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Genetic variation of resistant *Staphylococcus aureus* isolates and their susceptibility to the antimicrobial activity of some plant and algal extracts

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ABSTRACT

This study aimed to determine the antibacterial properties of different plant species and some volatile oils, and study genetic variation of some infectious microbial isolates. Materials and methods: A total of 32 bacterial clinical specimens were randomly collected from thirty-two patients (22 Males and 10 Females) attending the outpatient clinic of the Dermatology and Venereology of Tanta University Hospitals, Egypt. *Staphylococcus aureus* isolates were identified biochemically, their antibiotic susceptibility was assessed using fifteen selected antibiotic discs using the disc diffusion method to detect the multi-drug resistant (MDR) isolates. The agar well diffusion method was used to screen the resistant *S. aureus* isolates for susceptibility to the investigated natural extracts. The most effective extract was identified by calculating its minimum inhibitory concentration (MIC). The most effective natural extracts were chemically characterized using GC-MS and FT-IR analysis. SDS-PAGE was performed to evaluate genetic variation among MDR *S. aureus* isolates. Key results: Nine isolates were selected among 32 bacterial isolates and identified as MDR *S. aureus* isolates and the isolate No. 9 was proved to be the most MDR one, recording 53% resistance. Chebulic, safsaf willow, *Jania*, and Nile's roses were the most effective natural inhibitors with inhibition zone diameters of 29.67, 26, 30 and 24.67 mm, respectively. 2-Pentanone, 4-hydroxy-4-methyl-2-pentanone was the most common component in all extracts with a presence rate of 90.87, 85.90 and 85.61% for safsaf willow, Nile's roses and Jania, respectively. MIC was 25 mg/ml for the three selected extracts against the nine *S. aureus* isolates. Twenty-one bands were detected among the nine *Staphylococcus aureus* isolates, only six bands appear commonly in all *S. aureus* isolates.

Key words: *Staphylococcus aureus*, MDR, GC-MS, FT-IR, SDS-PAGE, RAPD, Jania, *Salix* extract.

1. Introduction

One of the biggest concerns for world health today is antibiotic resistance. The emergence and fast spread of germs resistant to several drugs pose a danger to our capacity to treat prevalent infectious diseases. One of the primary causes of drug resistance is thought to be the indiscriminate use of commercial antibiotics. The World Health Organization (WHO) estimates that by 2050, drug-resistant illnesses could claim 10 million lives annually and seriously harm the world economy (WHO, 2020). Finding natural alternatives with antimicrobial qualities that could be used to create innovative and effective antibiotics is therefore vital.

The pathogen *Staphylococcus aureus* is an adaptable human pathogen that can cause a variety of diseases in addition to being a commensal bacterium. *S. aureus* can be found in up to 40% of healthy human populations; the

most common places to find it are in the nose, throat, skin, and intestinal system (Esposito *et al.*, 2014). Children and the elderly, immuno-compromised individuals, including those with allelic variants of some genes that code for innate immunity factors, patients with chronic severe underlying diseases like diabetes, hepatitis, and HIV, and residents of industrialized nations are among the populations most likely to carry *S. aureus* carriage (Sivaraman *et al.*, 2009). Apart from its high virulence, *S. aureus* has demonstrated its ability to rapidly adapt and acquire resistance against almost all antibiotics that are used to eradicate it. Just a year after penicillin was initially used in clinical practice, resistance to the antibiotic. Antibiotic use is unregulated in Egypt, where most antimicrobial drugs are sold over the counter. As a result, the administration of the incorrect antibiotic and the improper dosage and duration of treatment frequently results in antibiotic therapy being unsuccessful. Antibiotic resistance, particularly the frequency of MRSA and MRSH (methicillin-resistant *S. haemolyticus*), has so alarmingly increased in Egypt (Nicolau and Silberg, 2017; Pfaller *et al.*, 2019; Martellosio *et al.*, 2023). Recently, a genomic analysis was carried out on Egyptian isolates of *S. aureus*, adding 56 new genome sequences to publicly available data sources (Henriksen *et al.*, 2018).

Since ancient times, people have utilized plants as medicine, and this tradition is still in place today. It is estimated that 80 percent of people in poor nations utilize medications made from plants (Tajbakhsh and Soleimani, 2018). Since medications produced from plants are safer than those made synthetically (Nisar *et al.*, 2018), the future will see an increase in their usage. It is projected that the global commerce in medical plants and their products will be worth USD 5 trillion by 2050 (Zahra *et al.*, 2020). Plants generate a wide range of chemicals that boost their resistance to environmental stressors but are not utilized in their basic metabolism. These bioactive secondary metabolites, which include phenols, terpenes, and alkaloids, offer protection from microorganisms and herbivores (Azmir *et al.*, 2013; Nazir *et al.*, 2020). Many plant components, including leaves, roots, flowers, fruits, peels, bark, seeds, stems, rhizomes, and more, have a high concentration of bioactive chemicals that have the potential to be used medicinally (Ifesan *et al.*, 2013). Natural products have been used extensively in the past to cure a wide range of illnesses. It has been suggested that almost half of all currently prescribed drugs are made of natural ingredients (Bilia *et al.*, 2017). So, the present study aimed to evaluate the antimicrobial effect of some natural extracts against the resistant S. aureus isolates, and to investigate the genetic variation among the collected isolates.

2. Materials and Methods

Isolation and characterization of bacteria from the infected patients

Twenty-three different bacterial isolates were collected in screw-capped containers from different patients at different departments in the outpatient clinic of Tanta University, Egypt. They were placed right away in nutritional agar slants and subsequently moved to the bacteriology lab at Botany Department, Faculty of Science, Tanta University, Egypt. Mannitol salt agar plates were streaked with pathogenic bacteria using sterile swabs, and the plates were then incubated at 37ºC for the entire night. By repeatedly cultivating the suspected colonies on nutrient agar media, they were purified. Suspected colonies of varying morphologies that had been grown on various media were selected, grown in isolation, and then repeatedly subcultured on a nutrient agar medium to purify them. Pure culture of each bacterial isolate was maintained as follows: Weekly subculture on soft nutrient agar and storage at 4ºC for short-term maintenance, suspending the bacterial suspension in 30% (v/v) glycerol broth and storage at -80ºC for long-term maintenance (Murray *et al*., 2003).

Biochemical identification of the selected bacterial isolates

Catalase test was applied to differentiate between different types of Gram-positive (*Staphylococci and Streptococci*), if catalase was positive, other tests including detection of β-hemolysis on blood agar, coagulase test, and mannitol fermentation were performed. Positive results allowed the identification of the bacterial isolate as *S. aureus*.

The identification of *S. aureu*s bacterial isolates

Some biochemical tests were used to select *S. aureu*s bacterial isolate, like growth on mannitol salt agar, catalase activity, coagulase test, DNase agar test, nitrate reduction test, liquification of gelatin, starch hydrolysis test, and gelatin hydrolysis test.

Antibiotic susceptibility test of *S. aureu*s bacterial isolate

Using fifteen specially chosen antibiotic discs (Bioanalyse, Turkey) from various antibiotic classes, the Standard Disc Diffusion Method (Bauer *et al.*, 1966) was used to determine the antibiotic susceptibility of *S. aureus* isolates. Nutrient agar medium was placed into sterile Petri dishes, and a 100 µL of the nutrient broth culture of bacterial isolates that had been cultured overnight at 37°C for 24 hours under aseptic conditions was swabbed. Using sterile forceps, antibiotic discs were gently pressed onto the surface of the inoculation plates to achieve good contact with the medium. The zone of inhibition (mm) for each antibiotic disc was evaluated after an overnight incubation at 37±0.2°C for 16-18 hours. The isolates were categorized as sensitive, intermediate, or resistant for each antibiotic by consulting performance standards for antimicrobial susceptibility testing (CSLI, 2013).

Screening the effect of plant extracts on the isolated bacteria

Preparation of the plant extracts

The aerial parts of seven dried plant species (tamarisk, Egyptian henbane, elagga, cactus, goose foot, aloe vera, safsaf willow) were cut into small pieces using a sharp knife. Eleven ground spices (cinnamon, clove, cumin, ginger, chilli pepper, rosmery, tumeric, lesser galangel, chebulic and valerian), in addition to five algal species powders (Gulfweed, Tangle, Red coralline, Ulva and Fucus) and two hydrophytes (Coriander well and Niles roses) were also used. They were placed in a blender and crushed into powders with an average particle size of 2 mm. For the extraction, 20 grams of the plant powders or 30 g of the algal powders were extracted using 200 ml of solvents for 48 hours. The solvents employed in the extraction were acetone and ethanol (200 ml) separately for 24 hours at room temperature. After that, the extracts were filtered using Whatman's No. 1 filter paper. To acquire the crude extract, the resulting filtrate was concentrated in a rotary evaporator at 35°C. The unrefined extracts were stored at 4°C until they were needed (Djeussi *et al.*, 2013).

Screening for antibacterial activity of plant extracts

In accordance with the National Committee for Clinical Laboratory Standards, the *S. aureus* isolates were evaluated for their susceptibility to various plant extracts using the agar well diffusion method to calculate the inhibition zone diameter (NCCLS, 1993). With DMSO, the unrefined plant extracts were suspended. For every plant extract, a suspension was prepared at a concentration of 100 mg/ml. A sterile glass rod was used to spread a 100 µL of each broth culture evenly across three well-dried plates of nutrient agar (replicate) after each bacterial isolate had been subcultured in nutrient broth for an entire night. The plates were then allowed to dry for fifteen minutes. Using a sterile cork borer, 8 mm diameter wells were created in the nutritional agar surface. Using an automated pipette, a 50 µL of each extract suspension was placed into the wells. The plates were then incubated for 24 hours at 37 °C. Following the incubation period, the diameter of the inhibition zones was determined in millimeter (mm). These inhibition zones were then contrasted with a positive control (Rifampin) and a negative control (50 µL DMSO).

Determination of MIC of the investigated extracts

The optimal extracts exhibiting antimicrobial activity were selected, and the agar diffusion method was then employed to evaluate the extracts to ascertain the minimum inhibitory concentration (MIC). The minimum inhibitory concentration (MIC) is the lowest amount of antibiotic that, following an overnight incubation period, can prevent a microbe from growing visibly. Plant extracts suspended in DMSO at concentrations of 25, 50, 100, and 200 mg/ml were used to calculate the MICs (Nascimento *et al*., 2000).

Chemical characterization of the selected plant extracts

Gas chromatography-mass spectrometry analyses of the plant extracts

The active components in the examined extract were identified using gas chromatographic mass spectrometry at Tanta University's Scientific Research Center & Measurements (SRCM); utilizing a heated FID-equipped Perkin Elmer Clarus 580/560S kind of equipment. The following are the conditions: Oven: initial temperature of 50°C for 4 minutes, ramp 10°C/min to 140°C, hold for 5 minutes, ramp 10°C/min to 270°C, hold for 3 minutes; injection temperature of 270°C; volume of 1 µL; split ratio of 20:1, carrier gas of He; solvent delay of 4.00 minutes; transfer temperature of 180°C; source temperature of 200°C. Scan: Column (Elite-5MS, 30 m, 0.25 mm ID), 50 to 550 Da. The relationship between their biological activities and the current effective ingredients was ascertained (Manzan *et al*., 2003).

Fourier transform infrared spectroscopy analysis of the plant extracts

FT-IR analysis was used to analyze *Salix subserrata, Terminalia chebula, Jania* and *Nile's roses* acetone extracts in the Central Lab of Biochemistry, Tanta University, Egypt using FT-IR spectrophotometer Perkin-Elmer 1430. The measurements were carried out at infra-red spectra between 400 and 4000 nm (Sherbrok *et al.*, 1984).

Protein profile analysis

The denaturating SDS polyacrylamide gel electrophoresis (SDS-PAGE) was performed to evaluate genetic variation among nine *S. aureus* isolates according to the procedures described by Laemmli (1970).

Random amplified polymorphic DNA technique

The procedures of DNA extraction for the total genomic of nine *S. aureus* isolates was performed according to manufacturer protocol of GeneJET Genomic DNA Purification Kit (K0721/ Thermo fisher). In the Eppendorf tube, RAPD analysis was performed using Ready – To- Go ®- RAPD analysis kit (100 reactions, pack of 6 primers, 27-9502-01, GE Healthcare) according to manufacturer protocol. The list of the included primers is included Table 1.

Table 1. Sequence of the selected random primers for finger printing of the selected isolates.

RAPD Primers	Primer sequences
RAPD analysis primer 1	(5'-d[GGTGCGGGAA]-3')
RAPD analysis primer 2	(5'-d[GTTTCGCTCC]-3')
RAPD analysis primer 3	(5'-d[GTAGACCCGT]-3')
RAPD analysis primer 4	(5'-d[AAGAGCCCGT]-3')
RAPD analysis primer 5	(5'-d[AACGCGCAAC]-3')

Inter-simple sequence repeats technique

Inter-Simple Sequence Repeat (ISSR) was applied as a molecular fingerprinting technique via five specific primers designed for *S. aureus* isolates (Table 2). DNA extraction procedures for total genomic of nine *S. aureus* isolates was performed according to manufacturer protocol of GeneJET Genomic DNA Purification Kit (K0721/ Thermo Fisher).

Table 2. Inter-Simple Sequence Repeat (ISSR) primer features.

Primers	Sequences	Annealing temperature (°C)	Reference
1	$(CT)_8G$	46.8	Sahu *et al.*, 2018
2	$GACA)_4$	47.4	
3	$(GA)_9T$	48.8	
4	$G(CT)_8$	48.8	
5	$(GACA)_4G$	49.0	

Data analysis

Through totalab software analysis (www.totalab.com. Ver.1.0) the obtained amplified bands were scored as 0, 1 for absence and presence.

Statistical analysis

Means ± SD (standard deviation) and percentages were among the descriptive statistical analyses carried out with Microsoft Excel 2023 (Microsoft Corp., USA). The Statistical Package for the Social Sciences (SPSS v. 26, USA) software was used to perform additional statistical analyses. To determine the significance of the findings between tasting

bacterial isolates and various extracts, a one-way ANOVA test was performed. P values below 0.05 were regarded as significant.

4. Results

Isolation and morphological characteristics of bacteria isolated from the infected patients

Thirty-two patients (10 female and 22 male) attending the outpatient clinic of Tanta University Hospitals' Dermatology and Venereology provided a total of 32 bacterial clinical specimens, which were then randomly collected in screw-capped containers. The specimens were subsequently transferred to the Botany and Microbiology Department's Laboratories of Bacteriology and Mycology, Faculty of Science, Tanta University, Egypt. Six isolates were isolated from wounds (18.75%), five isolates were isolated from pus (15.63%), three isolates were isolated from blood (9.38%), ten isolates were isolated from sputum (31.25%), four isolates were isolated from urine (12.50%), and four isolates were isolated from trachea (12.50%). The isolated bacteria were selected and purified according to different morphological characteristics such as color, opacity, shape (form), and margin. The morphological characteristics of pure bacterial isolate on nutrient agar media (cell shape, motility, and Gram stain), and color, edge, and the surface of the colony were observed. Growth of the bacterial isolates on three different media (Nutrient agar, Mannitol salt agar, and Blood agar) incubated overnight at 37º C was also detected.

Biochemical identification of *S.aureus* isolates

Using different biochemical tests (catalase, coagulase, and Dnase) and culture on Nutrient agar and Mannitol salt agar, *S. aureus* isolates were selected among 32 bacterial isolates. Nine isolates were catalase, coagulase, and Dnase positive, non-haemolytic on Mannitol salt agar, and Gram-positive. These isolates were identified as isolates, 3 isolates isolated from wounds, 2 isolates from trachea, and 1 isolated from pus, blood, urine, and sputum.

Antibiotic susceptibility of clinical bacterial isolates

Using the disk diffusion approach, fifteen antibiotic disks were used to screen *S. aureus* bacterial isolates for antibiotic resistance. The effectiveness of the fifteen tested antibiotics against the chosen bacterial isolates was screened, as shown in Fig. 1. Out of all the bacterial isolates, 87.5% possessed the highest resistance to cefepime, clindamycin, ampicillin, and oxacillin. Conversely, imipenem, nitrofurantoin, and tetracycline showed the lowest resistance (0 and 3.13%).

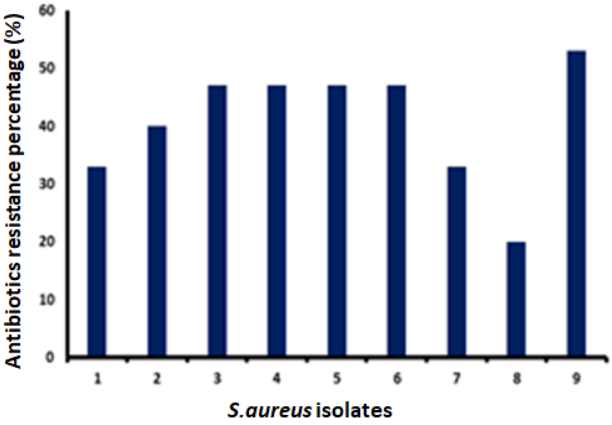

Fig. 1. Antibiotic resistance pattern of *S. aureus* isolates against various antibiotic groups.

All of the examined bacterial isolates had resistance to cephalosporin (87.5 and 46.9%). Conversely, 56.25 and 59.38 percent of the examined bacterial isolates were resistant to the penicillin medications. However, of the eighteen bacterial isolates, seventy-nine percent were resistant to beta-lactams. The tested bacterial isolates are also resistant to sulphonamides group trimethoprim (sxt), which recorded 9.38 percent, and some antibiotics such as the macrolide antibiotic erythromycin (18.8%), quinolone antibiotic ciprofloxacin (12.5%), ansamycin antibiotic rifampin (21.88%), and prototypical broad spectrum (31.25%). Based on the results acquired, it was determined that bacterial isolate No. 9 exhibited the highest level (53%) of multi-drug resistance.

Antibacterial activities of different plant extracts against *S. aureus* isolates

Table 3 illustrates the antibacterial activities of different plant extracts against *S. aureus* isolates. Seven different desert plants (Aloe vera, Cactus, Egyptian henbane, Elagga, Goosefoot, Safsaf willow, and Tamarisk) were evaluated using agar

well diffusion methods for their antibacterial activity. Using the agar well diffusion method, the effectiveness of acetone-extracted desert plants against *S. aureus* isolates was evaluated by measuring the diameter of the inhibitory zones at a concentration of 100 mg/ml in DMSO. Rifampin served as the positive control in this analysis, while DMSO served as the negative control. There was no inhibitory impact seen in the DMSO negative control. The inhibition zones in the Rifampin-positive controls ranged from 11 to 38 mm. According to the findings, Aloe vera, Cactus, and Elagga had no inhibitory effect on *S. aureus* isolates. The Safsaf willow acetone extract recorded 26.00 mm inhibition zone against the isolate number nine.

Table 3. Preliminary survey for antimicrobial effect of different plant and algal extracts from different habitats against various *S. aureus* isolates. Data are presented as mean of 3 replicas ± SD.

Desert plant extracts	*S. aureus* isolates								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Tamarisk	16.00±1.00	16.00±0.00	16.33±0.58	15.00±1.00	16.00±1.00	20.67±0.58	0.00±0.00	17.33±0.58	15.67±0.58
Elagga	0.00±0.00	0.00±0.00	11.00±1.00	0.00±0.00	12.0±0.00	20.0±0.00	10.67±0.58	12.67±0.58	11.00±1.00
Aloe vera	14.00±1.00	10.00±1.00	12.00±1.00	0.00±0.00	0.00±0.00	10.33±0.58	00±0.00	00±0.00	00±0.00
Goose-foot	12.00±0.00	12.67±0.58	9.00±0.00	11.33±0.58	15.00±1.00	13.00±1.00	19.67±0.58	12.00±0.00	10.67±0.58
Cactus	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	9.67±0.58	17.33±0.58	11.00±0.00	17.00±1.00	9.67±0.58
Egyptian henbane	11.00±0.00	11.33±0.58	19.67±0.58	12.33±0.58	13.67±0.58	13.67±0.58	12.33±0.58	14.67±0.58	25.67±0.58
Safsaf willow	21.00±1.00	15.00±1.00	18.00±0.00	20.00±0.00	21.00±1.00	19.33±0.58	19.67±0.58	15.00±1.00	26.00±0.00
Spices extracts									
Rosemary	20.33±0.58	17.67±0.58	22.00±1.00	15.67±0.58	22.00±0.00	21.00±0.0	20.00±1.00	16.67±0.58	20.67±0.58
Turmeric	8.90±0.17	16.00±1.00	14.33±0.58	18.33±0.58	0.00±0.00	16.67±0.58	14.330.58	12.000.00	22.670.58
Cumin	17.33±0.58	13.33±0.58	14.00±0.00	0.00±0.00	0.00±0.00	22.33±0.58	14.67±0.58	23.67±0.58	26.67±0.58
Chili pepper	12.67±0.58	13.33±0.58	16.00±1.00	12.67±0.58	0.00±0.00	15.33±0.58	15.33±0.58	12.33±0.58	19.33±0.58
Ginger	15.33±0.58	19.33±1.15	26.33±0.58	16.33±0.58	13.00±0.00	26.00±1.00	17.33±0.58	19.33±0.58	15.33±0.58
Cinnamon	12.00±1.00	18.33±0.58	17.33±0.58	18.33±0.58	19.33±0.58	18.67±0.58	12.33±0.58	17.33±1.15	18.67±0.58
Clove	22.33±0.58	25.33±0.58	25.67±0.58	22.33±0.58	22.67±0.58	20.00±0.00	20.00±0.00	18.67±0.58	25.33±0.58
Lesser galangel	15.67±0.58	23.00±0.58	16.00±0.58	19.00±0.58	13.67±0.58	15.67±0.58	15.00±0.58	14.00±0.58	19.67±0.58
Chebulic	18.67±0.58	25.67±0.58	25.00±0.00	29.67±0.58	22.67±0.58	25.00±0.00	22.67±0.58	20.67±0.58	29.33±0.58
Sheep's sorrel	19.67±0.58	20.00±0.00	21.00±0.00	21.00±0.00	20.67±0.58	21.00±0.00	18.00±0.00	19.67±0.58	25.67±0.58
Valerian	18.67±0.58	17.00±0.00	29.67±0.58	16.67±0.58	19.67±0.58	19.00±0.00	17.33±0.58	22.67±0.58	24.67±0.58
Different algae									
Gulfweed	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	14.67±0.58	15.33±0.58	10.33±0.58	0.00±0.00	0.00±0.00
Tangle	11.33±0.58	0.00±0.00	0.00±0.00	15.33±0.58	14.67±0.58	22.67±0.58	12.67±0.58	19.67±0.58	13.33±0.58
UIVA	18.33±0.58	19.67±0.58	13.67±1.15	0.00±0.00	15.00±0.00	22.67±0.58	26.67±0.58	19.67±0.58	13.00±0.00
Jania	30.00±0.00	27.33±0.58	14.00±1.00	0.00±0.00	15.67±0.58	15.67±0.58	13.67±1.53	15.33±0.58	23.00±0.00
Fucus	10.00±0.00	13.00±0.00	17.67±0.58	12.00±0.00	13.67±0.58	14.67±0.58	14.00±0.00	14.00±1.0	14.67±0.58
Hydrophytes extracts									
Coriander well	12.00±0.00	12.67±0.58	16.67±0.58	14.00±0.00	12.33±0.58	11.33±0.58	12.00±0.00	12.00±0.00	11.67±0.58
Nile's roses	14.67±0.58	14.00±0.00	18.33±0.58	15.33±0.58	16.67±0.58	14.33±0.58	14.00±0.00	24.67±0.58	15.67±0.58

Lesser galangel, Chebulic, Chilli pepper, Cinnamon, Clove, Cumin, Ginger, Rosemary, Sheep`s sorrel, Turmeric, and Valerian different spices extracts were tested for their antibacterial activity. While all the spices that were examined exhibited antibacterial action against the isolates of *S. aureus*, chebulic extracts were the most successful. Isolates 4 and 9 showed the highest inhibition zone by acetone extract, with inhibition zones of 29.67 and 29.33 mm, respectively.

Five different algal extracts (Gulfweed, Tangle, *Ulva*, *Jania* and *Fucus*) were tested for their antibacterial activity against *S. aureus* isolates. Gulfweed was the less effective algal extract as it affected only three bacterial isolates. The most effective algal extract was *Jania*. The maximum inhibition zone by acetone extract of *Jania* was observed on isolate number one with inhibition zones 30 mm.

Nile's roses and Coriander well are two hydrophyte plants tested for their antibacterial activity against *S. aureus* isolates. The results revealed that Nile's rose and Coriander well acetone extracts have highly significant antibacterial activity against *S. aureus* isolates. The maximum inhibition zone by acetone extract of Nile's roses was observed on isolate number three with inhibition zone of 24.67 mm.

Minimum inhibitory concentrations of the four selected plant extracts

In this experiment, acetone extracts of safsaf willow, chebulic, coralline and Niles roses were tested for their MIC against *S. aureus* isolates. Therefore, various concentrations (25, 50,100 and 200 mg/ml) were prepared and assayed against nine *S. aureus isolates*. Table 4 showed that the antibacterial activity increased significantly by increasing the selected plant acetone extract concentration from 25 to 200 mg/ml. Among the *S. aureus* bacterial isolates, 11 isolates were inhibited at MIC of 50 mg/ml.

Chemical characterization of plant extracts

GC-MS analysis of acetone extract of *Salix subserrata*

The GC-MS chromatograms in Table 5 and Fig. 2 displayed the main components found in the acetone extract of safsaf willow. with different levels of abundance (%). The main components in acetone extract of safsaf willow were2-Pentanone, 4-hydroxy-4-methyl (90.87%), p-Xylene (29.95%), o-Xylene (26.47%), Undecanal (5.60%), Oxirane, decyl (5.38%), Tetradecanal (5.17%), Oxirane, dodecyl (5.17%), and Oleic Acid (21.64%). The components of acetone extracts of safsaf willow expressed significant pharmacological activities.

Table 4. Determination of MIC of different natural extracts against the 9 selected *S. aureus* isolates.

Natural extracts		Nile's roses extract			
		Mean of inhibition zone (mm) against the 9 tested isolated			
		25	50	100	200
Nile's roses		11.33±0.58	13.33±0.58	14.00±0.00	14.33±0.58
Safsaf willow		18.33±0.58	22.00±0.00	25.00±0.00	26.67±0.58
Jania		25.00±0.00	27.67±0.58	30.00±0.00	31.67±0.58
Chebulic		25.67±0.58	29.00±0.00	30.12±0.58	30.33±0.58
ANOVA	**F-value**	22.625	22.531	55.833	9.563
	P-value	< 0.001**	< 0.001**	< 0.001**	< 0.001**

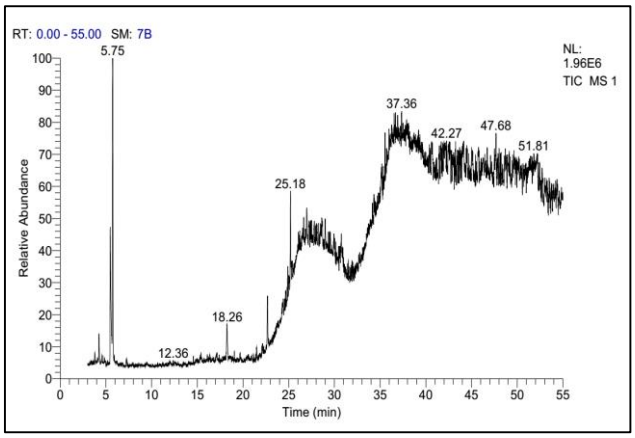

Fig. 2. GC-MS analysis profile of acetone extract of *Salix subserrata*.

Fourier transform infrared spectroscopy analysis of selected acetone plant extract.

The tabulated values of the various functional groups and their corresponding characteristic group frequency ranges are very helpful in the molecular diagnosis of vibrational frequencies or absorption bands. As seen in **Fig. 3**, FT-IR analysis was used to determine the safety of *Salix subserrata, Jania,* Nile's roses and *Terminalia chebula* acetone extract in the current study. The information revealed that there were no harmful cyano groups (C≡N) or acetylenic groups (C≡C).

Table 5. GC-MS analysis of acetone extract of *Salix subserrata.*

Rt	Compound Name	Probability	Area %
5.47	2-Pentanone, 4-hydroxy-4-methyl	90.87	11.67
5.75	o, p-Xylene	29.95	17.88
18. 26	Undecanal	5.60	3.14
18. 26	Oxirane, decyl	5.38	3.14
18. 26	Tetradecanal	5.17	3.14
22. 68	Undecanal	6.78	3.59
24. 89	Oleic Acid	21.64	1.63
24. 89	Octan-2-one, 3,6-dimethyl	8.55	1.63
24. 89	Octanoic acid, 7-oxo	5.87	1.63
24. 89	Pentadecanoic acid	4.73	1.63
24. 89	10-Undecenoic acid, octyl ester	4.18	1.63
25. 18	Oxirane, tetradecyl	16.30	5.49
25. 18	Oxirane, dodecyl	13.13	5.49
25. 18	Pentadecanal-	10.59	5.49
26. 96	Octan-2-one, 3,6-dimethyl	4.86	2.72
26. 96	Pentadecanoic acid	4.48	2.72
26. 96	Heptadecanoic acid, heptadecyl ester	3.52	2.72
30. 76	Oleic Acid	25.97	3.34
30. 76	Dodecanoic acid, 3-hydroxy	3.22	3.34
35. 19	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	13.69	1.44
35. 54	Z-8-Methyl-9-tetradecenoic acid	10.27	1.45
35. 97	3-Trifluoroacetoxypentadecane	5.52	1.62
40. 64	Z-8-Methyl-9-tetradecenoic acid	10.56	1.98
41. 47	Estra-1,3,5(10)-trien-17$_F$-ol	5.53	2.56
42. 57	7-Methyl-Z-tetradecen-1-ol acetate	17.99	1.06
42. 63	9-Hexadecenoic acid	7.45	2.66
43. 25	12-Methyl-E,E-2,13-octadecadie n-1-ol	4.07	2.40
43. 47	3-Trifluoroacetoxypentadecane	4.72	2.09
43. 70	E-8-Methyl-9-tetradecen-1-ol acetate	5.77	1.22
44. 07	Z-8-Methyl-9-tetradecenoic acid	10.27	1.17
44. 12	Cyclopentadecanone, 2-hydroxy	5.85	1.15
47. 51	Dodecanoic acid, 3-hydroxy	4.82	1.90
47. 68	1,2-15,16-Diepoxyhexadecane	4.21	4.46

The protein fingerprinting patterns of the investigated *S. aureus* isolates

Table 6 and Fig. 4 represented bands of nine *S. aureus* isolates. Twenty-one bands appear with a molecular weight range from (13:112 kD). Only six bands appear with all *S. aureus* isolates; while other bands were expressed differently among isolates, which was related to their habitat, nutrition, environmental and other host-pathogen interaction factors.

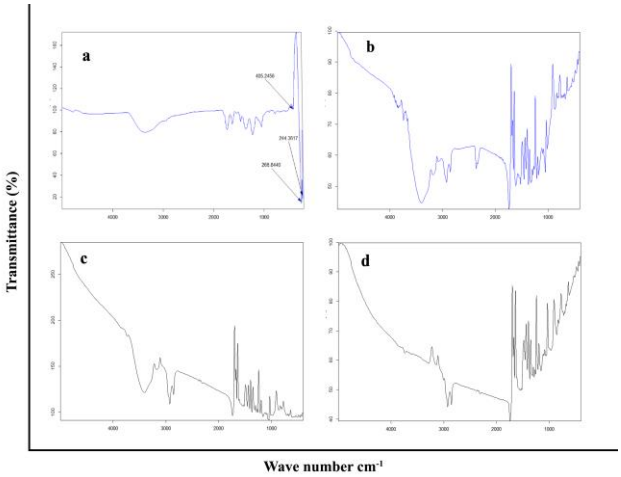

Fig. 3. FT-IR analysis of the selected natural acetone extracts. a) FT-IR chromatogram of *Terminalia chebula*; b) FT-IR analysis of *Salix subserrata;* c) FT-IR analysis of Nile's roses; and d) FT-IR analysis of *Jania sp*.

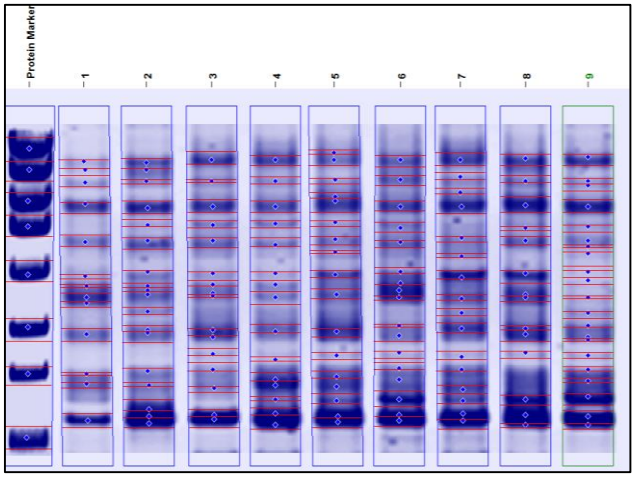

Fig. 4. Protein fingerprinting patterns for nine *Staphylococcus aureus* isolates.

Table 6. Total amplified bands for nine Protein fingerprinting patterns of *Staphylococcus aureus* isolates.

		***Staphylococcus aureus* isolates**								
Band No.	**M.W**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	**112**					**+ve**				
2	**86**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**
3	**81**	**+ve**					**+ve**			
4	**60**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**
5	**50**					**+ve**		**+ve**		**+ve**
6	**44**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**
7	**42**		**+ve**	**+ve**	**+ve**	**+ve**	**+ve**		**+ve**	**+ve**
8	**40**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**
9	**35**					**+ve**				**+ve**
10	**34**							**+ve**		

		***Staphylococcus aureus* isolates**								
Band No.	**M.W**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
11	**31**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**
12	**30**		**+ve**					**+ve**		
13	**30**		**+ve**				**+ve**		**+ve**	
14	**29**	**+ve**	**+ve**	**+ve**			**+ve**	**+ve**	**+ve**	
15	**29**			**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**
16	**27**			**+ve**			**+ve**			**+ve**
17	**25**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**			**+ve**
18	**23**			**+ve**						
19	**20**				**+ve**					
20	**15**	**+ve**	**+ve**	**+ve**						
21	**13**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**

5. Discussion

Nowadays, antibiotic resistance is a serious worldwide health issue. Our capacity to treat common infectious diseases is in jeopardy from the emergence and rapid spread of multidrug-resistant bacteria. Drug resistance is thought to be mostly caused by the indiscriminate use of commercial antibiotics. By 2050, drug-resistant illnesses might kill 10 million people annually and seriously harm the world economy, according to the World Health Organization (WHO, 2020). Over the years, interest in using plants and their components as antimicrobial agents has grown due to the inherent defenses that plants have against a broad range of invaders, such as bacteria and fungi. Nowdays, the hunt for naturally occurring, potent antibiotics is intensifying due to the rise in bacterial resistance to conventional antibiotics (Cole *et al.,* 2001). Research on herbal plants, as evidenced by the volume of publications in the field of phytochemicals for medicinal uses, stretches back to the time of Hippocrates, approximately the fifth century B.C., and has made a significant contribution to our understanding of the antimicrobial activities of phytochemicals (Terreni *et al.,* 2021).

Ginger had the largest inhibition zones among all the plant extracts in our investigation, making it the most effective. The flowering plant known as ginger (Zingiber officinale) is native to India, China, Southeast Asia, the West Indies, Mexico, and many other parts of the world (Khan *et al.*, 2019). Around the world, ginger is used as a spice and flavoring, and its numerous health advantages include pharmacological effects, antioxidant, antibacterial, anti-inflammatory, anti-nociceptive, anti-mutagenic, and hepato-protective properties (Nirmal *et al.,* 2013).

Ginger is an effective antibacterial agent against Gram-positive bacteria (Sawant *et al.,* 2021). Based on a study conducted by Wang *et al.* (2020) Ginger possesses notable inhibitory zones against *Escherichia coli* and *S. aureus*. Ginger has direct antibacterial activity and can be used to treat a variety of bacterial infections, according to a different study that also revealed these antibacterial qualities (Handayani *et al.*, 2018). Using water, ethanol, n-hexane, and ethyl acetate as solvents, researchers examined the antibacterial activity of ginger extracts and found that they had a considerable antibacterial effect (Mohammed *et al.,* 2019). In comparison, Naseer *et al.* (2021) revealed that there were no appreciable variations in the antibacterial properties of ginger's ethanol and water extracts against *S. aureus* and *S. pyogenes*.

Terpene and phenolic compounds are two of ginger's primary active components. Gingerols, shogaols, and paradols are some of the phenolic components found in ginger. Gingerols, which include 6-gingerol, 8-gingerol, and 10-gingerol, are the most prevalent phenolic chemicals found in fresh ginger (Nouwen *et al.*, 2004). Matching shogaols could be produced from the gingerols by heat treatment or long-term preservation. These shogaols could become paradols after hydrogenation (Mao *et al.,* 2019). Other phenolic compounds in ginger include quercetin, 6-dehydrogingerdione, zingerone, and gingerenone-A. In addition, ginger includes several terpene components that are important components of ginger essential oils, such as α-curcumene, α-farnesene, β-bisabolene, β-sesquiphellandrene, and zingiberene. In addition to these, ginger contains lipids, polysaccharides, raw fibers and organic acids (Tanweer *et al.,* 2020).

Among the evaluated desert plants, willows (*Salix subserrata* L.) showed the strongest antibacterial activity. Generally speaking, cellulose, hemicellulose, and lignin make up the majority of the biomass of willows, with flavonoids and other polyphenols being minor components (Yan *et al.,* 2021). Rates (2001) found that when broilers were fed *Salix alba* L. bark extract, the amount of beneficial lactobacilli

increased and the quantity of harmful bacteria (*Escherichia coli*, Enterobacteriaceae) on the cecal microbial population dropped. Additionally, it was discovered that three different *Salix* spp. bark extracts had bactericidal activity against *S. aureus*, with no discernible distinctions between these species. These results agree with our obtained results.

Terminalia chebula was the most powerful plant among the spices. Numerous studies have looked into *T. chebula*'s possible antibacterial properties. *T. chebula* exhibited antibacterial efficacy against human pathogenic pathogens that were both Gram-positive and Gram-negative. In order to destroy *Shigella sp*., methyl gallate, which was isolated from *T. chebula*, demonstrated its antibacterial action by producing extracellular and intercellular disintegration of *Shigella dysenteries* and cytoplasmic content leaking (Grace and Sankari, 2017).

Conclusion

Many infected cases had complicated healing problems due to the presence of multi-drug resistant *S. aureus* isolates, that could be treated with the promising antimicrobial activity of safsaf willow acetone extract with obviously low MIC of 25 mg/ml. These resistant isolates were genetically analyzed; their fingerprints were found to have only 6 common bands and other 15 variable bands due to different gene expression activities, related to their different physiological, environmental and host-pathogen interaction factors.

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