doi.org/10.36721/PJPS.2023.36.2.SP.699-706.1

Antimicrobial activity of $AgNO_3$ nanoparticles synthesized using *Valeriana wallichii* against ESKAPE pathogens

Razaullah Shah[1], Ifrah Sarosh[2], Rabia Shaukat[3], Khaloud Mohammed Alarjani[4], Rabab Ahmed Rasheed[5], Dina S. Hussein[6], Kifayat Hussain[7], Khunsha Anosh Ali[8], Sayab Khan[1], Muhammad Kamil Khan[1], Zeeshan Niaz[1]* and Junaid Ahmad[9]

[1]Department of Microbiology, Hazara University Mansehra, Pakistan
[2]BHU Pounta, Multan, Pakistan
[3]THQ Mushtaq Lang Jalalpur Pirwala, Multan, Pakistan
[4]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh Saudi Arabia
[5]Histology and Cell Biology Department, Faculty of Medicine, King Salman International University, South Sinai, Egypt
[6]Department of Chemistry, College of Sciences and Health, Cleveland State University, Cleveland, USA
[7]Department of Pathology, Rehman College of Allied Health Sciences, Peshawar, Pakistan
[8]Department of Medical Lab Technology, Sarhad University of Science and Information Technology, Peshawar, Pakistan
[9]Department of Microbiology, Immunology, Infectious Disease, Transplantation and Related Disease, University of Rome for Vergata, Italy

Abstract: The emergence of multidrug-resistant ESKAPE infections has emerged as a serious public health threat. Nosocomial infections are most often caused by ESKAPE bacteria. To combat multidrug-resistant ESKAPE, the research team used *Valeriana Wallichii* extracts and nanoparticles. The well diffusion technique was used to test antimicrobial activity on Muller Hinton agar medium. The FTIR, SEM and XRD techniques were used to characterize the nanoparticles synthesized in an environmentally benign manner. Both NPs performed better than extracts made with methanol and water in this investigation. The smallest zones of inhibition were shown against *A. baumannii and Enterobacter cloacae,* whereas the largest zones of inhibition were seen against *E. faecium*. However, NPs synthesized from shoot extracts exhibited remarkable effects against all MDR ESKAPE infections, with zones of inhibition of 23, 20, 12, 18, 22 and 14mm, respectively. Although *E. faecium*. had the largest inhibitory zone in both methanolic root and shoot extracts (19mm and 22mm, respectively), *K. pneumonia* and *E. cloacae* had the smallest zones when tested with these solvents. Water-based extracts inactivated multidrug-resistant bacteria. Our research show that extracts and nanoparticles have stronger antibacterial efficiency because biologically active substances including Terpenoids, Alkaloids, Phenol and Pholobutannins affect people and microbe.

Keywords: Silver nanoparticles, Multidrug-resistance, Urinary tract infection, Nanotechnology, Biological compound and Minimum inhibitory concentration.

INTRODUCTION

As the leading problem in countries both developed and developing, infectious illnesses caused by MDR microorganisms owing to incorrect antimicrobial usage need the urgent development of new medicines (Tacconelli *et al*., 2018). The advent of MDR bacteria like ESKAPE, which are characterized by their resistance to several treatments, has been related to an increase in both morbidity and death. So, ESKAPE infections are a major problem now (Marcucci *et al*., 2023). Because of this, ESKAPE infections are a top priority in terms of antibiotic activity screening and assessment (Vaou, Stavropoulou, Voidarou, Tsigalou, & Bezirtzoglou, 2021). *Staphylococcus aureus, Enterococcus faecium, Enterobacter* spp, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are all known to cause illness. The unique physical and chemical properties of nanoparticles make them a promising candidate for use as antibacterial agents. Bactericidal infections treated with silver nanoparticles have been discovered. Nanosilver and medicinal plants are used to cure infections (Khan *et al*., 2019). Like medicinal plants, silver nanoparticles have discovered new biological uses thanks to their innate therapeutic characteristics. Medicinal plants have been utilized for millennia in most nations, but in Asia, many different plants are used to treat various infectious ailments (Suroowan *et al.*, 2017).

For many years, people have relied on *V. wallichii* to combat bacterial illnesses. *V. Jatamensi* is one of the most extensively distributed plant families in the world's temperate regions, with over 250 different species (Jugran *et al.,* 2021). The Ayurvedic herb *V. Jatamansi* is widely used for a variety of conditions. Iridoids, liganoids, valeriandoids, and valepotriates are only some of the chemical components discovered in the plant's rhizomes and roots. *In vitro* and *in vivo* studies have shown that it possesses antimicrobial, antioxidant and anticancer effects (Kalhori *et al*., 2023). More study of this plant is needed to deduce its molecular mechanism and develop secure

**Corresponding author:* e-mail: themicrobiolgist@gmail.com

medical applications in contemporary medicine. The *Valeriana* plant family has a long history of use in traditional medicine in Pakistan. Hilly regions, especially those in Pakistan's north, are where you'll find *valeriana* plants (Hassan *et al*., 2019).

MATERIALS AND METHODS

Collection of plant samples

The Konsh, Bhaleja and Allai districts in the hazara area provided the freshest roots and shoots (stems and leaves) of *V. wallichii*. Distilled water was used to clean the plant samples before analysis.

Grinding and slicing

The plant was cut up and dried for two weeks in an outbuilding. After being dried, the shoots and roots were rinsed with distilled water, ground into a powder, and then sterilized with 15% hydrogen peroxide. The powdered sample was stored in a container at room temperature (Jamal *et al*., 2023).

Plant extract preparation

Methanol and water were used as solvents in the extraction procedure. Flaked rhizomes and shoots weighing 15 g were steeped in mixtures of 200mL each of methanol and water. Both solutions were kept in a shaker incubator at 120 rpm and room temperature for 72 hours. The resulting liquid was filtered twice, first with muslin cloth and then with Whatman filter paper. After adding methanol to the powder, it was left at room temperature for many hours. The remedy was baked at 60 degrees Celsius for 48 to 72 hours. The crude extract solution, which was semisolid, was then allowed to sit at room temperature (Gupta *et al.*, 2012).

Identification of plants

To identify plants, seedpods, fruit, flowers, branches and leaves were gathered which aids in the identification process. Field notes were taken for the major macroscopic characteristics of the fresh specimens. Ocular micrometer and good hand lens (10 x magnifications) was used to observed the morphological traits at the site. For further identification of plants, materials were transferred to the department of botany Hazara University Mansehra.

Bacterial samples collection

The ESKAPE pathogens were procured from the Hazara University Mansehra Department of Microbiology. These pathogenic strains were grown overnight in LB broth.

Silver nanoparticles green synthesis

In a beaker ranging in colour from light yellow to dark brown, we first combined 0.50961g of silver nitrate powder, 10ml of root and shoot extract and 90ml of distilled water. The beakers containing the nanoparticles were then wrapped with perforated aluminum foil and heated to 60°C for 48 hours.

Characterization

Nanoparticles were analyzed using XRD, SEM and FT-IR. By using XRD, we learned about the composition, crystallinity and size of the AgNPs; by using FTIR, we learned about the likely functional groups and chemical compositions involved in the bioreduction of the AgNPs; and by using SEM, we learned about the morphology, size, and shape of the AgNPs.

Antibacterial activity

The well diffusion technique and MHA medium were employed to test for antibacterial activity. The MHA medium upon which the bacterial lawn was grown We employed antibiotic discs (Flusidic Acid, Chloramphenicol, Doxycycline, Tazobactam, Cefotaxime, Ceftazidime and Norfloxacine) as positive controls and 7 wells (6 mm) for the shoot and root extract (methanol) and 1 for the negative control (DMSO).

Preparation of nanoparticles and other solvent extract dilution

DD water and DMSO were used to dilute the extracts and nanoparticles. Root and stem extracts were dissolved in a combination of DMSO and DD water to a concentration of 1ml. These dilutions of the solvent extract were also made in a 1:60 ratio, using 60 mg of methanol in 1 mL of DMSO+ DD water (500l total). These dilutions were utilized at a concentration of 100l in each of the six wells of the petri plates (two for nanoparticles and four for extracts) to determine the bacterial zone of inhibition.

Minimum Inhibitory concentrations (MICs) determination

The MICs were tested using MDR ESKAPE bacteria. In the LB dilution experiment, antibacterial activity of nanoparticles and plant extracts was evaluated. Concentrations of 2mg, 4mg, 6mg, and 8mg/mL of Ag nanoparticles and plant extracts were used in the test. Each microplate has 30 bacteria and 10mL of LB broth. After being incubated for a whole night, the bacteria in one test tube will be compared to the control microorganisms in the other. After each concentration was incubated, a spectrophotometer measured the optical density (OD_{600}) of the sample in each test tube. Using increasingly diluted extracts and nanoparticles, the MIC for each pathogen was determined (Owuama, 2017).

Phytochemical analysis

A plant extract measuring 3 mL was steeped in a solvent measuring 50 mL for 24 hours before being filtered through paper. Secondary plant metabolites were analyzed using both plant and methanolic extract. A shift in color indicated the existence of the metabolites. Afterwards, it underwent a battery of biological tests for a wide range of compounds, including phlobatannins, phenol, alkaloids, terpenoids, glycosides, polysaccharides, and anthraquinones.

STATISTICAL ANALYSIS

For data tabulation, Microsoft Excel was used for statistical analysis and graphical representation of data, latest version of GraphPad Prism was used.

RESULTS

Antibacterial activity of V. wellichii extract and NPs

This research was conducted to learn more about the bactericidal effects of *V. wallichii* and synthesized nanoparticles against MDR ESKAPE (table 1). The microbiology department of Hazara University Mansehra provided the study's preserved samples of MDR ESKAPE pathogens. Against these pathogenic strains, researchers examined the efficacy of several plant extracts and nanoparticles produced from plant components as antibacterial agents (fig. 1).

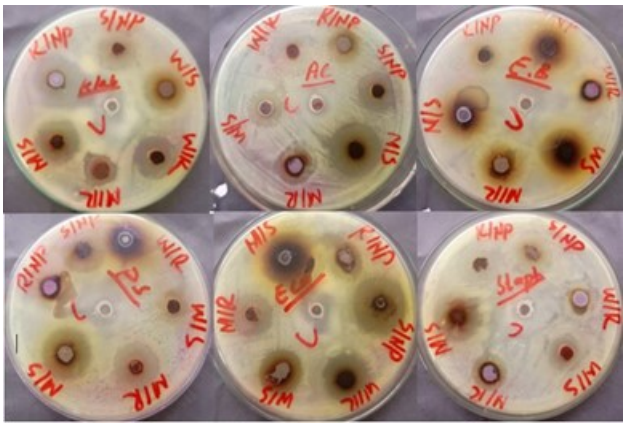

Fig. 1: Inhibition of Pathogens in the ESKAPE group by *V. wellichii* extract and NPs.

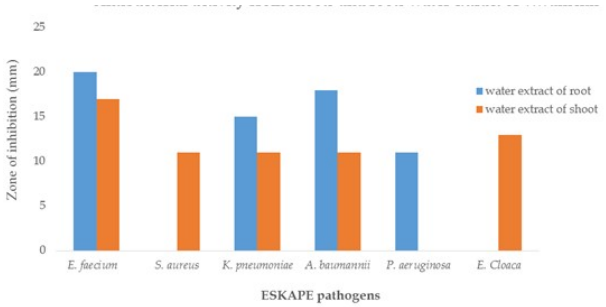

Fig. 2: Antibacterial activity from shoots and roots water extract of *V. wallichii* plant.

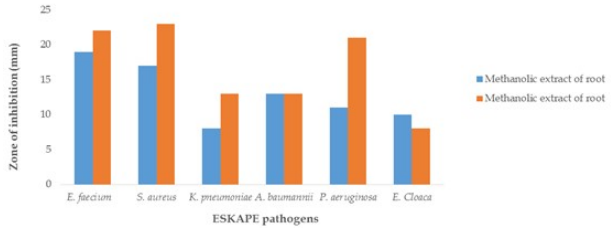

Fig. 3: Methanolic extract of *V. wallichii* plants shows antibacterial action in both shoots and roots.

Water extract of roots and shoots

Root and shoot extracts that dissolve in water are highly antibacterial and effective against all types of harmful bacteria. In tests with *E. faecium* and *A. baumannii*, a 100 mL dose of the root extract showed promising results. The 20mm ZOI against *E. faecium* was the greatest, followed by the 18mm ZOI against *A. baumannii*, and finally the 15mm ZOI against *K. pneumoniae* (fig. 2).

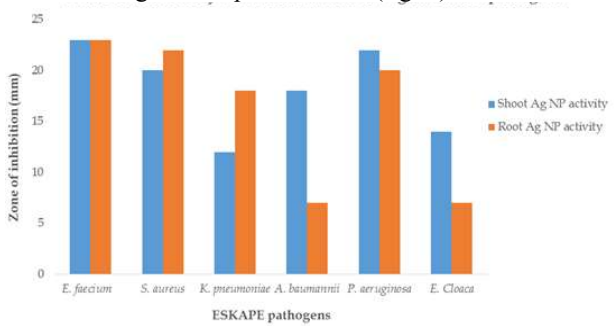

Fig. 4: SNPs in roots and shoots have antibacterial effect against MDR bacteria.

Methanolic root and shoot extract

The extract of root and shoot were effective against MDR collected strains (fig 3). The largest zone of inhibition (ZOI) was seen (13) against M. furfur. The shoot extract shows the highest activity (ZOI of 22mm) against *E. faecium* and the lowest activity (ZOI of 8mm) against *P. aeruginosa*. The methanol extract of the plant exhibits superior activity over the aqueous one. Changes in activity were seen at both higher and lower concentrations of crude extract.

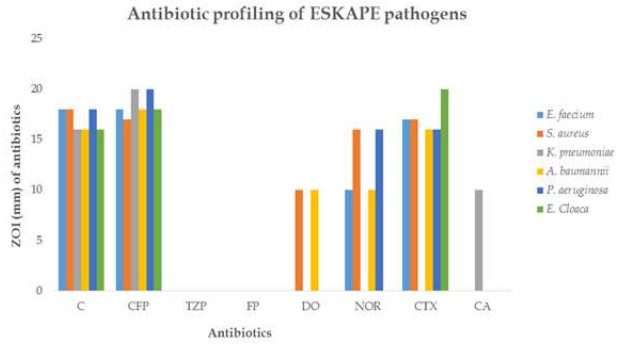

Fig. 5: Antibiotics effective against ESKAPE bacteria and their zone of inhibition.

Silver nanoparticle of roots and shoots activity

Silver nanoparticles made from root and shoot extract were effective against all MDR bacteria. At a concentration of 100l, the ZOI of Ag NP of root was highest (23mm) against *E. faecium*, while it was lowest (7 mm) against *E. cloacae* and *A. baumannii*. At a concentration of 100l, the highest ZOI (23 mm) of Ag NP of shoot was seen against *E. faecium*, while the lowest ZOI (12mm) was observed against *K. pneumoniae* (fig. 4).

Table 3: Plant extracts with antibacterial action against MDR ESKAPE

S. No.	Extract Solvent	Plant component	Zone of Inhibition					
			E	*S*	*K*	*A*	*P*	*E*
1	Water	Roots	20mm	0mm	15mm	18mm	11mm	0mm
2	Water	Shoots	17mm	11mm	11mm	11mm	0mm	13mm
3	Methanol	Roots	19mm	17mm	8mm	13mm	11mm	10mm
4	Methanol	Shoots	22mm	23mm	13mm	13mm	21mm	8mm
5	AgNPs	Roots	23mm	22mm	18mm	7mm	20mm	7mm
6	AgNPs	Shoots	23mm	20mm	12mm	18mm	22mm	14mm

Key: E= *E. faecium*, S= *S. aureus*, K= *K. pneumoniae* A= *A. baumannii* P= *P. aeruginosa* E= *E. Cloaca*

Table 3: Plant extracts' micro dilution broth MICs for MDR ESKAPE

Pathogens	Plants components in 2-8mg-ml					
	M.R	M.S	W.S	R-NPs	S-NPs	W.R
E	4mg	2mg	4mg	2mg	2mg	2mg
S	4mg	2mg	6mg	2mg	2mg	N.D
K	8mg	6mg	6mg	4mg	6mg	4mg
A	8mg	6mg	6mg	4mg	6mg	4mg
P	N.D	2mg	N.D	2mg	2mg	6mg
E	8mg	8mg	6mg	8mg	6mg	N.D

Descriptions= ND means Not detected E= *E. faecium*, S= *S. aureus*, K= *K. pneumoniae* A= *A. baumannii* P= *P. aeruginosa* E= *E. Cloaca*

Table 3: Different chemicals detected in the *V. wallichii* roots and shoots extract

Chemical Compound	Aqueous extract		Methanolic extract	
Phenol	_ _	_ _	++	++
Terpenoids	++	++	++	++
Phlobutannis	_ _	_ _	++	++
Anthraquinones	++	++	_ _	_ _
Glycosides	_ _	_ _	_ _	_ _
Alkaloids	++	++	++	++
Carbohydrates	++	++	_ _	+

Description: -- -- = No chemical detected and ++=presence of chemical

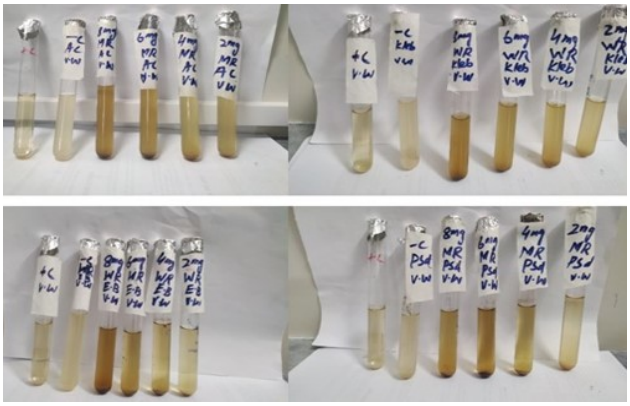

Fig. 6: Extracts and NPs from *V. wallichii* showed a MIC in LB broth dilutions against ESKAPE.

Antibiotics sensitivity test

Tazobactam (TZP110), fusidic acid (FP10), norfloxacin (NOR1), cefotaxime (CTX30), and ceftazidime (CA30) were all evaluated for sensitivity using a disc. We standardized the found ZOI in comparison to the CLSI book 2021 (fig. 5).

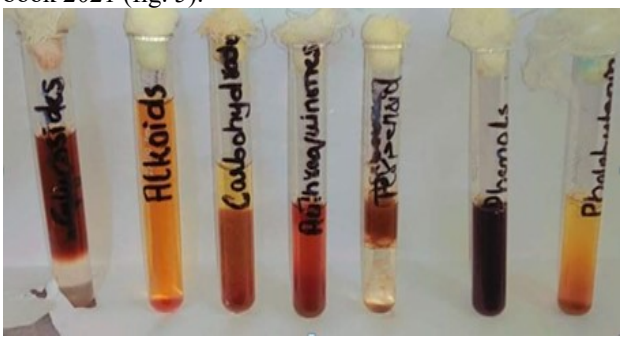

Fig. 7: Analyzed Chemicals in *V. wallichii*, starting on the left to right.

Minimum inhibitory concentration

Inhibition of bacterial growth was achieved by employing a concentration of plant extract below its MIC. We employed six distinct MDR ESKAPE isolates and dilutions of extracts and NPs at 2, 4, 6 and 8mg/ml in LB

broth (table 3.2). Inhibitory doses of methanolic roots extract were 6 and 8mg/ml, however P. aeruginosa was resistant (fig. 3.6). However, no minimal inhibitory concentrations (MICs) were detected for S. aureus or E. cloacae in water root extracts and no inhibitory action was detected against P. aeruginosa in water shoot extracts at the quantities tested.

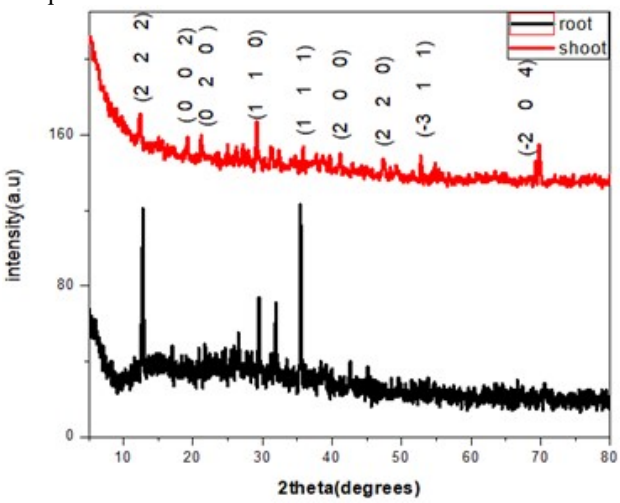

Fig. 8: Display the XRD pattern of nanoparticles synthesized from *V. wallichii.*

Phytochemical analysis

Several plant metabolites were analyzed in *V. wallichii* methanolic and aqueous extracts. High concentrations of alkaloids and terpenoids were discovered in water and methanolic extracts of the roots and shoots, respectively. Both the water and methanol tests revealed no difference for pholobutannins, whereas the tests for carbohydrates and anthraquinones showed positive findings for water but negative results for methanol. Glycosides were not discovered since neither extract caused a noticeable change in color.

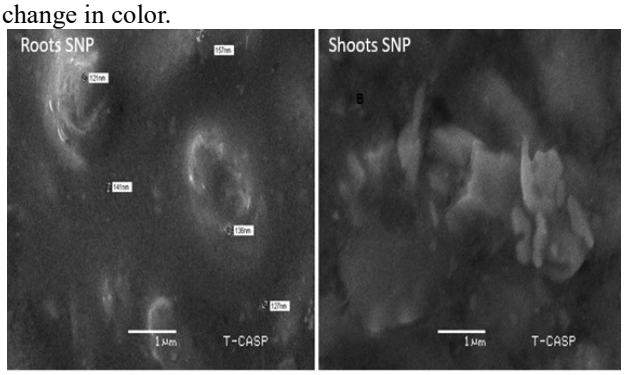

Fig. 9: Display the SEM images of roots and shoots nanoparticles of *V. wallichii.*

XRD analysis

Particle structure and crystallinity were verified by X-ray diffraction from 0 to 80 degrees at a scan rate of 0.02 degrees per second (fig. 3.8). 1250°, 29.32°, 31.00°, 32.11°, 36.10° The X-ray diffraction (XRD) patterns of silver's FCC structure (JCPDS file: 00-006-0349) were attributed to reflections from the (222), (002), (020), (110), and (111) planes. Additionally, peaks with 2Theta values of 11, 18 and 70 degrees coexisted. They line up with silver nitrate's FCC crystalline planes. Nanoparticles of silver nitrate were produced, as shown by the findings. Plant extract contains nitrate ions, which react with silver ions to produce silver nitrate nanoparticles.

SEM analysis

Typical nanoparticle sizes of 127nm, 136nm, 141nm, 121 nm and 157nm were successfully produced, as shown by SEM pictures. Nanoparticle sizes in plant roots range from 126nm to 532nm, whereas those in shoots range from 120nm to 429nm (fig. 9).

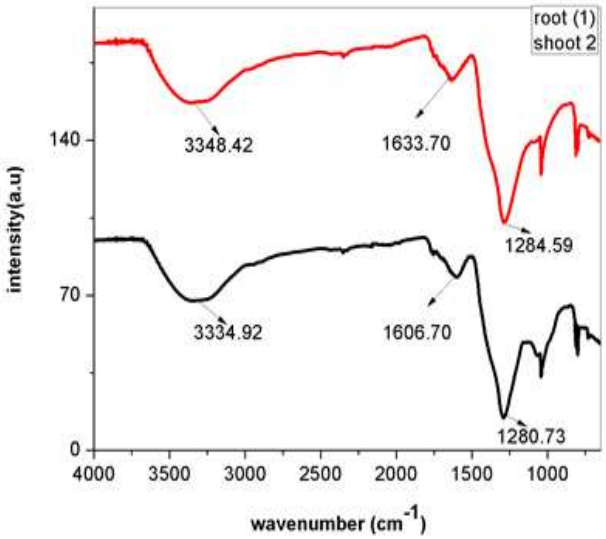

Fig. 10: Display FTIR analysis of roots and shoots nanoparticles from *V. wellichii*

FTIR analysis

The existence of distinct functional groups causes absorption bands to appear in a variety of places. Vibrational stretching of C-H alkenes, N-H amines and C=O carboxyl groups account for the broad 3334.92 nm absorption band from the roots and 3348.42 nm band from the shoots. The bands at 1606.70 were identified as the bending vibration of N-H amines and C=C of alkenes in both the roots and the shoots. The C-N stretching vibration of amines and the C-O and C-OH stretching vibration of anhydrites are to blame for this (fig. 10).

DISCUSSIONS

Nosocomial infections were responsible for 75,000 fatalities in the United States in 2011 (Santajit & Indrawattana, 2016). Antimicrobial-resistant pathogen infections are a major cost and resource drain for healthcare systems across the world. Due to diagnostic ambiguities and expensive treatment costs, people have begun to lose faith in traditional medicine, despite the fact that death and morbidity rates are quite high (Dunachie,

Day & Dolecek 2020). The most efficient way to accomplish this objective is by the green synthesis of silver NPs. Various plant extracts, such as *V. wallichii*, have been used in the past for the antibacterial activity of numerous human pathogenic bacteria, including MDR pathogens; however, no studies involving the use of MDR ESKAPE have been reported.

The biggest ZOI (20mm) was seen against *E. faecium*; however our water root extracts were effective against all MDR ESKAPE bacteria. The aqueous extract of *Piper auritum* (20mg/ml) did not exhibit the inhibitory action previously described, however, when compared to all other research (Perez Gutierrez *et al.,* 2012). In this study, the largest zone of inhibition for Extract from water shoots showed a 17mm detection range against *E. faecium* and a 13mm detection range against *E. cloacae*.

The lowest zone of inhibition (ZOI) found in this extract was 11mm and it was effective against the bacteria *S. aureus*, *K. pneumoniae* and *A. baumannii*. The antibacterial activity of the crude extract and other plant fractions was recently evaluated against 11 pathogens, such as the ones that cause typhoid fever, Shigellosis and pyogenic infections in humans, such as *Salmonella typhi* and *Shigella sonnei*. Our results show that the aqueous shoots extract has greater antibacterial activity than those reported by Khan *et al*. (2023), who found that the maximum antibacterial activity against *S. typhi, Shigella*, and *S. aureus* was 8mm, 10mm and 10.5mm, respectively. Another investigation found that the antibacterial activity of the leaf aqueous extract was the lowest, especially against *K. pneumoniae* (Man, Santacroce, Iacob, Mare & Man, 2019). This research proposes that a plant extract might be the most effective therapy for these problems, but that more testing is needed before it can be made available to the public.

It was also shown that an extract of the roots and shoots, preserved in methanol, is effective against multidrug-resistant bacteria such *E. faecium, P. aeruginosa* and *S. aureus* (fig. 3). The results showed that of the three solvent extracts examined, the methanol extract was the most efficient against all of the species. The largest zone of inhibition (ZOI) was seen (13) against *M. furfur*. The *S. aureus* and *P. mirabilis* also showed ZOI=12mm, followed by *C. albicans* and *A. niger* and finally *M. furfur*. Growth of *E. faecium* and *K. pneumoniae* was likewise significantly inhibited (ZOI= 11 mm), but that of *P. aeruginosa* and *S. epidermidis* was inhibited to a lesser extent (ZOI= 9 mm). Oluduro (2012) used the agar well diffusion technique to show that the methanolic extract was more efficient than the water extract against the tested organism. This may be due to the fact that methanolic extract contains more of the antioxidant polyphenols such flavonoids and anthraquinones (Kuri-GarcAa & GuzmA, 2017).

AgNPs have been shown to successfully limit microbial growth in prior research. When compared to *E. coli* and *C. albicans*, *P. aeruginosa* showed the largest zone of inhibition. Our results show that AgNPs are the least effective against *K. pneumonia* of all the microorganisms we examined. *P. aeruginosa* showed the greatest sensitivity to AgNPs among the microorganisms examined, whereas *K. pneumonia* showed the greatest resistance. Root AgNPs had the greatest effect on *P. aeruginosa* and *K. pneumoniae* (Nasar *et al*., 2022). Our results suggest that all of the species we studied are sensitive to SNPs in their roots and branches. This is due to silver's superior penetrative abilities compared to those of aqueous or methanolic extracts, which are often used for similar purposes. Shoot single nucleotide polymorphisms have been shown to be very susceptible to *P. aeruginosa*.

The plant extracts and NPs were effective against *S. aureus*, but the bacterium proved resistant to *V. wallichii* aqueous root extracts. Roots and shoots NPs were the most effective, with MICs of 2-4mg/ml, as predicted (Sindhu, Singh, Shirkhedkar, & Panichayupakaranant, 2022). Inhibitory doses of methanolic roots extract were 6 and 8mg/ml, however *P. aeruginosa* was resistant. *A. baumannii* was effective against *K. pneumoniae* at both 4mg and 6mg doses. Most Gram-negative bacteria were more resistant to the plant extracts than *E. faecium* and *S. aureus* (Piatek *et al.,* 2020). The SNPs found in the roots and shoots showed the most promise in decreasing bacterial concentrations by 2mg/ml against *E. faecium, S. aureus* and *P. aeruginosa*. A methanolic extract of the shoots was effective against *E. coli, S. aureus* and *P. aeruginosa* at 2mg/ml and 6mg/ml, respectively, while an extract of the roots was effective against *K. pneumoniae, A. baumannii, and E. cloacae* at 8mg/ml and 6mg/ml, respectively. The aqueous extracts of both the roots and the shoots showed strong antibacterial activity against both *E. faecium* (MIC 2 and 4mg/ml) and *S. aureus* (MIC 6mg/ml).

Based on traditional medical applications identified via ethno botanical research, six plants were selected for pilot tests (Joshi *et al*., 2020). High levels of alkaloids, saponin, and combination anthraquinone and cyanogenetic glycosides were all found in Finbriata. The bactericidal S. sparganophora and the antibacterial *V. amygdalina* were found in *G. brevis. V. amygdalina* stands apart from other plants of its kind due to the strength of its compounds that are responsible for a number of biological processes and the intense bitterness of its taste (Ajao *et al.,* 2021). *C. argentea* only had tannins and alkaloids, but *A. spinosus* had anthraquinones, alkaloids and cardenolide. To verify the medicinal properties of these plants, we analysed their phytochemical constituents (Kasim *et al*., 2012). *V. wallichii* have tannins and saponins in addition to alkaloids and flavonoids. These extracts' anti-inflammatory effects may stem from the plant's high

flavonoid concentration. Traditional treatments for inflammatory diseases, such as scorpion stings and liver protection, have been shown to be effective in scientific investigations.

CONCLUSION

The antimicrobial resistance in bacteria may create It is urgent that we find a solution to the growing problem of antibiotic resistance in bacteria. Plants with medicinal properties are utilised to combat harmful microorganisms. In this research, MDR ESKAPE-related illnesses were treated using plants like *V. wallichii.* Results showed that green chemistry silver nanoparticles were more effective against bacteria than plant extracts. More work in nanotechnology is required to develop nanomedicines from this plant extract that might cure various HAIs and other potentially fatal illnesses. Quantitative phytochemical study is necessary to identify the most physiologically active chemicals in this plant.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Researchers supporting project number (RSP2023R185), King Saud University, Riyadh, Saudi Arabia.

REFERENCES

Ajao AA-n, Mukaila YO and Sabiu S (2021). Wandering through southwestern Nigeria: An inventory of Yoruba useful angiosperm plants. *Heliyon,* **8** (1): 1-10.

Dunachie SJ, Day NP and Dolecek C (2020). The challenges of estimating the human global burden of disease of antimicrobial resistant bacteria. *COMICR,* **57** (22): 95-101.

Gupta A, Naraniwal M and Kothari V (2012). Modern extraction methods for preparation of bioactive plant extracts. *IJONAS,* **1**(1): 8-26.

Joshi B, Panda SK, Jouneghani RS, Liu M, Parajuli N, Leyssen P and Luyten W (2020). Antibacterial, antifungal, antiviral and anthelmintic activities of medicinal plants of Nepal selected based on ethnobotanical evidence. *eCAM,* **25** (12)**:** 1-14.

Jugran AK, Bhatt ID and Rawal RS (2021). Cultivation and Utilization of *Valeriana jatamansi* Jones for Conservation Planning and Management. *In*: Medicinal Plants: Domestication, *Biotechnol. Reg. Med.*, **28**(11): 113-138.

Kasim L, Ayodele O, Effedua H, Adejumo O, Ekor M and Fajemirokun T (2012). Antimicrobial activity of six selected plants against some strains of pathogenic organisms. *JMA,* **4**(3): 54-59.

Khan SU, Anjum SI, Ansari MJ, Khan MHU, Kamal S, Rahman K and Khan SU (2019). Antimicrobial potentials of medicinal plant's extract and their derived silver nanoparticles: A focus on honey bee pathogen. *Saudi J. Biol. Sci.,* **26**(7): 1815-1834.

Kuri-Garcaa A and Guzma S (2017). Phenolic profile and antioxidant capacity of *Cnidoscolus chayamans*a and *Cnidoscolus aconitifolius*: A review. *J. Med. Plant Res.,* **11**(45): 713-727.

Man A, Santacroce L, Iacob R, Mare A and Man L (2019). Antimicrobial activity of six essential oils against a group of human pathogens: A comparative study. *Pathogens,* **8**(1): 1-15.

Nasar MQ, Shah M, Khalil AT, Kakar MQ, Ayaz M, Dablool AS and Shinwari ZK (2022). Ephedra intermedia mediated synthesis of biogenic silver nanoparticles and their antimicrobial, cytotoxic and hemocompatability evaluations. *Inorg. Chem. Commun,* **137** (96): 109252.

Owuama CI (2017). Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method. *Afr. J. Microbiol. Res.,* **11**(23): 977-980.

Perez Gutierrez RM, Flores Cotera LB and Gonzalez AMN (2012). Evaluation of the antioxidant and anti-glication effects of the hexane extract from Piper auritum leaves *in vitro* and beneficial activity on oxidative stress and advanced glycation end-product-mediated renal injury in streptozotocin-treated diabetic rats. *Molecules,* **17**(10): 11897-11919. .

Santajit S and Indrawattana N (2016). Mechanisms of antimicrobial resistance in ESKAPE pathogens. *Biomed Res. Int.,* **12**(2): 1-8

Suroowan S, Javeed F, Ahmad M, Zafar M, Noor MJ, Kayani S and Mahomoodally MF (2017). Ethnoveterinary health management practices using medicinal plants in South Asia: A review. *Vet. Res. Commun.,* **41**(12): 147-168.

Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL and Carmeli Y (2018). Discovery, research and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis,* **18**(3): 318-327.

Marcucci C, Rademacher M, Kamecki F, Pastore V, Bach HG, Ricco RA, Wagner ML, Knez D, Gobec S, Colettis N and Marder M (2023). Biological evaluation of valeriana extracts from Argentina with potent cholinesterase inhibition for the treatment of neurodegenerative disorders and their comorbidities-The case of *Valeriana carnosa* Sm. (Caprifoliaceae) studied in mice. *Pharmaceuticals.* **16**(1): 129.

Kalhori MR, Soleimani M, Alibakhshi R, Kalhori AA, Mohamadi P, Azreh R and Farzaei MH (2023). The potential of miR-21 in Stem Cell Differentiation and its application in tissue engineering and regenerative medicine. *SCRR.* **11**(4):1-20.

Jamal G, Parveen R, Khan MA, Srivastava V, Mustafa S, Ahmad S and Husain SA (2023). A Review on

Valeriana wallichii: Chemical composition and pharmacological research. *Curr. Tradit. Med.* **9**(4):55-71.

Khan TA, Al Nasr IS, Koko WS, Ma J, Eckert S, Brehm L, Ben Said RS, Daoud I, Hanachi R, Rahali S and van de Sande W (2023). Evaluation of the antiparasitic and antifungal activities of new synthetic piperlongumine-type cinnamide derivatives: Booster effect by halogen substituents. *Chem. Med. Chem*, **22**(12): 44-77.