International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2023.872.880

Research Article
A Comparative Study of Pentahydrate Copper Sulphate and *Acacia nilotica* (L.) Extract Against Bacterial and Fungal Isolates

[1]Lienda Bashier Eltayeb, [1]Abeer Abd Alla Ellhassan Babekir, [2]Rehab Ali, [3]Naveed Ahmed, [4]Anass Mohamed Abaas, [5]Nasir Abdelrafie Hamad, [3]Muhammad Absar and [6,7]Zeshan Ali

[1]Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Prince Sattam bin Abdulaziz University, Al-Kharj 11942, Riyadh, Saudi Arabia
[2]Department of Drug and Toxicology, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
[3]Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia
[4]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Al-Jouf University, Sakaka 72388, Saudi Arabia
[5]Department of Biochemistry, Faculty of Medicine, National University of Sciences and Technology, Suhar, Oman
[6]College of Food Science and Engineering, Bohai University, Jinzhou 121013, China
[7]Department of Biosciences, COMSATS University Islamabad, Sahiwal, Punjab 57000, Pakistan

Abstract

Background and Objective: One of the most important issues currently challenged by public health organizations worldwide is Antimicrobial Resistance (AMR). The current study aimed to evaluate the antimicrobial effects of *Acacia nilotica* extracts and Copper Sulphate ($CuSO_4$) on different clinical bacterial and fungal isolates. **Materials and Methods:** Using standard microbial methods, four bacterial species (*Staphylococcus aureus*, vancomycin-resistant staphylococci, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *E. coli*) and fungus species were obtained from clinical samples of various infections. By using colonial morphology, Gram's stain and metabolic assays in accordance with accepted microbial practices, the strains were identified. The strains were subcultured onto Sabouraud dextrose agar, MacConkey agar and nutrient agar blood agar before being kept at 37°C for roughly 18 to 24 hrs to determine purity. The antibacterial activity was screened using the paper disc diffusion technique. **Results:** The *Acacia nilotica* extracts have shown the maximum antibacterial activity against *Pseudomonas* species followed by vancomycin sensitive *S. aureus*, vancomycin resistant *S. aureus* while, the least activity was observed against *Bacillus subtilis* at the concentration of 750 μg mL^{-1}. *Candida albicans* and *Rhizopus* have shown sensitivity against tested drugs. The antimicrobial activity of $CuSO_4$ against tested bacterial species revealed the highest sensitivity as well as for fungal species. *Penicillium* species were completely resistant against both studied extracts. **Conclusion:** Results of the present study have reported a recommended use of *Acacia nilotica* extracts compared with $CuSO_4$ for their potential antimicrobial effects as significant antibacterial as well as antifungal agents.

Key words: Antimicrobial activity, *Acacia nilotica*, pentahydrate copper sulfate, multi-drug resistance isolates, vancomycin-resistant staphylococci, bactericidal

Citation: Eltayeb, L.B., A.A.A.E. Babekir, R. Ali, N. Ahmed and A.M. Abaas *et al*., 2023. A comparative study of pentahydrate copper sulphate and *Acacia nilotica* (L.) extract against bacterial and fungal isolates. Int. J. Pharmacol., 19: 872-880.

Corresponding Author: Lienda Bashier Eltayeb, Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Prince Sattam bin Abdulaziz University, Al-Kharj, Riyadh, Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A wide range of antibiotics are used in both human and animal medicinal procedures, however, bacterial resistance has significantly reduced their effectiveness[1,2]. One of the most important issues presently confronting public health organizations around the globe is microbial resilience[3]. Although bacterial resistance may develop naturally, it has been accelerated by a long history of using antibiotics inappropriately to treat viruses and other illnesses that are not brought on by bacteria. As antibiotics lose their effectiveness, treating increased resistance is becoming more difficult[4,5]. How to transform the prescribing and use of antibiotics has been the subject of a recent study[2]. Finding novel compounds to lessen and cure bacterium resistance is one of the objectives of pharmaceutical firms. One of the accessible option sources that could address the issue of bacterium tolerance is natural preparations. The haphazard use of antibiotics in the treatment of contagious illnesses has led to a rise in multiple drug resistance[6,7], a serious issue that has compelled researchers to look for novel antibacterial compounds. Natural preparations are thought to be one of the main sources of yet-to-be-identified antibacterial compounds in nature. In order to manage contagious illnesses in a different manner, it is necessary to use therapeutic herbs[8-11].

The shrub *Acacia nilotica* has a wide range of uses and can be used to cure a wide range of illnesses[12,13]. The plant's bark is frequently used to treat respiratory manifestations, diarrhea, leukoderma and bleeding. The *Acacia nilotica* contains many bioactive components, like tannin, phlobatannin, catechin, gallic acid, pyrocatechol, protocatechuic acid, 5-epigallocatechin-7gallate, epigallocatechin and 7-digallate. Additionally, human illnesses caused by *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Klebsiella* spp., are treated with its delicate foliage and buds[14,15]. A well-known bioflavonoid with biochemical effects is quercetin. Antioxidant, anti-inflammatory, anti-mutagenic, anti-cancer, anti-microbial and antiviral properties are all positive effects[16].

On the other hand, the Environmental Protection Agency of USA has approved the use of antimicrobial copper since 2008[17]. Copper surfaces or surfaces coated with this metal, have been shown to have a 90-95% lower bacterial load[18,19]. Furthermore, recent evidence indicates that physiological Cu can be used as potential antimicrobial agents[20]. The current study was designed to examine the antibacterial and antifungal effects of natural extract of *A. nilotica* compared to copper sulphate salt in a trial to treat and control some of the multidrug resistant bacteria considered dangerous to human health.

MATERIALS AND METHODS

Study area: An *in vitro* experimental study conducted on *Acacia nilotica* herbal extract and pentahydrate copper sulfate solution against clinical bacterial isolates at Clinical Laboratory of Faculty of Applied Medical Sciences, Department of Medical Laboratory Science, Prince Sattam bin Abdul-Aziz University, Al-Kharj Kingdom of Saudi Arabia; during the period from December, 2021 to April, 2022.

Ethical consideration: An ethical approval was granted from the Ethical Committee of Prince Sattam bin Abdul-Aziz University. Permission for bacterial isolates were collected and granted by the authorities of Al-Kharj hospital for children and maternity.

About 6 bacterial isolates includes: (*Staphylococcus aureus*, vancomycin resistant staphylococci, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *E. coli* and *Klebsiella pneumoniae* that were isolated from clinical samples of different infections using standard methods and four fungal species (*Candida albicans*, *Aspergillus niger*, *Rhizopus* species and *Penicillium*) were isolated from air contaminant samples and identified using microscopy and lactophenol cotton blue staining method. The bacterial strains were identified by using the morphology of colonies, Gram staining of prepared smears and the metabolic assays in accordance with accepted microbial practices. To check for purity, the strains were sub-cultured onto nutritional agar, blood agar, MacConkey agar and sabouraud dextrose agar and then kept at 37°C for roughly 18 to 24 hrs.

Preparation of extracts

Copper sulphate preparation: The broth dilution method was used to determine the MIC-Cu. To make a 50 g L^{-1} stock solution, copper (II) sulfate pentahydrate ($CuSO_4$ $5H_2O$) (Merck Millipore, Germany) was used. This stock solution was filter-sterilized prior to use to achieve the final concentrations. Cu^{2+} concentrations were ranged from 62.5 to 500 mg mL^{-1} using two-fold dilution series to give concentrations as 16, 8, 4 and 2% (500, 250, 125 and 62.5 mg mL^{-1}), respectively[21].

***Acacia nilotica* plant extracts:** The 1000 g of *Acacia nilotica* was collected from the local market and identified and authenticated by the taxonomists of medicinal and aromatic plants. Air-dried ground plant's material (20 g of *A. nilotica*) was extracted for 6 hrs at room temperature in an orbital shaker (Gallenkamp, UK) or under reflux on a water bath using each of the solvents-absolute methanol, absolute ethanol, aqueous ethanol and aqueous methanol (200 mL). The leftovers and the samples were sorted using Whatman

filtration paper. The same brand-new liquid and preparations were used to separate the leftovers twice. Using a rotating evaporator (Büchi rotavapor R-144, Flawil, Switzerland), the mixed extracts were condensed and released from the liquid at decreased pressure and 5°C. To determine the output, the desiccated crude condensed extracts were measured and kept in a refrigerator (-4°C) until they were used for analysis[22,23].

Detection of vancomycin resistant staphylococci VRS: Utilizing vancomycin screens and adhering to National Committee for Clinical Laboratory Standards (NCCLS), the identification for VRSA was performed. The antibiotic panels were selected based on the Clinical and Laboratory Standards Institute (CLSI) guidelines. The antimicrobial sensitivity testing of the staphylococci strains was determined using the modified Kirby-Bauer disc diffusion method[24,25] on Muller Hinton agar (MHA) using the standard zone sizes of inhibition to define sensitivity or resistance against ciprofloxacin, rifampicin, chloramphenicol, gentamicin, penicillin and vancomycin.

Using clean brushes, a solution made from each strain that was equal to 0.5 McFarland standards was evenly distributed on the MHA plate that contained NaCl (4%) and vancomycin (6 g mL^{-1}). For 24 hrs, all of the dishes were kept at 35°C. Zones of suppression found above 12 mm were considered as sensitive, 11-12 mm as intermediate and <12 mm as resistant. Furthermore, the presence of growth (>1 colony) around the disk was also considered as vancomycin-resistant.

Calculation of the multiple antibiotic resistance (MAR) index: A method for evaluating possible risks and looking for antibiotic-resistant microbes is the MAR index. This study utilized 14 antibacterial compounds. The MAR index was computed by dividing the total number of antimicrobials used during the study by the number of antimicrobials to which the strain imparted resistance.

Antimicrobial susceptibility testing for extract

Disc diffusion method: Using copper sulphate and Mueller Hinton culture, the disc diffusion technique was utilized to test the antimicrobial activity of *Acacia nilotica* plant preparations. The research was carried out in accordance with the recommendations of the National Council for Clinical Laboratory Standards. The bacterial suspension was decreased (turbidity = McFarland standard 0.5) using clean physiological solution. The MHA surface was evenly swabbed with 100 µL of bacterial solution and the inoculum was given 5 min to dry. The surface of the MHA was covered with 6 mm diameter sterilized filter paper plates that had been immersed in 20 L of a solution of each extract at various concentrations and serially reduced as 40, 20, 10 and 5% (750, 375, 187.5 and 93.75 mg mL^{-1}, respectively). And for copper sulphate, 16, 8, 4 and 2 (500, 250, 125 and 62.5 mg mL^{-1}).

Distilled water-soaked filter paper was certainly used as a reference for both samples for quality assurance. The infected dishes underwent a 24 hrs inversion incubation period at 37°C. The inhibitory zones' widths (mm) were determined. The *Escherichia coli* K12 ATCC 10798, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25932, *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 strains were used as control. Quality control was achieved at each step during research through sterilization purification, subculture and susceptibility test which was conducted twice.

To find the lowest inhibitory dose, a 10 mL sample from each tube that showed no microbe activity in the MIC Cu test was grown on MHA and kept at 37°C for 18 to 24 hrs. The minimum bactericidal concentration was determined to be the least amount of copper that produced no growth (MBC-Cu).

Statistical analyses: Data was analyzed using the software SPSS. One-way ANOVA and Pearson correlation coefficient (r) ($p \leq 0.05$) was used. Diameter zones of inhibition of extracts are reported as Mean±Standard Deviation.

RESULTS

The results demonstrated the samples' high level of multidrug resistance (Table 1). All of the Enterobacteriaceae strains were incredibly resistant to amoxicillin and imipenem. Current research revealed that the examined genotypes were becoming more resistant to the use of common antibiotics, demonstrating the need for tracking systems to track drug resistance (Table 1 and 2).

Antibacterial activity of synthesized *Acacia nilotica* seeds and copper sulphate penta-hydrate $CuSO_4 \cdot 5H_2O$) were studied amongst both Gram-positive and Gram-negative pathogenic bacteria and the opportunistic pathogenic yeast *C. albicans*, *A. niger*, *Rhizopus* species and *Penicillium* species were used to investigate antifungal potential with different concentration as 32, 16, 8, 4 and 2% (1000, 500, 250, 125 and 62.5 mg mL^{-1}) for copper sulfate. *Acacia nilotica* showed maximum zone of inhibition against *Pseudomonas* species (18 mm) followed by vancomycin sensitive *S. aureus* (VSSA), Vancomycin-Resistant *S. aureus* (VRSA) and least was observed against *Bacillus subtilus* at 750 µg mL^{-1} concentration, while *Candida albicans* and *Rhizopus* only showed sensitivity against *Acacia nilotica* MIC (15 mm), while *Aspergillus niger*. All data were summarized in (Table 3).

Table 1: Antimicrobial susceptibility pattern of predominant isolated bacteria

Bacterial isolates	Gentamicin (GM) 10 µg	Penicillin (P) 30 µg	Amoxicillin (A) 10 µg	Vancomycin (VAN) 30 µg	Erythromycin 15	Azithromycin ATH 15	Chloramphenicol (CL) 30 µg	Piperacillin/tazobactam TZP 75	Ciprofloxacin (CIP) 15	Nalidixic acid (NA) 10	Colistin (CO) 25	Ceftriaxone	Cefixime (CFM) 5	Rifampicin (RIF) 30
Vancomycin sensitive *S. aureus*	17	9	11	19	16	10	15	22	19	11	8	25	18	11
Vancomycin resistant *S. aureus*	11	8	10	10	12	10	16	19	20	11	9	12	14	8
Bacillus subtilis	18	9	11	22	10	ND	ND	18	12	ND	7	23	18	ND
Pseudomonas species	7	7	9	7	8	6	10	6	10	5	5	10	18	6
Escherichia coli	16	10	13	24	9	11	15	23	25	10	10	22	14	18
Klebsiella pneumoniae	9	14	12	9	10	9	23	14	22	6	5	25	15	27

ND: Not determined, inhibition zone diameter in millimeters (mm) of the Isolates

Table 2: Multiple antibiotic resistance (MAR) index for clinical bacterial isolates

Bacterial species	Total number of antibiotics	Antibiotics resistant profile	MAR index (%)
Vancomycin sensitive *S. aureus*	14	6	42.9
Vancomycin resistant *S. aureus*	14	8	57.14
Bacillus subtilis	10	3	21.42
Pseudomonas aeruginosa	14	13	92.9
Escherichia coli	14	6	42.9
Klebsiella species	14	7	50.0

Table 3: Zone of inhibition of crude *Acacia nilotica* extract against bacterial and fungal species

	Inhibition zone diameter (mm) of crude *Acacia nilotica* extract				
Bacterial species	400 mg mL^{-1} ±SD	200 mg mL^{-1} ±SD	100 mg mL^{-1} ±SD	50 mg mL^{-1} ±SD	25 mg mL^{-1} ±SD
Vancomycin sensitive *S. aureus*	17.6±1.2	16.3±0.7	13.3±1.4	11.6±0.5	8±0.00
Vancomycin resistant *S. aureus*	16±0.8	13±0.6	11±1.3	9±1.0	5±1.00
Pseudomonas species	18±0.4	16±0.3	12±0.8	10±0.7	6±0.05
Bacillus subtilis	15±1.3	14±1.1	11±1.5	10±0.9	5±1.5
Escherichia coli	16±1.4	16±1.3	15±0.7	13±0.4	9±0.2
Klebsiella pneumoniae	9±1.2	8±0.7	R	R	R
Candida albicans	20±0.6	19±1.9	17±1.6	15±0.9	10±0.85
Aspergillus niger	6±1.3	R	R	R	R
Rhizopus species	9±0.7	8±1.5	8±0.5	6±0.29	R
Penicillium species	7±0.4	R	R	R	R

R: Resistant strain and ±SD: Standard deviation

Table 4 demonstrate Copper Sulphate ($CuSO_4 \cdot 5H_2O$) antimicrobial activity against bacterial species, there were comparable result among VSSA and VRSA revealed highest sensitivity (29 and 28 mm), respectively, as well as fungal species *Candida albicans*, *Rhizopus* and *Aspergillus niger* the MIC were (32, 30 and 28 mm). *Penicillium* species were completely resistant against both studied extractions.

Figure 1 displays antimicrobial pattern of Vancomycin sensitive *S. aureus*, *Vancomycin sensitive S. aureus* in *Acacia nilotica*, *Pseudomonas* species against copper sulphate and *Acacia nilotica*.

The antimicrobial MIC and MBC values of copper sulfate and *A. nilotica*, where the great antimicrobial activity was defined as MIC values of 250 and 800 mg mL^{-1} or less for copper sulfate and *A. nilotica*, respectively. Report of MBC/MIC ratio of *Acacia nilotica* in which the MBC-Cu equivalent to 1000 µg mL^{-1} of copper displayed the greatest bactericidal effect against bacterial isolates, while MBC-Cu equivalent to 500 µg mL^{-1} of copper displayed the greatest fungicidal. For *A. nilotica* 1600±0.00 µg mL^{-1} was revealed the best bactericidal against vancomycin sensitive *S. aureus* and *Pseudomonas*. All data displayed in Table 5-7.

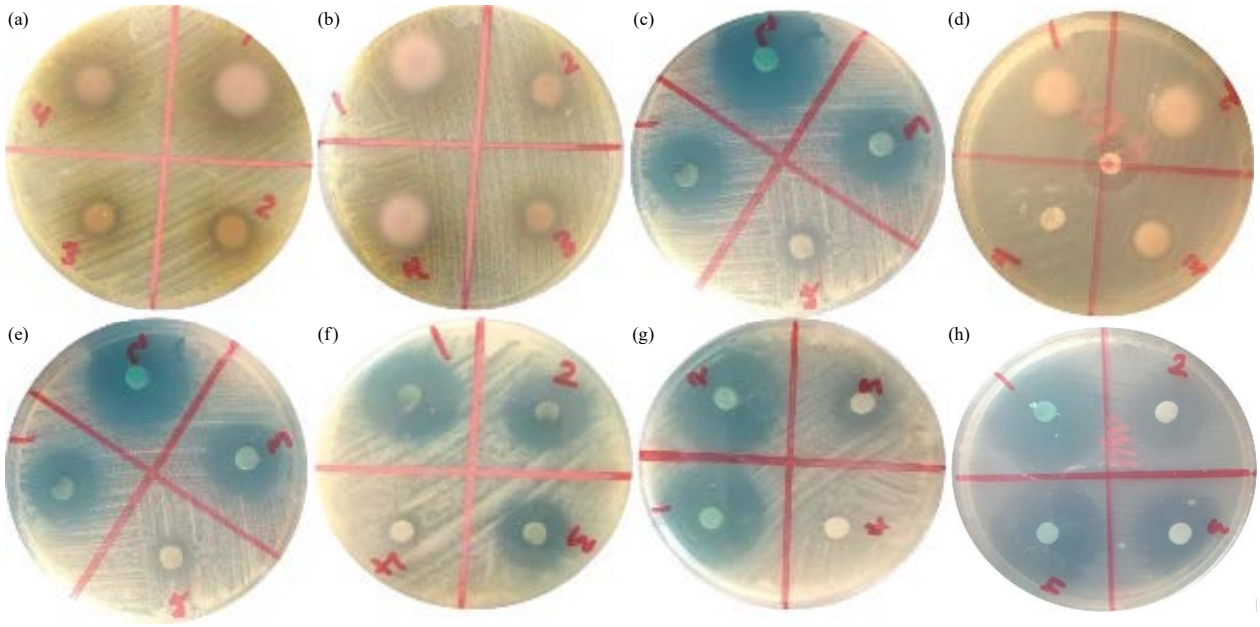

Fig. 1(a-h): (a) Vancomycin sensitive *S. aureus* (VSSA) in *A. nilotica*, (b) Vancomycin resistance *S. aureus* (VSRA) in *A. nilotica*, (c) Vancomycin resistance *S. aureus* in copper sulphate (d) Pseudo-monas species in *A. nilotica*. (e) *E. coli* in copper sulphate, (f) *Pseudomonas* species in copper sulphate, (g) *Aspergillus* species in copper sulphate and (h) *Rhizopus* species in copper sulphate

Table 4: Zone of inhibition of copper sulphate against bacterial and fungal species

	Inhibition zone diameter (mm) of copper sulphate				
Bacterial species	1000 mg mL^{-1}	1000 mg mL^{-1}	1000 mg mL^{-1}	1000 mg mL^{-1}	1000 mg mL^{-1}
Vancomycin sensitive *S. aureus*	29±2.03	27±1.4	24±2.3	20±1.5	14±0.2
Vancomycin resistant *S. aureus*	28±1.5	25±1.9	20±2.01	15±1.4	10±1.0
Pseudomonas species	10±1.9	9±1.6	8±1.4	8±0.9	R
Bacillus subtilis	17±1.03	8±1.09	6±0.25	R	R
Escherichia coli	23±0.5	20±0.9	19±1.02	15±1.4	14±0.9
Klebsiella pneumoniae	14±0.2	11±0.9	10±0.5	7±0.30	R
Fungal species					
Candida albicans	32±1.04	29±1.10	27±0.7	22±0.54	19±0.4
Aspergillus niger	28±2.08	18±2.98	17±0.43	9±1.7	8±0.15
Rhizopus species	30±1.46	28±0.45	13±1.05	11±1.6	10±0.25
Penicillium species	5±1.00	R	R	R	R

R: Resistant strain and ±SD: Standard deviation

Table 5: Minimum bactericidal and fungicidal concentrations of copper sulfate and *Acacia nilotica* against nosocomial pathogens

Bacterial species	MIC copper sulfate (Mean±SD)	MIC *Acacia nilotica* (Mean±SD)
Vancomycin sensitive *S. aureus*	125±0.00	800±0.00
Vancomycin resistant *S. aureus*	250±0.00	400±0.00
Pseudomonas species	125±0.00	400±0.00
Bacillus subtilis	250±0.00	100±0.00
Escherichia coli	125±0.00	200±0.00
Klebsiella pneumoniae	250±0.00	400±0.00
Candida albicans	250±0.00	100±0.00
Aspergillus niger	500±0.00	400±0.00
Rhizopus species	500±0.00	200±0.00
Penicillium species	250±0.00	400±0.00

Table 6: Report of MBC/MIC of *Acacia nilotica*

Bacterial species	MBC-*A. nilotica* (μg mL^{-1}) Mean±SD	*A. nilotica* MBC/MIC antimicrobial activity	Antibacterial activity of *A. nilotica*
Vancomycin sensitive *S. aureus*	1600±0.00	2	Bactericidal
Vancomycin resistant *S. aureus*	800±0.00	4	Bacteriostatic
Pseudomonas species	800±0.00	2	Bactericidal
Bacillus subtilis	400±0.00	4	Bacteriostatic
Escherichia coli	1600±0.00	8	Bacteriostatic
Klebsiella pneumoniae	1600±0.00	4	Bacteriostatic
Candida albicans	200±0.00	2	Bactericidal
Aspergillus niger	1600±0.00	4	Bacteriostatic
Rhizopus species	1600±0.00	8	Bacteriostatic
Penicillium species	1600±0.00	4	Bacteriostatic

Table 7: Report of MBC/MIC of copper sulphate

Bacterial species	MBC-Cu (μg mL^{-1}) Mean±SD	Cu MBC/MIC antimicrobial activity	Antimicrobial activity of Cu
Vancomycin sensitive *S. aureus*	1000±0.00	8	Bactericidal
Vancomycin resistant *S. aureus*	1000±0.00	4	Bacteriostatic
Pseudomonas species	500±0.00	4	Bactericidal
Bacillus subtilis	500±0.00	4	Bacteriostatic
Escherichia coli	1000±0.00	8	Bacteriostatic
Klebsiella pneumoniae	1000±0.00	4	Bacteriostatic
Candida albicans	500±0.00	2	Bactericidal
Aspergillus niger	500±0.00	1	Bactericidal
Rhizopus species	500±0.00	2	Bactericidal
Penicillium species	1000±0.00	4	Bacteriostatic

DISCUSSION

In the present study, some Gram-positive bacteria (*S. aureus*) were found to be resistant to vancomycin, an essential antibiotic for treating Gram-positive bacterial infections. Significant advances have been made in the knowledge of the molecular mechanisms and genetic characteristics of vancomycin resistance in Gram-positive bacteria. Even through this, it is still uncertain where the resistant strains emerged. The existence of genes encoding *vanA*, *vanH*, *vanR*, *vanS* and *vanX* homologues in secondary metabolic products of glycopeptide-producing organisms is a prospective resistance source[26]. Glycopeptides, either alone or in combination with another antibacterial, are commonly the only treatment option available for infections triggered by multi-resistant strains of staphylococci, streptococci and enterococci. Vancomycin-resistant gram-positive bacteria can develop and spread antibiotic resistance to all antibiotics. As a result, understanding the molecular and structural vancomycin resistance mechanisms may point to alternative solutions to this significant matter[27].

The crude extract of *A. nilotica* hindered pathogenic isolates with a mean zone of inhibition against *Pseudomonas* species (18 mm) followed by VSSA and VRSA and the least was observed against *Bacillus subtilis* (15 mm) at 750 μg mL^{-1} concentration. While, *Candida albicans* and *Rhizopus* were only show sensitivity against *Acacia nilotica* MIC (15 mm), while *Aspergillus niger*. All data were summarized in Table 1. Table 2 demonstrate Copper Sulphate ($CuSO_4 \cdot 5H_2O$) antimicrobial activity against bacterial species, there are comparable result among vancomycin sensitive *S. aureus* and vancomycin resistant *S. aureus* revealed the highest sensitivity (29 and 28 mm), respectively, as well as fungal species *Candida albicans*, *Rhizopus* and *Aspergillus niger* the MIC were 32, 30 and 28 mm). *Penicillium* species were completely resistant against both studied extractions.

It seems like an innovative and hopeful concept to use copper as an option to avoid nosocomial infections[28]. In different laboratory-based studies, the biocidal impact of copper as a touch surface has been thoroughly examined and it appears to have a possible use in healthcare infection prevention and control efforts[15,20,29]. The antiseptic properties of copper are being used in a variety of other contexts in saddition to as a touch surface[20]. According to this study's *in vitro* evaluation of the antimicrobial efficacy of copper sulphate salts to inactivate an important nosocomial pathogen, bacteria with a wide pattern of antibiotic resistance were among the bacteria that were inhibited from growing at a copper concentration as low as 800 g mL^{-1} in 80% of the isolates from various biological fluids from Algerian patients.

Additionally, a greater copper content of 1600 g mL^{-1} should guarantee deactivation, which means it stops all isolates from multiplying. Copper is known to produce the following antibacterial effects without being mutagenic: Copper has been demonstrated to exhibit the following antibacterial properties lacking being mutagenic: Deactivation of enzymatic pathways, the generation of reactive oxygen species, precipitation of microbial proteins, bacterial cell wall modification and destruction or modification of nucleic acid synthesis[20]. These effects are still being studied as part of copper's antibacterial mechanisms. According to a previous study, copper is active against both Gram-positive and Gram-negative bacteria that can cause illness[30]. Gram-negative isolates had smaller MBCs-Cu than Gram-positive isolates. The one-way ANOVA study did not reveal any variations that were statistically significant between various bacterial types. Contrarily, a large disparity between Gram-negative and Gram-positive microbes was found.

Considering the findings of this study, there is a critical need to conduct phytochemical studies and clinical trials to understand how *A. nilotica* and copper sulphate extracts effectively kill microbial species. More purification, identification and characterization are required for the discovery of novel antibacterial compounds derived from *A. nilotica* for therapeutic medicinal applications. It's mandatory to authenticate phenotypic resistance patterns revealed, because some non-inherited resistance is linked with certain mechanisms including biofilm formation, a stationary growth phase or microbial persistence. Ethanol and chloroform extracts from *Acacia nilotica* were not used, furthermore only fruits were studied so comprehensive research for another part of *Acacia* such as leaves, roots, seeds, bark, flowers, gum and immature pods is essential.

CONCLUSION

Current study reported a simple, safe, environment-friendly and economical biological herb of *Acacia nilotica* extraction compared with copper sulphate, which was assessed for its antimicrobial action and demonstrated significant antibacterial as well as antifungal activity against nosocomial microorganisms. The multidrug resistant nosocomial microbes are significantly inhibited by the copper sulphate compounds' antimicrobial action. The research supported the copper sulfate compounds' efficacious (bactericidal or bacteriostatic) action. According to this research, copper resistance and the trait of drug resistance are presumably related in some way. The possibility for tolerance to emerge, as has occurred with antibiotics, is one worry about using copper sulphate as a sterilizing agent. According to this research, 43.75% of Enterobacteriaceae, 80% of staphylococci and one type of *P. aeruginosa* were deemed copper-resistant bacteria. This research confirms earlier findings that *S. aureus* and the majority of Gram-negative bacteria also carry a multi-copper oxidase.

SIGNIFICANCE STATEMENT

The haphazard use of antibiotics in the treatment of contagious illnesses has led to a rise in multiple drug resistance, a serious issue that has compelled researchers to look for novel antibacterial compounds. Natural preparations are thought to be one of the main sources of yet-to-be-identified antibacterial compounds in nature. In order to manage contagious illnesses in a different manner, it is necessary to use therapeutic herbs such as *Acacia nilotica*, compared to an edible, powerful oxidizing agent (pentahydrate copper sulphate). The current study aimed to evaluate the antimicrobial effects of *Acacia nilotica* extracts and Copper Sulphate ($CuSO_4$) on different clinical bacterial and fungal isolates. Results recommended use of *Acacia nilotica* extracts compared with $CuSO_4$ for their potential antibacterial significant as well as antifungal agents.

ACKNOWLEDGMENTS

This publication was supported by the Deanship of Scientific Research at Prince Sattam Bin Abdul Aziz University. Authors appreciated to Department of Medical Laboratory Sciences, College of Applied Medical Sciences at Prince Sattam bin Abdul-Aziz University and Al Kharj Maternity and Children Hospital for providing a clinical bacterial isolates.

REFERENCES

1. Rabaan, A.A., S. Alhumaid, A. Al Mutair, M. Garout and Y. Abulhamayel *et al*., 2022. Application of artificial intelligence in combating high antimicrobial resistance rates. Antibiotics, Vol. 11. 10.3390/antibiotics11060784.
2. Zeb, S., M. Mushtaq, M. Ahmad, W. Saleem and A.A. Rabaan *et al*., 2022. Self-medication as an important risk factor for antibiotic resistance: A multi-institutional survey among students. Antibiotics, Vol. 11. 10.3390/antibiotics11070842.

3. Zeshan, B., M.I. Karobari, N. Afzal, A. Siddiq and S. Basha *et al*., 2021. The usage of antibiotics by COVID-19 patients with comorbidities: The risk of increased antimicrobial resistance. Antibiotics, Vol. 11. 10.3390/antibiotics11010035.
4. Rabaan, A.A., K. Eljaaly, S. Alhumaid, H. Albayat and W. Al-Adsani *et al*., 2022. An overview on phenotypic and genotypic characterisation of carbapenem-resistant *Enterobacterales*. Medicina, Vol. 58. 10.3390/medicina58111675.
5. Yusof, N.Y., N.I.I. Norazzman, S.N.W. Ab Hakim, M.M. Azlan and A.A. Anthony *et al*., 2022. Prevalence of mutated colistin-resistant *Klebsiella pneumoniae*: A systematic review and meta-analysis. Trop. Med. Infect. Dis., Vol. 7. 10.3390/tropicalmed7120414.
6. Mustafai, M.M., M. Hafeez, S. Munawar, S. Basha and A.A. Rabaan *et al*., 2023. Prevalence of carbapenemase and extended-spectrum β-lactamase producing *Enterobacteriaceae*: A cross-sectional study. Antibiotics, Vol. 12. 10.3390/antibiotics12010148.
7. Rasool, Z., H. Noreen, A. Anjum, A. Rizvi and A.A. Rabaan *et al*., 2022. Genotypic and phenotypic characterization of erythromycin-resistant *Staphylococcus aureus* isolated from bovine mastitis and humans in close contact. Trop. Med. Infect. Dis., Vol. 8. 10.3390/tropicalmed8010026.
8. Afzal, M., A.S. Khan, B. Zeshan, M. Riaz and U. Ejaz *et al*., 2023. Characterization of bioactive compounds and novel proteins derived from promising source *Citrullus colocynthis* along with *in-vitro* and *in-vivo* activities. Molecules, Vol. 28. 10.3390/molecules28041743.
9. Ahmed, N., M.I. Karobari, A. Yousaf, R.N. Mohamed and S. Arshad *et al*., 2022. The antimicrobial efficacy against selective oral microbes, antioxidant activity and preliminary phytochemical screening of *Zingiber officinale*. Infect. Drug Resist., 15: 2773-2785.
10. Ramzan, M., M.I. Karobari, A. Heboyan, R.N. Mohamed and M. Mustafa *et al*., 2022. Synthesis of silver nanoparticles from extracts of wild ginger (*Zingiber zerumbet*) with antibacterial activity against selective multidrug resistant oral bacteria. Molecules, Vol. 27. 10.3390/molecules27062007.
11. Tariq, F., N. Ahmed, M. Afzal, M.A.U. Khan and B. Zeshan, 2020. Synthesis, characterization and antimicrobial activity of *Bacillus subtilis*-derived silver nanoparticles against multidrug-resistant bacteria. Jundishapur J. Microbiol., Vol. 13. 10.5812/jjm.91934.
12. Singh, B.N., B.R. Singh, R.L. Singh, D. Prakash, B.K. Sarma and H.B. Singh, 2009. Antioxidant and anti-quorum sensing activities of green pod of *Acacia nilotica* L. Food Chem. Toxicol., 47: 778-786.
13. Deshpande, S.N., 2013. Preliminary phytochemical analysis and *in vitro* investigation of antibacterial activity of *Acacia nilotica* against clinical isolates. J. Pharmacogn. Phytochem., 1: 23-27.
14. Jamadar, D. and K.J. Sannapapamma, 2018. GCMS analysis and colour fastness properties of silk yarn dyed with *Acacia nilotica* pods. Pharma Innovation, 7: 474-479.
15. Kaneria, M., Y. Baravalia, Y. Vaghasiya and S. Chanda, 2009. Determination of antibacterial and antioxidant potential of some medicinal plants from Saurashtra Region, India. Indian J. Pharm. Sci., 71: 406-412.
16. Raghavendra, M.P., S. Satish and K.A. Raveesha, 2006. *In vitro* evaluation of anti-bacterial spectrum and phytochemical analysis of *Acacia nilotica*. J. Agric. Technol., 2: 77-88.
17. Grass, G., C. Rensing and M. Solioz, 2011. Metallic copper as an antimicrobial surface. Appl. Environ. Microbiol., 77: 1541-1547.
18. Singh, B.N., B.R. Singh, B.K. Sarma and H.B. Singh, 2009. Potential chemoprevention of n-nitrosodiethylamine-induced hepatocarcinogenesis by polyphenolics from *Acacia nilotica* bark. Chem. Biol. Interact., 181: 20-28.
19. Singh, R. and S. Arora, 2009. *In vitro* evaluation of peroxyl radical scavenging capacity of water extract/fractions of *Acacia nilotica* (L.) Willd. Ex Del. Afr. J. Biotechnol., 8: 1270-1272.
20. Benhalima, L., S. Amri, M. Bensouilah and R. Ouzrout, 2019. Antibacterial effect of copper sulfate against multi-drug resistant nosocomial pathogens isolated from clinical samples. Pak. J. Med. Sci., 35: 1322-1328.
21. Sacoto-Figueroa, F.K., H.M. Bello-Toledo, G.E. González-Rocha, L.L. Machuca, C.A. Lima, M. Meléndrez-Castro and G.A. Sánchez-Sanhueza, 2021. Molecular characterization and antibacterial activity of oral antibiotics and copper nanoparticles against endodontic pathogens commonly related to health care-associated infections. Clin. Oral Invest., 25: 6729-6741.
22. Sadiq, M.B., J. Tarning, T.Z.A. Cho and A.K. Anal, 2017. Antibacterial activities and possible modes of action of *Acacia nilotica* (L.) Del. against multidrug-resistant *Escherichia coli* and *Salmonella*. Molecules, Vol. 22. 10.3390/molecules22010047.
23. Adwan, G., B. Abu-Shanab and K. Adwan, 2010. Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug-resistant *Pseudomonas aeruginosa* strains. Asian Pac. J. Trop. Med., 3: 266-269.
24. Ross, J.E., N.E. Scangarella-Oman, R.K. Flamm and R.N. Jones, 2014. Determination of disk diffusion and mic quality control guidelines for GSK2140944, a novel bacterial type II topoisomerase inhibitor antimicrobial agent. J. Clin. Microbiol., 52: 2629-2632.
25. Nassar, M.S.M., W.A. Hazzah and W.M.K. Bakr, 2019. Evaluation of antibiotic susceptibility test results: How guilty a laboratory could be? J. Egypt. Public Health Assoc., Vol. 94. 10.1186/s42506-018-0006-1.

26. Bibi, Y., S. Nisa, F.M. Chaudhary and M. Zia, 2011. Antibacterial activity of some selected medicinal plants of Pakistan. BMC Complementary Altern. Med., Vol. 11. 10.1186/1472-6882-11-52.
27. Selim, S., 2022. Mechanisms of gram-positive vancomycin resistance (Review). Biomed. Rep., Vol. 16. 10.3892/br.2021.1490.
28. Ahmed, N., K. Tahir, S. Aslam, S.M. Cheema and A.A. Rabaan *et al*., 2022. Heavy metal (arsenic) induced antibiotic resistance among extended-spectrum β-lactamase (ESBL) producing bacteria of nosocomial origin. Pharmaceuticals, Vol. 15. 10.3390/ph15111426.
29. Mohamed, E.H., Y.S. Alghamdi, S.M. Abdel-Hafez, M.M. Soliman and S.H. Alotaibi *et al*., 2020. Susceptibility assessment of multidrug resistant bacteria to natural products. Dose-Response, Vol. 18. 10.1177/1559325820936189.
30. Ramalechume, C., P. Shamili, R. Krishnaveni and C.M.A. Swamidoss, 2020. Synthesis of copper oxide nanoparticles using tree gum extract, its spectral characterization, and a study of its anti-bactericidal properties. Mater. Today Proc., 33: 4151-4155.