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ORIGINAL ARTICLE

Green Synthesis of Silver Nanoparticles from Flowers of *Helianthus annus* and *Tagetes erecta* and their Antibacterial Activity against MDR Pathogens

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ABSTRACT

Background: Multidrug Resistant (MDR) organisms are of major concern to healthcare sector as increasing antimicrobial resistance has made it obligatory to focus on alternative options. Investigating the medicinal benefits of nanoparticles synthesized from biological sources is relevant in this regard.

Objectives: Main purpose of the current study was to explore antibacterial potential of silver nanoparticles (AgNPs) synthesized from flower extracts of *Helianthus annus* and *Tagetes erecta* against MDR bacteria.

Methodology: MDR bacteria were isolated from sewage water of hospitals and characterized by Gram staining and biochemical analysis. From aqueous and methanolic extracts of *H. annus* and *T. erecta* flowers, silver nanoparticles were prepared and then characterized and confirmed by visual analysis, FTIR spectroscopy, and UV-Visible spectroscopy. Antibacterial activity of crude extracts and their nanoparticles was evaluated by well diffusion and disk diffusion methods, respectively.

Results: Bacterial isolates included MDR *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella* spp., *Shigella* spp. and *Proteus* spp. as well as Methicillin-Resistant *Staphylococcus aureus* were identified. Instant color change in extract solutions upon addition of silver nitrate confirmed the formation of nanoparticles. FTIR and UV-Visible spectroscopy showed response of biomolecules present in flower extracts during formation of silver nanoparticles, indicating the presence of different phytochemicals. Antibacterial activity of crude extracts increased greatly after synthesis of nanoparticles and they produced zones of inhibition against all of the MDR bacteria included. Methanolic extracts and their nanoparticles possessed more antibacterial power compared to aqueous extracts.

Conclusion: Our findings suggest the presence of potent antibacterial components in flowers of *H. annus* and *T. erecta* which necessitates further evaluation.

Keywords
Antibiotic resistance, Helianthus annus, Multidrug Resistance, Nanoparticles, MDR, Tagetes erecta.

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INTRODUCTION

Multidrug Resistant (MDR) organisms are the principal cause for the morbidity and mortality worldwide. In terms of contagious diseases, MDR pathogens are the obligatory reason for annual mortality[1]. Due to acquired chromosomal mutations or transposon-mediated transfer, microbes incorporate genes that make them intrinsically resistant to

antibiotics[2]. Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Extensive-Spectrum Beta-Lactamase (ESBL) producers have attained the ability to dissolve penicillins and cephalosporins. Members of several bacterial genera have acquired ESBL genes and produce enzymes that enable them to demonstrate resistance against multiple antimicrobial agents[3]. The mechanism of resistance in microbes may involve metabolic rearrangements in some pathogens[4]. Bacteria acquire antibiotic resistance owing to the modification of extracellular proteins and DNA, as well as formation of thick biofilms[5]. Rapidly increasing resistance against known antibiotics necessitates the exploration of alternative drug sources to combat microbial diseases. Phytochemicals present in plants such as vitamins (A, C, E, and K), flavonoids, terpenoids, saponins, enzymes, pigments, and minerals have been reported to possess antimicrobial, antioxidant and anti-cancerous activities[6]. According to the World Health Organization (WHO), approximately 80% of people rely on medicinal plants for treatment of ailments[7].

Recently, nanotechnology has extended biological systems for the production of nanoscale materials by using superficial techniques[8]. Regarding biomedical approaches, nano techniques are being used in tissue engineering, drug delivery, and biosensor production[5]. In phyto-nanotherapy, the effect of plants is combined with metal nanoparticles which are equivalent to synthetic drugs, with the advantage of being associated with less side effects[9]. Nanoparticles clasp more surface atoms which enhances their functional aptitude.

Among metals employed for nanoparticle synthesis, silver holds great promise owing towards exhibition of high level of toxicity against microorganisms and representation of the minimal harmful effects on mammals[10]. Silver has been incorporated in many ointments and creams where it acts as an antimicrobial agent to prevent infections from burns and wounds[11]. Silver Nanoparticles (AgNPs) synthesized from flower extracts have exhibited antimicrobial activity against several pathogens[12]. They were detected as a reservoir of Ag^+ ions, which lead to deprivation of cell membrane by incorporating themselves into bacterial proteins[13]. This biosynthesis-based approach is attractive because AgNPs synthesis could be done extra-cellularly by using cell-free extracts of biomolecules loaded in free amino acids and organic acids. AgNPs suspension was shown to demonstrate intrinsic antibacterial activity against MDR pathogens as well, and it has been established that the colloidal AgNPs could eradicate growth of pathogenic bacteria[14]. With reference to *Helianthus annus*, ozonated vegetable oils like canola, olive, and sunflower are used against bacterial infections[15]. *Tagetes erecta* flower possesses many pharmacological properties and is also used as food colorant as it possesses many bioactive compounds which are the key determinants for its antioxidant and antibacterial activity[16]. In view of these observations, the present study aimed at exploring the antibacterial potential of AgNPs synthesized from *Helianthus annus* and *Tagetes erecta* flower extracts against MDR pathogens.

MATERIALS AND METHODS

Isolation and Characterization of Bacteria

Sewage water was collected from Nishtar Hospital and Family Hospital, Multan, Pakistan, in air-tight containers. Serial dilutions of sewage water were plated onto nutrient agar to obtain pure isolates, which were then characterized based on Gram staining, growth on McConkey, EMB and mannitol salt agar, as well as a series of biochemical tests including MR-VP, TSI, citrate utilization, starch hydrolysis, gelatin hydrolysis, and indole production. Using Kirby-Bauer disk diffusion method, MDR status of isolates was ascertained. For this purpose, isolated organisms were cultured on Mueller-Hinton agar plates and incubated at 37ºC for 18-24hrs after placement of antibiotic disks. Antibiotics used for this purpose included Vancomycin (30µg), Imidazole (20µg), Imipenem (30µg), Augmentin (10µg), Ceftazidime (30µg) and Methicillin (30µg). Subsequently, zones of inhibition were measured using CLSI guidelines so as to establish status of bacteria as sensitive (S), intermediate (I) or resistant (R) to the antibiotics used.

Preparation of Flower Extracts

Floral part of two plants *Helianthus annus* and *Tagetes erecta,* were collected from field area of Multan in sterile plastic bags and transported to the laboratory of Department of Microbiology and Molecular Genetics, The Women University, Multan. The collected floral parts were washed several times with running water followed by distilled water in last step to remove dust particles. For

aqueous extraction, 20g of fresh petals of each flower were added to 60ml of distilled water and boiled on slow heat for 1hr with continuous stirring. Then the extract was filtered, allowed to cool down at room temperature and stored at 4ºC for future use. For the preparation of methanolic extracts, petals were oven dried at 45ºC for 3-5 days and grinded into fine powder later on. Oven-dried powder (20g) of each flower was added separately to 60ml of methanol and mixed with continuous shaking in shaking incubator at 15,000rpm for 72hrs. Then, it was evaporated to obtain concentrated solution and stored at 4ºC for further use.

Synthesis and Characterization of Silver Nanoparticles

Stock solutions of flower extracts were added to 5mM colorless silver nitrate solution separately in dark room to minimize photoactivation of silver. Color change observed after 5min and 10min served as an indication for successful synthesis of nanoparticles. These solutions were centrifuged 2-3 times at 12,000rpm for 15min, supernatant was discarded every time to obtain purified particles and pellet was oven-dried at 45-50°C[12]. Characterization of these Silver Nanoparticles (AgNPs) was done by UV-Visible spectroscopy and Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectrum was recorded in the range of 500-4000cm^{-1}. Various modes of vibration were observed in transmittance mode. The reduction of Ag^{+} to Ag° was monitored by measuring the UV-Visible spectrum within the range of 400-800nm at a resolution of 10nm in the UV-Vis spectrophotometer including methanol as control.

Analysis of Antibacterial Activity of Flower Extracts and AgNPs

For the assay of antibacterial potential of crude floral extracts as well as synthesized AgNPs, two alternative approaches were employed: well diffusion and disk diffusion. All the analyses were performed in triplicates so as to minimize chances of error. For well diffusion analysis to check antibacterial activity of crude extracts, holes of 6mm were punched on Mueller Hinton agar plates after swabbing isolates on them. Extract solution (20µl) was added to each well and plates were left at room temperature for 1hour to allow diffusion of extracts followed by incubation at 37ºC for 24hrs. Afterwards, zones of inhibition were measured and recorded in mm. For AgNPs activity analysis, filter paper disks were made by soaking those in AgNPs solution for 30min and subsequently drying for 5-10min. These discs were placed onto Mueller Hinton agar plates after swabbing of isolates and incubated at 37ºC for 24hrs, subsequent to which, zones of inhibition were measured in mm.

RESULTS

Bacterial Isolates and their MDR Status

Isolated organisms included *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella* spp., *Shigella* spp. and *Proteus* spp. whose identity was ascertained following the scheme provided by Bergey's Manual of Systematic Bacteriology. MDR status of these isolates was established based on resistance to more than one of the antibiotic drugs tested as documented in Table **1**. With the exception of *S. aureus,* all pathogens were MDR. *S. aureus* was however, resistant to methicillin. Majority of the isolates demonstrated resistance against vancomycin and augmentin whereas, the most effective drug against majority of the pathogens was ceftazidime.

Table 1. Antibiotic Sensitivity Profiles of Bacteria Isolated from Sewage Water.

Test Organism	Vancomycin	Imidazole	Imipenem	Ceftazidime	Augmentin	Methicillin
E. coli	S	R	S	R	R	I
S. aureus	S	I	I	S	S	R
P. aeruginosa	R	I	R	S	R	I
K. pneumoniae	R	I	R	S	R	S
Salmonella spp.	R	S	S	I	I	R
Shigella spp.	S	I	R	S	R	I
Proteus spp.	R	I	S	R	R	I

Characterization of AgNPs Synthesized from Floral Extracts

Silver Nanoparticles (AgNPs) synthesized from *H. annus* and *T. erecta* floral extracts were characterized based on visual analysis, FTIR spectroscopy and UV-Visible spectroscopy. Visual analysis on the basis of color change is used as a convenient indication for successful synthesis of AgNPs and the reason for the observation of this color change is surface plasmon resonance. For *H. annus,* the color of solution changed from colorless to dark yellow 5min after the addition of silver nitrate solution while that of *T. erecta* changed to dark green (Fig. **1**). After 10min, the color became dark as resonance increases with the passage of time. FTIR analysis was performed to evaluate the presence of characteristic phytochemicals in floral extracts, which act as reducing and capping agents. FTIR bands for *H. annus* were observed in different regions with the broadest absorbance band observed at 3404.30cm^{-1} (Fig. **2**). FTIR bands for *T. erecta* were also observed at different values but the broadest band was observed at 3401cm^{-1} (Fig. **3**). UV-Visible spectroscopy analysis was performed to establish reduction of Ag^{+} to Ag^{o} which is characteristic feature of silver demonstrating maximum absorbance in the range of 425nm. Absorbance for both flowers was between 1.0 and 1.5, in the range of 400-450nm which confirmed the presence of AgNPs (Fig. **4**).

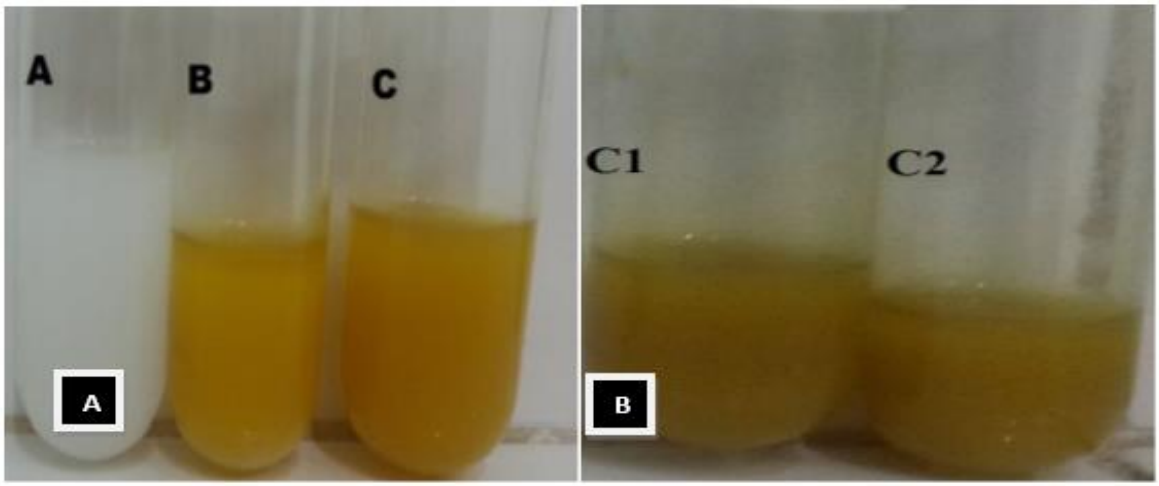

Figure 1. Visual analysis of AgNPs synthesized using (A) *H. annus* and (B*) T. erecta* floral extracts.

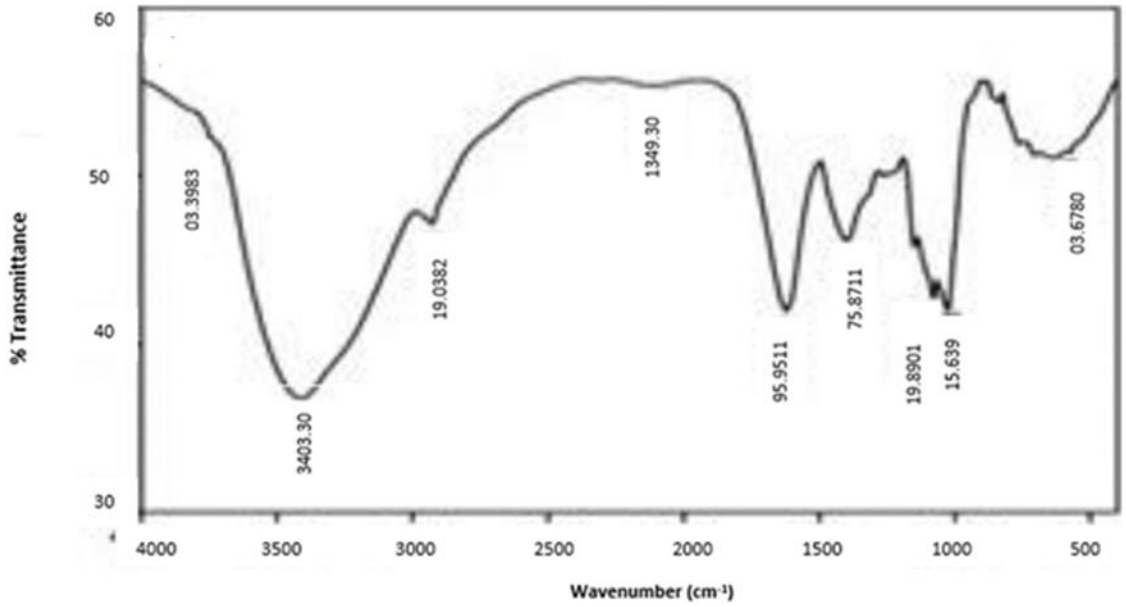

Figure 2. FTIR analysis of AgNPs synthesized from *H. annus* flowers.

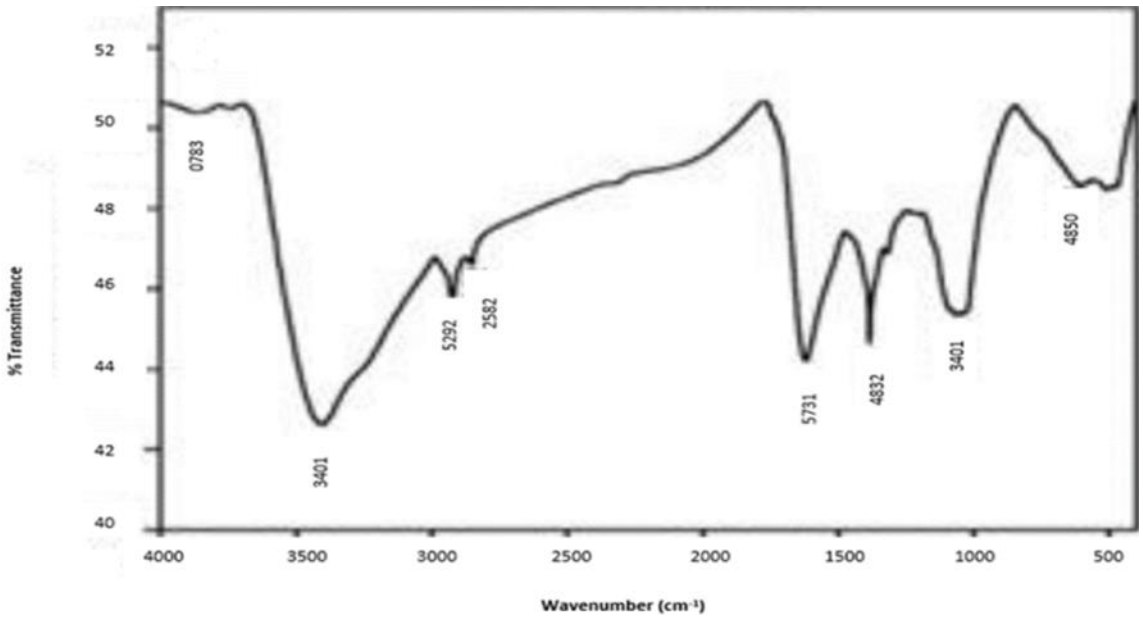

Figure 3. FTIR analysis of AgNPs synthesized from *T. erecta* flowers.

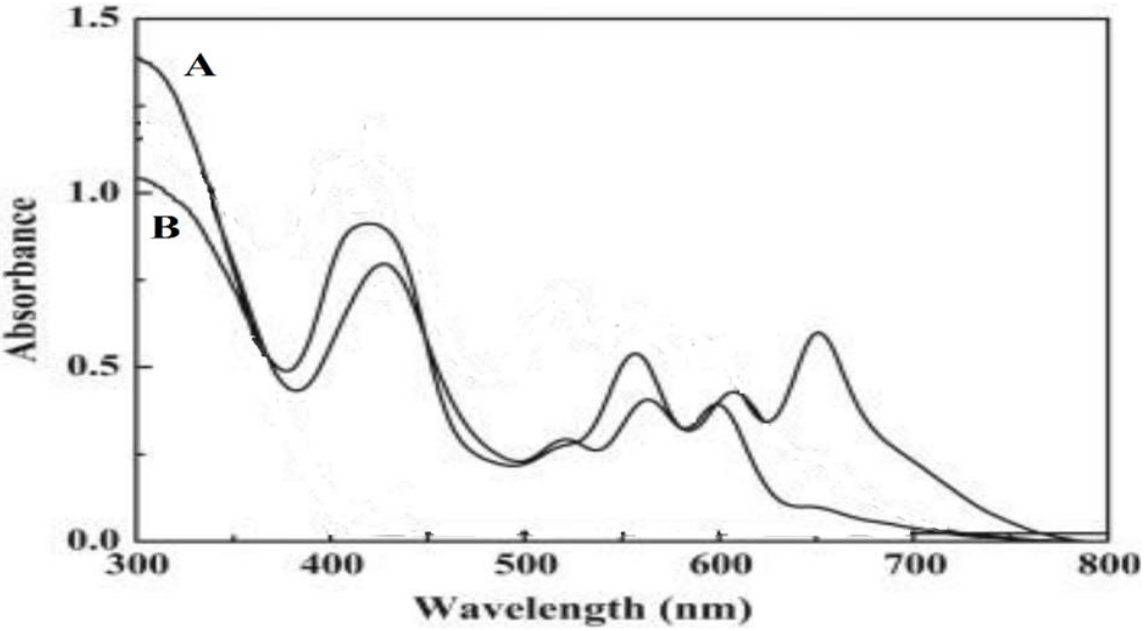

Figure 4. UV-Visible spectrum of AgNPs synthesized from (A) *H. annus* and (B) *T. erecta* flowers.

Antibacterial Activity of Crude Floral Extracts and their AgNPs

In general, the antibacterial activity of crude floral extracts was enhanced after synthesis of their AgNPs, although exceptions existed (Table **2**). This observation is reinforced by the fact that aqueous floral extract of *H. annus* was completely ineffective against *E. coli* and *Salmonella* spp. while that of *T. erecta* was not efficacious against *P. aeruginosa*. However, these demonstrated antibacterial effect against aforementioned pathogens subsequent to the synthesis of AgNPs. It was further documented that crude extract as well as AgNPs of *H. annus* flowers exhibited stronger bactericidal effect against most of the MDR isolates (Fig. **5**). However, *T. erecta* flowers were much more effective against *E. coli* as compared to *H. annus* flowers. Likewise, in comparison with corresponding extract or AgNPs of *H. annus* flowers, crude aqueous extract of *T. erecta* flowers possessed stronger

bactericidal activity against *Salmonella* spp. and AgNPs synthesized from its methanolic extract displayed more potency against *Shigella* spp. On the whole, methanolic extracts of both flowers as well as AgNPs synthesized from it were more effective against MDR isolates in comparison with aqueous extract and AgNPs synthesized from it, and the difference was noteworthy in some cases. The strong antibacterial potential of both flowers was revealed by the observation that AgNPs synthesized from methanolic extract of *H. annus* completely cleared the growth of *S. aureus*, and *K. pneumoniae* and those of *T. erecta* cleared growth of *Shigella* spp.

Table 2. Antibacterial Activity Assay of *H. annus* and *T. erecta* Floral Extracts and their Synthesized AgNPs.

Test organism / Type of extract	AgNPs of *T. erecta* (Zone of inhibition in mm)	AgNPs of *H. annus* (Zone of inhibition in mm)	Crude extract of *T. erecta* (Zone of inhibition in mm)	Crude extract of *H. annus* (Zone of inhibition in mm)
E. coli				
Aqueous extract	17	12	08	00
Methanol extract	13	15	19	06
S. aureus				
Aqueous extract	14	18	12	17
Methanol extract	14	Clear	16	16
P. aeruginosa				
Aqueous extract	10	16	00	14
Methanol extract	11	16	04	14
K. pneumoniae				
Aqueous extract	13	17	06	15
Methanol extract	12	Clear	12	16
***Salmonella* spp.**				
Aqueous extract	12	15	07	00
Methanol extract	14	19	06	08
***Shigella* spp.**				
Aqueous extract	14	15	09	10
Methanol extract	Clear	19	09	12
***Proteus* spp.**				
Aqueous extract	08	10	15	16
Methanol extract	12	12	14	19

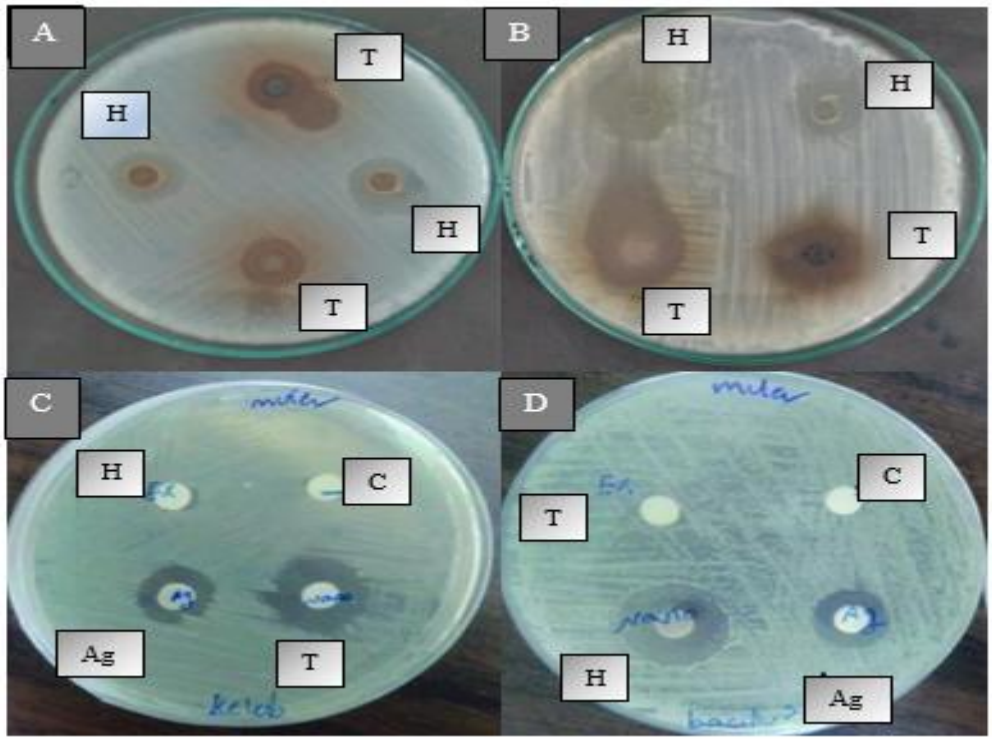

Figure 5. Evaluation of antibacterial activity of extracts by well diffusion and disk diffusion methods: A) Crude extracts against Methicillin-resistant *S. aureus*, T= *T. erecta*, H= *H. annus*; B) Crude extracts against *Proteus* spp., T= *T. erecta*, H= *H. annus;* C) AgNPs against *E. coli*, T= *T. erecta*, H= *H. annus*, Ag=$AgNO_3$ solution, C=Control; C) AgNPs against *P. aeruginosa*, T= *T. erecta*, H= *H. annus*, Ag=$AgNO_3$ solution, C=Control.

DISCUSSION

Due to its multi-targeted action, silver has already been established as a promising agent in overcoming antibiotic resistance because bacterial cell membranes have been documented to be harmed by AgNPs owing to structural changes, and this property can be used to enhance susceptibility of bacteria towards antibiotics[17]. Silver Nanoparticles (AgNPs) have specific optical, electrical, and thermal properties, as well as a large surface area to volume ratio, allowing them to associate with bacterial surfaces optimally, resulting in superior antimicrobial activity[18]. Binding of silver ions to bacterial cell macromolecules causes structural alterations and deformations as well as degradation of essential enzymes by interaction with thiol groups, cumulating in the form of oxidative stress[19]. Plant-based compounds possess pharmacologically significant attributes as their secondary metabolites act as anti-oxidant, anti-microbial, anti-cancer, anti-inflammatory agent etc[20]. Furthermore, various parts of plants are non-toxic, economic, eco-friendly and safe for the synthesis of nanoparticles with an added advantage of being environment friendly[21]. In the present study, nanoparticles were synthesized using *T. erecta* and *H. annus* whose flowers are used for ornamental, cosmetic and medicinal purposes.

Characterization of AgNPs synthesized from *T. erecta* and *H. annus* flower extracts relied upon spectroscopic analysis. Visual analysis in terms of color change serves as a simple spectroscopic signature marking the successful synthesis of nanoparticles[12]. Color change observed after addition of silver nitrate solution to crude flower extracts was rapid and instantaneous and the intensity of color increased with the passage of time due to shift in surface plasmon resonance. Several previous studies have documented change in the color of solution as an indication for formation of nanoparticles from various plant sources and it occurs due to reduction of silver ions and excitation of surface plasmon vibrations which are characteristic attributes of silver[22-24]. To identify the functional groups responsible for reduction of silver ions to AgNPs, FTIR spectrum was generated in the range of 500-

4000cm^{-1}. FTIR spectrum for *T. erecta* produced peaks in the range of 557.45-3862.58cm^{-1} and *H. annus* produced the broadest band at 3404.30cm^{-1}. These narrow peaks correspond to functional groups like flavonoids, saponins, terpenoids etc. These observations were in accordance to those reported earlier for some other plant sources[25]. The presence of narrow peaks implies that nanoparticles synthesized were of small size within the range of 1-100nm. Presence of AgNPs was further confirmed by UV-Visible spectroscopy and maximum absorbance was observed at 425nm. This is characteristic attribute of silver which is used to confirm the presence of AgNPs, and has been reported previously by other researchers as well[26].

Two different types of extracts were prepared from flowers of *T. erecta* and *H. annus* i.e. aqueous and methanolic. Methanolic extract was more effective against MDR organisms possibly due to the presence of hydrophobic phytochemicals in the solution. Effectiveness of methanolic extract compared to other solvents has been reported for *Aegle marmelos* fruit as well[27]. Silver Nanoparticles (AgNPs) of *H. annus* possessed greater antibacterial activity as compared to *T. erecta* particularly against gram-positive bacteria. This was probably due to difference in cell wall composition as thin wall of gram-negative bacteria leads to poor penetration of silver nanoparticles[28, 29]. Additional factors determining antibacterial activity of AgNPs include availability of particles in the medium, their electrostatic interactions and bacterial sensitivity to them[30, 31]. We observed a general increase in the antibacterial potential of both floral extracts subsequent to the synthesis of AgNPs which is in line with previous reports[32]. Plant-AgNPs exhibited antibacterial activity against all the pathogens tested but particularly against *S. aureus*, *Shigella* spp. Similar to these findings, zones of inhibition have been shown by synthesized nanoparticles against pathogens like *S. aureus*, *B. cereus*, and P. *aeruginosa*[33]. Additionally, antibacterial potential of plant-AgNPs has been reported against MDR pathogens including *E. coli*, *K. pneumoniae* and *A. baumannii*[34]. The exact mechanism underlying antibacterial potential of AgNPs has not been deciphered yet. Theories proposed in this regard implicate altered membrane permeability, generation of reactive oxygen species, release of membrane lipopolysaccharides as well as disintegration of membrane potential[35-38].

CONCLUSION

Inappropriate use of antibiotics has become significant and predominant problem as it is leading towards rapid increase in resistance among pathogens. The combined effect of plant and metal nanoparticles is equivalent to synthetic drugs with lower side effects. Biosynthesis-based approach is attractive and effective as demonstrated by antibacterial potential of *H. annus* and *T. erecta* flowers against MDR organisms in this study which must be explored by further research.

CONFLICTS OF INTEREST

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None.

LIST OF ABBREVIATIONS

AgNPs	Silver Nano Particles
CLSI	Clinical and Laboratory Standards Institute
FTIR	Fourier Transform Infrared
MDR	Multi Drug Resistant

REFERENCES

1. Zahlane K, Ouafi AT, Barakate M. The clinical and epidemiological risk factors of infections due to multi-drug resistant bacteria in an adult intensive care unit of University Hospital Center in Marrakesh-Morocco. J Infect Public Health. 2020; 13(4):637-43.
2. Li B, Webster TJ. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. J Orthop Res. 2018; 36(1):22-32.
3. Shah AS, Karunaratne K, Shakya G, Barreto I, Khare S, Paveenkittiporn W, *et al*. Strengthening laboratory surveillance of antimicrobial resistance in South East Asia. BMJ. 2017; 358-63.
4. Zampieri M, Enke T, Chubukov V, Ricci V, Piddock L, Sauer U. Metabolic constraints on the evolution of antibiotic resistance. Mol Syst Biol. 2017; 13(3):917-25.

5. Zheng JX, Wu Y, Lin ZW, Pu ZY, Yao WM, Chen Z, *et al*. Characteristics of and virulence factors associated with biofilm formation in clinical *Enterococcus faecalis* isolates in China. Front Microbiol. 2017; 8:2338-45.
6. Shad AA, Ahmad S, Ullah R, AbdEl-Salam NM, Fouad H, Rehman NU, *et al*. Phytochemical and biological activities of four wild medicinal plants. Sci World J. 2014; 1-8.
7. Nandhini DU, Sakthinathan B, Kumaresan S. Ethanobotany of medicinal herbs in indigenous folklore of siruvani region of Tamil Nadu. J Pharmacog Phytochem. 2017; 6(5):2078-81.
8. Kaegi R, Sinnet B, Zuleeg S, Hagendorfer H, Mueller E, Vonbank R, Boller M, *et al*. Release of silver nanoparticles from outdoor facades. Environ Pollut. 2010; 158(9):2900-5.
9. Yallapu MM, Ebeling MC, Khan S, Sundram V, Chauhan N, Gupta BK, *et al*. Novel curcumin-loaded magnetic nanoparticles for pancreatic cancer treatment. Mol Cancer Ther. 2013; 12(8):1471-80.
10. Sahayaraj K, Rajesh S. Bionanoparticles: Synthesis and antimicrobial applications. Science against microbial pathogens. Comm Curr Res Technol Adv. 2011; 23:228-44.
11. Singhal G, Bhavesh R, Kasariya K, Sharma AR, Singh RP. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. J Nano Res. 2011; 13(7):2981-8.
12. Padalia H, Moteriya P, Chanda S. Green synthesis of silver nanoparticles from marigold flower and its synergistic antimicrobial potential. Arab J Chem. 2015; 8(5):732-41.
13. Chakraborty S, Chelli VR, Das RK, Giri AS, Golder AK. Bio-mediated silver nanoparticle synthesis: Mechanism and microbial inactivation. Toxicol Environ Chem. 2017; 99(3):434-47.
14. Ghodake G, Kim M, Sung JS, Shinde S, Yang J, Hwang K, *et al*. Extracellular synthesis and characterization of silver nanoparticles-Antibacterial activity against multidrug-resistant bacterial strains. Nanomat. 2020; 10(2):360-6.
15. Valacchi G, Lim Y, Belmonte G, Miracco C, Zanardi I, Bocci V, *et al*. Ozonated sesame oil enhances cutaneous wound healing in SKH1 mice. Wound Repair Regen. 2011; 19(1):107-15.
16. Siriamornpun S, Kaisoon O, Meeso N. Changes in colour, antioxidant activities and carotenoids (lycopene, β-carotene, lutein) of marigold flower (*Tagetes erecta* L.) resulting from different drying processes. J Funct Foods. 2012; 4(4):757-66.
17. Liu C, Luo L, Liu L. Antibacterial effect and mechanism of silver-carried zirconium glycine-N, N-dimethylenephosphonate as a synergistic antibacterial agent. Inorg Chem Commun. 2019; 107497-103.
18. Soumya KR, Mathew S, Sugathan S, Mathew J, Radhakrishnan EK. Studies on prevalence of biofilm associated genes and primary observation on sasX gene in clinical isolates of coagulase negative staphylococci (CoNS). APMIS. 2016; 124(4):319-26.
19. Akram FE, El-Tayeb T, Abou-Aisha K, El-Azizi M. A combination of silver nanoparticles and visible blue light enhances the antibacterial efficacy of ineffective antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA). J Clin Microbiol Antimicrob. 2016; 15(1):1-3.
20. Akram M, Hamid A, Khalil A, Ghaffar A, Tayyaba N, Saeed A, *et al*. Review on medicinal uses, pharmacological, phytochemistry and immunomodulatory activity of plants. Int J Immunopathol Pharmacol. 2014; 27(3):313-9.
21. Shahid M, Shahzad A, Malik A, Sahai A. Recent trends in biotechnology and therapeutic applications of medicinal plants. Dordrecht Springer. 2013.
22. Alsammarraie FK, Wang W, Zhou P, Mustapha A, Lin M. Green synthesis of silver nanoparticles using turmeric extracts and investigation of their antibacterial activities. Colloids Surf B: Biointerfaces. 2018; 171:398-405.
23. Gavamukulya Y, Maina EN, Meroka AM, Madivoli ES, El-Shemy HA, Wamunyokoli F, *et al*. Green synthesis and characterization of highly stable silver nanoparticles from ethanolic extracts of fruits of *Annona muricata*. J Inorg Organomet Polym Mater. 2020; 30(4):1231-42.
24. Tamilarasi P, Meena P. Green synthesis of silver nanoparticles (Ag NPs) using *Gomphrena globosa* (Globe amaranth) leaf extract and their characterization. Mat Today: Proc. 2020; 33:2209-16.
25. Yazdi ME, Khara J, Sadeghnia HR, Bahabadi SE, Darroudi M. Biosynthesis, characterization, and antibacterial activity of silver nanoparticles using *Rheum turkestanicum* shoots extract. Res Chem Intermed. 2018; 44(2):1325-34.
26. Jacob SJ, Prasad VS, Sivasankar S, Muralidharan P. Biosynthesis of silver nanoparticles using dried fruit extract of *Ficus carica*-Screening for its anticancer activity and toxicity in animal models. Food Chem Toxicol. 2017; 109:951-6.

27. Devi M, Devi S, Sharma V, Rana N, Bhatia RK, Bhatt AK. Green synthesis of silver nanoparticles using methanolic fruit extract of *Aegle marmelos* and their antimicrobial potential against human bacterial pathogens. J Tradit Complement Med. 2020; 10(2):158-65.
28. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnol. 2007; 18(22):225103-9.
29. Rasheed T, Bilal M, Iqbal HM, Li C. Green biosynthesis of silver nanoparticles using leaves extract of *Artemisia vulgaris* and their potential biomedical applications. Colloids Surf B: Biointerfaces. 2017; 158:408-15.
30. Chen SF, Li JP, Qian K, Xu WP, Lu Y, Huang WX, *et al*. Large scale photochemical synthesis of M@TiO2 nanocomposites (M= Ag, Pd, Au, Pt) and their optical properties, CO oxidation performance, and antibacterial effect. Nano Res. 2010; 3(4):244-55.
31. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against *Enterococcus faecalis* biofilm. J Endod. 2014; 40(2):285-90.
32. Abdel-Aziz MS, Shaheen MS, El-Nekeety AA, Abdel-Wahhab MA. Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract. J Saudi Chem Soc. 2014; 18(4):356-63.
33. Zarei Z, Razmjoue D, Karimi J. Green synthesis of silver nanoparticles from *Caralluma tuberculata* extract and its antibacterial activity. J Inorg Organomet Polym Mater. 2020; 30:4606-14.
34. Alharbi FA, Alarfaj AA. Green synthesis of silver nanoparticles from *Neurada procumbens* and its antibacterial activity against multi-drug resistant microbial pathogens. J King Saud Univ Sci. 2020; 32(2):1346-52.
35. Amro NA, Kotra LP, Wadu-Mesthrige K, Bulychev A, Mobashery S, Liu GY. High-resolution atomic force microscopy studies of the *Escherichia coli* outer membrane: structural basis for permeability. Langmuir. 2000; 16(6):2789-96.
36. Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, *et al*. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J Proteome Res. 2006; 5(4):916-24.
37. Panáček A, Kvitek L, Prucek R, Kolář M, Večeřová R, Pizúrová N, *et al*. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. J Phys Chem B. 2006; 110(33):16248-53.
38. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, *et al*. Antimicrobial effects of silver nanoparticles. Nanomed: Nanotechnol Biol Med. 2007; 3(1):95-101.

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