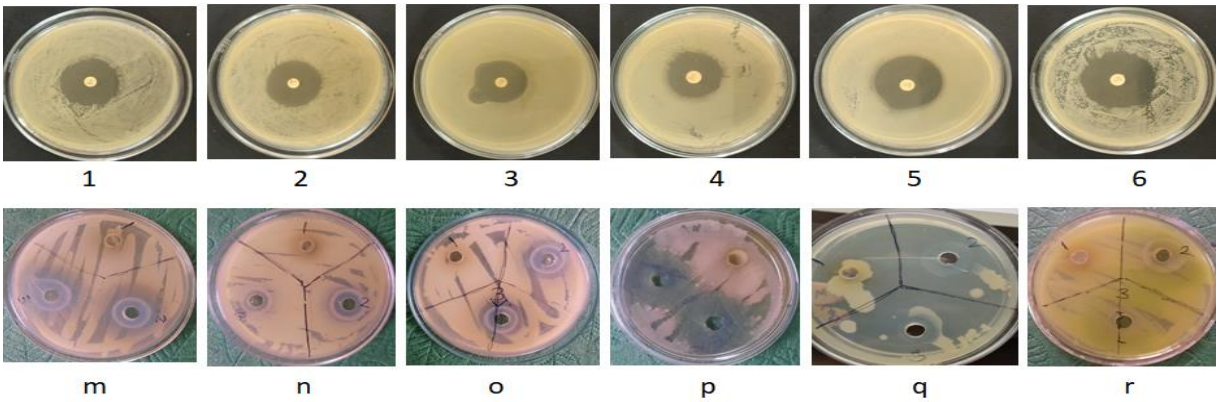

Figure 3: Zones of inhibition plant extract, Cu-Zn solution, Cu-Zn nanoparticle tested against six pathogens. (1-6 control), (m-r solutions) 1,m) *B. cereus* 2,n) *S. aureus* 3,o) *C. albicans* 4,p) *B. subtilis* 5,q) *E. coli* 6,r) *P. aeruginosa*

Table 3
Inhibition zone diameters (in mm) using leaf extract (*Lantana camara*) in different organic solvent tested against six pathogens.

Bacteria pathogens taken		Volume of different solvent (150µl)				
		Control	Methanol	Acetone	Butanol	Acetonitrophenol
1.	*E. coli*	-	21mm	19mm	23mm	12mm
2.	*Candida albicans*	-	18mm	19mm	20mm	13mm
3.	*Staphylococcus aureus*	-	18mm	22mm	17mm	18mm
4.	*Bacillus cereus*	-	13mm	11mm	11mm	13mm
5.	*Bacillus subtilis*	-	14mm	12mm	15mm	12mm
6.	*Pseudomonas aeruginosa*	-	19mm	18mm	15mm	16mm

Table 4
Inhibition zone diameters (in mm) dipped bandage in Cu-Zn nanoparticles solution against six pathogens.

S.N	Bacteria pathogens taken	Control	Bandage
1.	*E. coli*	-	28mm
2.	*Candida albicans*	-	27mm
3.	*Staphylococcus aureus*	-	29mm
4.	*Bacillus cereus*	-	31mm
5.	*Bacillus subtilis*	-	29mm
6.	*Pseudomonas aeruginosa*	-	33mm

In contradiction, a highest zone of inhibition was observed against *P.aeruginosa* resulting in 33mm size zone of inhibition which serves the purpose of study to prepare more efficient bandages doped with bimetallic nanoparticle solution and showing bigger zone of inhibition in comparison to normal bandages available on counter in

maximum medical shops. The Cu-Zn nanoparticles exhibited the potent activity against *Bacillus cereus* and *Pseudomonas aeruginosa* observed at 31mm and 33mm zone at 150μl. *S. aureus* and *Bacillus subtilis* were also more susceptible to Cu-Zn nanoparticles bandage with 29mm and 29mm of inhibition zone at 150μl concentration.

Results demonstrated that nanocomposite coated cotton gauze has a 78% increase in drying time and water absorbency (38%). Khatami et al[22] and Kumar et al[24] reported cotton wound bandages impregnated with nanoparticles of Ag and ZnO and mixed Ag/ZnO nanoparticles. Antimicrobial effect of bandages impregnated with liquid solution of Ag nanoparticles was more than that observed for ZnO and mixed Ag/ZnO nanoparticles; however, this difference was not very significant.

Antioxidant activity: The antioxidant potency of the plant extract, Cu, Zn, Cu-Zn bimetallic nanoparticle and ascorbic acid was used as a standard for DPPH assay. The scavenging activity of the NPs increased with increasing concentrations of NPs. The antioxidant activity of plant extract was observed to be 11.12%, 28.49%, 33.32%, 39.74%, 47.54%, 51.0%, 55.4%61.2% at 10µg, 30µg, 60µg, 90µg,120µg, 150µg, 180µg and 210µg/ml respectively. Similar to plant extract, Cu, Zn, Cu-Zn bimetallic nanoparticles also showed variation in percentage inhibition and was dose dependent observed to be (11.9%, 20.99%, 27.58%, 35.27%, 42.7%, 48.5%, 53.2%, 60.1%), (28.87%, 29.08, 33.91%, 35.04%, 47.89%, 53.9%, 59.2%, 65.4%), (40.80%, 43.37%, 54.83%, 61%, 67.23%, 72.4%, 75.9%, 83.1%) in figure 5. The investigation confirmed that the antioxidant efficacy of NPs was concentration-dependent.

Suresh et al[39] discovered that Ag-ZnO NPs had a higher DPPH free radical activity of 340 (IC_{50} µg/ml) than ascorbic acid, which had 289.74 (IC_{50} µg/ml). Ag-ZnO NPs and ascorbic acid have hydroxyl radical scavenging capabilities of 344.35 (IC_{50} µg/ml) and 316.10 (IC_{50} µg/ml) respectively.

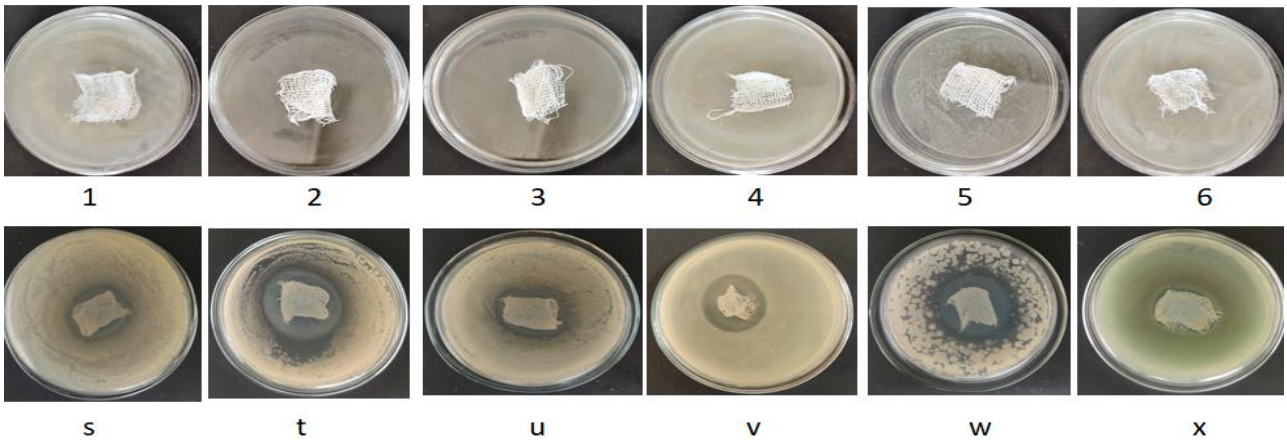

Figure 4: Zones of inhibition Cu-Zn dipped bandage tested against six pathogens. (1-6 control), (m-r solution dipped bandages) 1,s) *B. cereus* 2,t) *S. aureus* 3,u) *C. albicans* 4,v) *B. subtilis* 5,w) *E. coli* 6,x) *P. aeruginosa*

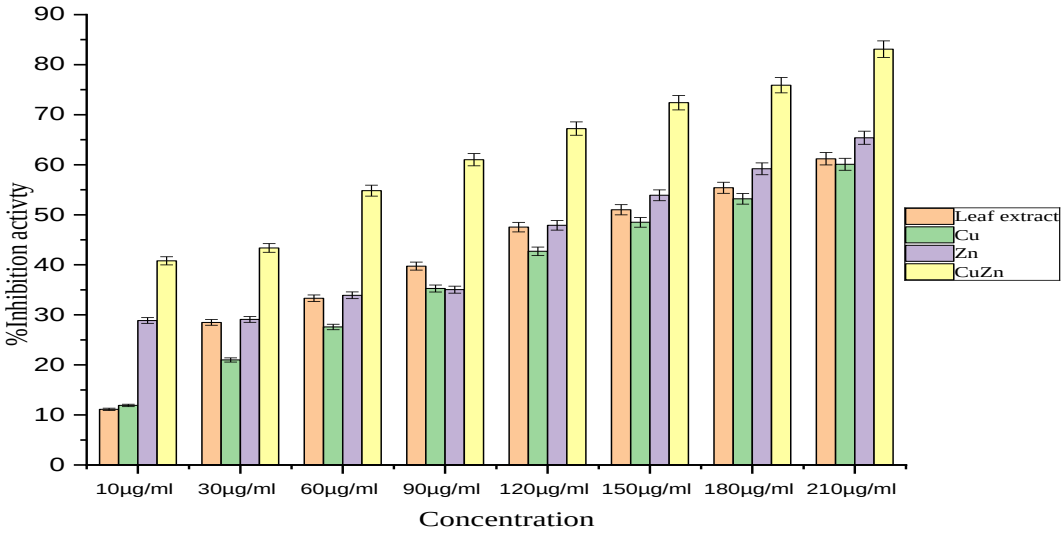

Figure 5: DPPH scavenging activity of plant extract, Cu, Zn, Cu-Zn nanoparticles

Hayat et al[14] found antioxidant activity against 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid [ABTS] at high concentrations of ZnO-NPs (400 µg/mL) biosynthesised from *F. officinalis* (41.67%) and *P. harmala* (39.79%). Lower activity was recorded at a minimum concentration of 50µg/ml of ZnO-NPs biosynthesised from *F. officinalis* (30.34%) and *P. harmala*.[34]

Conclusion

The current study clearly showed that the phytochemical composition of the leaf extract of *Lantana camara* varied depending on the solvent used. Compared to other solvents, the leaf extract extracted in methanol solvent had a higher concentration of extractable metabolites. Furthermore, due to variations in their phytochemical makeup, all solvent extracts exhibited significant antioxidant and antibacterial activity with varied discrepancies. Because it contains regulated reducing and stabilising agents, the leaves extract of *Lantana camara* is confirmed to be the ideal choice for the biosynthesis of copper zinc nanoparticles.

In comparison to the aqueous leaf extract, the biosynthesised Cu-Zn bimetallic nanoparticles demonstrated a potent DPPH radical scavenger. Gram-negative bacteria were more vulnerable to the antimicrobial activity of the copper zinc nanoparticles (NPs) produced using this green chemistry approach than Gram-positive bacteria. The produced gold nanoparticles had strong antioxidant and antibacterial activities, according to the data. According to the study, biosynthesised gold nanoparticles show great promise as a prospective contender for numerous medicinal applications and as a means of treating a wide range of illnesses.

References

1. Aljabali A.A., Akkam Y., Al Zoubi M.S., Al-Batayneh K.M., Al-Trad B. and Abo Alrob O., Synthesis of gold nanoparticles using leaf extract of Ziziphus zizyphus and their antimicrobial activity, *Nanomaterials*, **8**, 174 **(2018)**

2. Atacan K., Güy N., Ozmen M. and Özacar M., Fabrication of silver doped different metal oxide nanoparticles and evaluation of their antibacterial and catalytic applications, *Appl. Surf. Sci. Adv.*, **6**, 100156 **(2021)**

3. Behravan M., Panahi A.H., Naghizadeh A., Ziaee M., Mahdavi R. and Mirzapour A., Facile green synthesis of silver nanoparticles using Berberis vulgaris leaf and root aqueous extract and its antibacterial activity, *Int J Biological Macromol*, **124**, 148–154 **(2019)**

4. Bhakya S., Muthukrishnan S., Sukumaran M. and Muthukumar M., Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity, *Appl. Nanosci.*, **6**, 755–766 **(2016)**

5. Bhusal M., Pathak I., Bhadel A., Shrestha D.K. and Sharma K.R., Synthesis of silver nanoparticles assisted by aqueous root and leaf extracts of Rhus chinensis Mill and its antibacterial activity, *Heliyon*, **10(13)**, 1-13 **(2024)**

6. Borges I., Henriques P.C., Gomes R.N., Pinto A.M., Pestana M., Magalhaes F.D. and Goncalves I.C., Exposure of smaller and oxidized graphene on polyurethane surface improves its antimicrobial performance, *Nanomaterials*, **10(2)**, 349 **(2020)**

7. Chung I.M., Rahuman A.A., Marimuthu S., Kirthi A.V., Anbarasan K. and Padmini P., Green synthesis of copper nanoparticles using Eclipta prostrata leaves extract and their antioxidant and cytotoxic activities, *Exp Ther Med*, **14**, 18–24 **(2017)**

8. Daphedar A. and Taranath T.C., Green synthesis of zinc nanoparticles using leaf extract of Albizia saman (Jacq.) Merr. and their effect on root meristems of Drimia indica (Roxb.) Jessop, *Caryologia*, **71**, 91–102 **(2018)**

9. Davydova L., Menshova A., Shumatbaev G., Babaev V. and Nikitin E., Phytochemical Study of Ethanol Extract of Gnaphalium uliginosum L. and Evaluation of Its Antimicrobial Activity, *Antibiotics*, **13(8)**, 785 **(2024)**

10. Dejene A., Bogale R.F., Yadeta L., Gendo K.M., Kenasa G. and Berhanu A.L., Biogenic synthesis of copper oxide nanoparticles using Clausena anisata leaf and Euphorbia abyssinica bark extracts and its comparative study of antibacterial activities, *Results Chem.*, **8**, 101569 **(2024)**

11. Ezebo R.O., Okonkwo C.C., Ozoh C.N., Nwankwo C.A., Nwafor E.C., Esimai B.G. and Obienyem J.N., Phytochemical Screening and Antimicrobial Activity of Ethanol and Methanol Extracts of Lantana camara Leaf, *World News Nat. Sci.*, **37**, 151–163 **(2021)**

12. Harborne J.B., The biochemical systematics of flavonoids, In the flavonoids, Springer, US, 1056–1095 **(1975)**

13. Hasanin M.S., Hashem A.H., Al-Askar A.A., Haponiuk J. and Saied E., A novel nanocomposite based on mycosynthesized bimetallic zinc-copperoxide nanoparticles, nanocellulose and chitosan: characterization, antimicrobial and photocatalytic activities, *Electron. J. Biotechnol*, **65**, 45-55 **(2023)**

14. Hayat K., Din I.U., Alam K., Khan F.U., Khan M. and Mohamed H.I., Green synthesis of zinc oxide nanoparticles using plant extracts of Fumaria officinalis and Peganum harmala and their antioxidant and antibacterial activities, *Biomass Conv. Biorefin.*, **15(6)**, 1–15 **(2024)**

15. Iravani S. and Varma R.S., Sustainable synthesis of cobalt and cobalt oxide nanoparticles and their catalytic and biomedical applications, *Green Chem*, **22(9)**, 2643–2661, https://doi.org/10.1039/D0GC00885K **(2020)**

16. Kachare K., Shendage S., Matwal S., Walvekar M., Vhanbatte S., Chang J.Y. and Ghule A., Bio-mediated synthesized zinc oxide coated on cotton fabric for antibacterial and wound healing application, *Surf. Coat. Technol.*, **491**, 131171 **(2024)**

17. Karagoz Y., Attar A. and Arbak A., Heat transfer performance of sol-gel synthesized CuONP-doped coolant in diesel engines, *Case Stud. Therm. Eng.*, **37**, 102264, https://doi.org/10.1016/j.csite.2022.102264 **(2022)**

18. Kasthuri K., Kumar J.K., Rajkumar P., Kalpana S. and Balasubramani V., Feasible antibacterial nanomaterials: Copper oxide nanoparticles synthesized using dandelion leaf extract, *Inorg. Chem. Commun.*, **167**, 112804 **(2024)**

19. Khan M., Mahmood A. and Alkhathlan H.Z., Characterization of leaves and flowers volatile constituents of Lantana camara growing in central region of Saudi Arabia, *Arabian J Chem*, **9**, 764–774 **(2016)**

20. Khan S.T., Musarrat J. and Al-Khedhairy A.A., Countering drug resistance, infectious diseases and sepsis using metal and metal oxides nanoparticles: current status, *Colloids Surf. B: Biointerfaces*, **146**, 70–83 **(2016)**

21. Khatak S., Wadhwa N. and Jai, Monometallic zinc and bimetallic Cu-Zn nanoparticles synthesis using stem extracts of Cissus quadrangularis (Haddjod) and Proneness as alternative antimicrobial agents, *Biosci Biotechnol Res Asia*, **17(4)**, 763-774 **(2020)**

22. Khatami M., Varma R.S., Zafarnia N., Yaghoobi H., Sarani M. and Kumar V.G., Applications of green synthesized Ag, ZnO and Ag/ZnO nanoparticles for making clinical antimicrobial wound-healing bandages, *Sustainable Chem. Pharm*., **10**, 9–15 **(2018)**

23. Kumar P.V., Kala S.M. and Prakash K.S., Green synthesis of gold nanoparticles using Croton Caudatus Geisel leaf extract and their biological studies, *Mater Lett*, **236**, 19–22 **(2019)**

24. Kumar S.V., Bafana A.P. and Pawar P., Optimized production of antibacterial copper oxide nanoparticles in a microwave-assisted synthesis reaction using response surface methodology, *Colloids Surf A Physicochem Eng Asp*, **573**, 170–178, https://doi.org/10.1016/j.colsurfa.2019.04.063 **(2019)**

25. Kyene M.O., Droepenu E.K., Ayertey F., Yeboah G.N., Archer M.A., Kumadoh D. and Appiah A.A., Synthesis and characterization of ZnO nanomaterial from Cassia sieberiana and determination of its anti-inflammatory, antioxidant and antimicrobial activities, *Scientific African*, **19**, e01452 **(2023)**

26. Lu Q., Eid K., Li W., Abdullah A.M., Xu G. and Varma R.S., Engineering graphitic carbon nitride (g-C3N4) for catalytic reduction of CO2 to fuels and chemicals: strategy and mechanism, *Green Chem*, **23**, 5394–5428, https://doi.org/10.1039/D1GC01303C **(2021)**

27. Mashayekhi V., Haj Mohammad Ebrahim Tehrani K., Azerang P., Sardari S. and Kobarfard F., Synthesis, antimycobacterial and anticancer activity of novel indole-based thiosemicarbazones, *Arch. Pharm. Res*., **44**, 1–13 **(2021)**

28. Moodley J.S., Krishna S.B., Pillay K., Sershen and Govender P., Green synthesis of silver nanoparticles from Moringa oleifera leaf extracts and its antimicrobial potential, *Adv Nat Sci Nanosci Nanotechnol*, **9**, 015011 **(2018)**

29. Moon J.K. and Shibamoto T., Antioxidant Assays for Plant and Food Components, *J. Agric. Food Chem*., **57**, 1655–1666 **(2009)**

30. Nasrollahzadeh M., Shafei N., Nezafat Z., Sadat Soheili Bidgoli N., Soleimani F. and Varma R.S., Valorisation of fruits, their juices and residues into valuable (nano) materials for applications in chemical catalysis and environment, *Chem Rec*, **20(11)**, 1338–1393, https://doi.org/10.1002/tcr.202000078 **(2020)**

31. Natrayan L., Janardhan G., Nadh V.S., Srinivas C., Kaliappan S. and Velmurugan G., Eco-friendly zinc oxide nanoparticles from Moringa oleifera leaf extract for photocatalytic and antibacterial applications, *Clean Technol. Environ. Policy*, **27(10)**, 1–13 **(2024)**

32. Naz R. and Bano A., Phytochemical screening, antioxidants and antimicrobial potential of Lantana camara in different solvents, *Asian Pac J Trop Dis*, **3**, 480–486 **(2013)**

33. Palei N.N., Bhuvana M.P., Jayaraman R. and Dhar A.K., Antimicrobial and in vitro cytotoxicity effect of green synthesized silver nanoparticles using aqueous leaf extract of Senna hirsuta, *Bulletin of Pharmaceutical Sciences Assiut University*, **46(2)**, 1185–1195 **(2023)**

34. Perez C., Albert I., DeFay K., Zachariades N., Gooding L. and Kriegler M., A nonsecretable cell surface mutant of tumor necrosis factor (TNF) kills by cell-to-cell contact, *Cell*, **63(2)**, 251–258 **(1990)**

35. Rakhimol K., Thomas S. and Kalarikkal N., Casein mediated synthesis of stabilized metal/metal-oxide nanoparticles with varied surface morphology through pH alteration, *Mater Chem Phys*, **246**, 122803, https://doi.org/10.1016/j.matchemphys.2020.122803 **(2020)**

36. Rashid F., Javaid A., Ashfaq U.A., Sufyan M., Alshammari A., Alharbi M. and Khurshid M., Integrating pharmacological and computational approaches for the phytochemical analysis of Syzygium cumini and its anti-diabetic potential, *Molecules*, **27(17)**, 5734 **(2022)**

37. Saravanakumar K., Li Z., Kim Y., Park S., Keon K., Lee C.M. and Cho N., Fucoidan-coated cotton dressing functionalized with biomolecules capped silver nanoparticles (LB-Ag nps–FN–ocg) for rapid healing therapy of infected wounds, *Environ. Res.*, **246**, 118004 **(2024)**

38. Sathiyavimal S., Vasantharaj S. and Kaliannan T., Eco-biocompatibility of chitosan coated biosynthesized copper oxide nanocomposite for enhanced industrial (Azo) dye removal from aqueous solution and antibacterial properties, *Carbohydr Polym*, **241**, 116243, https://doi.org/10.1016/j.carbpol.2020.116243 **(2020)**

39. Suresh P., Doss A., Praveen Pole R.P. and Devika M., Green synthesis, characterization and antioxidant activity of bimetallic (Ag-ZnO) nanoparticles using Capparis zeylanica leaf extract, *Biomass Conv. Biorefin*, **14(14)**, 16451–16459 **(2024)**

40. Tiwari A.K., Jha S., Tripathi S.K., Shukla R., Awasthi R.R., Bhardwaj A.K. and Dikshit A., Spectroscopic investigations of green synthesized zinc oxide nanoparticles (ZnO NPs): antioxidant and antibacterial activity, *Discov. Appl. Sci.*, **6(8)**, 399 **(2024)**

41. Tiwari D.K., Schubert N.D., Cendejas L.M.V., Villegas J., Montoya L.C. and Garcia S.E.B., Interfacing carbon nanotubes (CNT) with plants: enhancement of growth, water and ionic nutrient uptake in maize (Zea mays L.) and implications for nanoagriculture, *Appl. Nanosci*., **4**, 577–591 **(2013)**

42. Varma R.S., Biomass-derived renewable carbonaceous materials for sustainable chemical and environmental applications, *ACS Sustain Chem Eng*, **7(7)**, 6458–6470, https://doi.org/10.1021/acssu schemeng.8b06550 **(2019)**

43. Vasantharaj S., Sathiyavimal S. and Saravanan M., Synthesis of ecofriendly copper oxide nanoparticles for fabrication over textile fabrics: characterization of antibacterial activity and dye degradation potential, *J Photochem Photobiol B Biol*, **191**, 143–149, https://doi.org/10.1016/j.jphotobiol.2018.12.026 **(2019)**

44. Yasir M., Singh J., Tripathi M.K., Singh P. and Shrivastava R., Green synthesis of silver nanoparticles using leaf extract of common arrowhead houseplant and its anticandidal activity, *Pharmacogn Mag*, **13**, S840–844 **(2017)**.

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