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Anti-*Staphylococcus aureus* Activity of the Aqueous Ethanolic Extract of *Solenostemma argel* Aerial Parts

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Abstract

Solenostemma argel (Asclepiadaceae) is a beneficial folk medicine used to treat many diseases, including the kidney, liver, and stomach diseases. The aim of this study was to assess the anti-*Staphylococcus aureus* activity of an aqueous ethanolic extract of *Solenostemma argel* (AEESA) and identify its phenolic and volatile compounds using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectroscopy (GC-MS). As revealed using the disc diffusion test and DNA cleavage analysis, *S. argel* extract was active against *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) was 31.6 g/mL. Sixteen phenolic compounds of AEESA were analyzed using HPLC, wherein the phenolic compounds: pyrocatechol (9519.95 µg/g), ferulic acid (3221.41 µg/g), chlorogenic acid (3221.41 µg/g), and gallic acid (2730.85 µg/g) were the most abundant. Additionally, naringenin (2262.80µg/g) and quercetin (1750.25µg/g) were the major flavonoids. GC-MS analysis of AEESA revealed 30 volatile compounds, the major percentages of 9,12-octadecadienoic acid (z,z)-, methyl ester (33.44%), ascorbic acid 2,6-dihexadecanoate (7.22%), and butylated hydroxytoluene(5.46%), followed by 2-Pentadecanone, 6,10,14-trimethyl (4.77%), 2-Pentadecanone, 6,10,14-trimethyl (3.35%), Stigmasta-5,22-dien-3-ol, and acetate (3.Beta; 3.35%). The application of *S. argel* extract in reducing the growth of biofilms would subsequently diminish the development of antibiotic resistance and promote the future uses of plant extract components.

Keywords: *Solenostemma argel*, Bioactive Compounds, GC-MS, HPLC, *Staphylococcus aureus*

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INTRODUCTION

Solenostemma argel Del. Hayenne (Family Asclepiadaceae) is a desert plant indigenous to Africa that is used in traditional medicine worldwide but is prominent in Saudi Arabia, Sudan, Chad, Egypt, Algeria, Libya, and Palestine.[1,2] Extensive research has shown that *S. argel* contains several chemicals and offers a range of bioactivities without causing poisoning.[2] Numerous researchers have examined the phytochemicals in *S. argel*.[3-6] It contains multiple components, and crystalline compounds have been examined in several parts of *S. argel* (leaves, stems, and flowers).[7] *Solenostemma argel* methanolic extract used as a source of bioactive substances with subsequent health advantages, such as antioxidant, antiinflammatory, anticancer, and antibacterial activities.[3] *Staphylococcus aureus* is a well-known bacterial pathogen that causes millions of serious infections (such as pneumonia, cardiovascular disease, and nosocomial bacteremia) worldwide.[8] *Staphylococcus aureus* is resistant to most antibiotics, even when used in conjunction with other medications. Excessive use of antibiotics to treat microbiological infections, such as *Staphylococcus aureus* may result in antibiotic resistance. To minimize the possible repercussions of antibiotic resistance, focusing on the extracts of halophytic wild plants as an alternative method to combat pathogenic microorganisms has become critical. Furthermore, information regarding the antibacterial properties of *S. argel* against the potentially harmful *S. aureus* is lacking. The current study used high-performance liquid chromatography(HPLC) and gas chromatography-mass spectroscopy (GC-MS) to analyze the aqueous ethanolic extract of *S. argel* (AEESA) against *S. aureus* and to identify the polyphenols and volatile components present.

MATERIALS AND METHODS

Plant material

Solenostemma argel was collected from Al-Ahsa, Saudi Arabia in March 2021. In order to prepare the fresh plant aerial parts for use in the experiments, they were cleaned, dried in an oven at 50°C until constant weight, and then ground to a fine powder.

Preparation of the *Solenostemma argel* extract

Using a Soxhlet apparatus at 80°C, 800 g of the dried aerial parts of *S. argel* has been extracted with 70% aqueous ethanol. The extraction process was continued until the solution became completely clear. The alcoholic extract was filtered, dried using reduced-pressure evaporation, and weighed (12.6 g).[9] For future studies, the dried extracts were stored at room temperature(25°C-30°C).

Disc diffusion assay

The *S. aureus* strain was obtained from King Faisal University College of Medicine. *Staphylococcus aureus* was cultivated in nutritional broth (Cat. no. 7014), and the disc diffusion experiment was performed according to the method of Rad.[10] The samples were grown at 37°C at 200 rpm until their turbidity at 600 nm reached 0.3. To inoculate 10 mL of nutritional broth, 100 μL of overnight bacterial culture were used. Five milliliters of each bacterial strain was then placed in separate nutrient agar plates, and each plate was gently mixed to ensure that the culture was dispersed evenly on the agar. Before applying the test solution discs to the agar, all the inoculation plates were kept uncovered in a biosafety cabinet to allow the residual liquid to sink into the agar. Four 6 mm diameter discs containing different test solutions were placed on the agar surface of each inoculation plate. Three of the discs contained AEESA test solutions extracted from aerial parts, whereas the positive control disc containing 10 μg of Meropenem was acquired from Condalab in Madrid, Spain. After each plate had been incubated at 37°C overnight, the diameters of the incubation zone were measured.

Calculating the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the extract against *S. aureus* was determined using the agar microdilution technique against *S. aureus*.[11] The bacterial strain was cultured in Mueller-Hinton broth for 24 h at 30°C. (MHB, Oxoid, UK). The bacterial suspension was adjusted with a sterile saline solution until it reached 1×10^7 CFU/mL. After that, a 96-well microtiter plate was loaded with 50 μL MHB and 50 μL inoculum. A 5 mg/mL stock solution

of plant extract in DMSO was prepared, and the wells were mixed with two-fold dilutions of the stock plant extract in MHB. Each well's capacity was increased up to 150 µL by adding MHB. The microplates were incubated for 24 h at 30°C (160 rpm) in a rotating agitator. MHB with the inoculum was used as a positive control, whereas MHB with the plant extract served as a negative control for maximal growth. p-Iodonitrotetrazolium (MP Biomedicals, Salon) was used to determine the inhibitory concentration. After incubation, each microtiter well received 10 L (0.1 mg/mL) of p-iodonitrotetrazolium violet, then the bacterial growth was assessed by reincubating at 30°C for 1 h. The color shift from violet to orange-red indicated that the cells were alive. The inhibitory concentration was comparable to that required for metabolically active cell oxidation. A Bio-Rad ELISA reader was used to measure the absorbance of the 96-well microtiter plates at 630 nm. Testing was performed in triplicates. The concentration of the first well that exhibited no turbidity was used to calculate the MIC.[12]

Anti-biofilm activity

To determine the anti-biofilm activity of the plant extract, bacterial strains were inoculated into trypticase soy broth (TSB) supplemented with 0.1% glucose (w/v). After 18 h of incubation at 30°C, cells were collected via centrifugation at 10,000 rpm for 10 min. PBS was used to wash the cell pellets. Specifically, 100µL of bacterial suspensions in TSB (OD_{600} = 0.1) were added to 96-well plates and incubated at 37°C for 24 h under static conditions. After 1 h of incubation with the plant extracts at the specified concentrations, 0.1% crystal violet was added to each well to stain the biofilms for 10 min. After adding 30% (v/v) acetic acid to each well to dissolve the crystal violet, the optical density of each sample was measured at 540 nm using a microplate reader (Rayto, Germany).[13,14] The tests were performed in triplicates.

Assay for DNA fragmentation

Staphylococcus aureus cells were injected into a 12-well plate. Following overnight incubation, the medium was replenished and IC_{50} doses of the test sample extracts were added. After 24 h of incubation, cells were centrifuged,

washed with PBS, and counted. The cells (5×10^7) were then treated for 2 h at 37°C with lysis buffer (10 mM Tris (pH 8), 10 mM EDTA, and 0.5% Triton X-100). RNase A (20µg /mL) was applied to genomic DNA for 1 h at 37°C, followed by proteinase K (50µg/mL) for 2 h at 50°C. DNA was extracted using phenol, chloroform, and isoamyl alcohol (25:24:1), washed with 70% ethanol, air-dried, soaked in distilled water, and separated on a 1.5% agarose gel with 10 mg/mL EtBr. The DNA fragmentation pattern was observed using a UV transilluminator and photographed using gel documentation equipment.

HPLC analysis of phenolic compounds in AEESA

High-performance liquid chromatography (HPLC) was performed using the Agilent 1260 series. Separation was carried out using a 4.6× 250 mm i.d., 5 m, Eclipse C18 column. The mobile phase had a flow rate of 0.9 mL/min and was composed of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B). The linear gradient was sequentially programmed into the mobile phase as follows: 12–15 min (82% A), 15–16 min (82% A), and 16–20 min (82% A), all of which were within acceptable ranges. A multi-wavelength detector was operated at 280 nm. For every one of the sample solutions, 5 L of injection volume was used. The column was maintained at a constant temperature of 40°C.

GC/MS analysis of volatile compounds in AEESA

The volatile compounds in the AEESA were analyzed, and the dried aqueous ethanolic extract (5 g) was dissolved in 30 mL distilled water (with warming) and further fractionated via extraction with 60 mL diethyl ether. The diethyl ether layer was concentrated on a water bath at 60°C to obtain diethyl ether fraction of aqueous ethanolic extract. The diethyl ether fraction was then analyzed using GCMS-QP2010 SE equipment (SHIMADZU, Japan) and a direct capillary column Rtx-5MS (30 m × 0.25 mm × 0.25 m film thickness). The column oven temperature was originally maintained at 60°C for 2 min before being raised by 10°C/min to 300°C for 4 min. The injector temperature was maintained at 300°C. Helium was used as a carrier gas at a constant flow rate of 2 mL/min. The extract sample(1 µl) was automatically injected using an autosampler

AOC-20S attached to the GC in split mode. Full scan mode m/z 20–500 EI mass spectra were collected at 70 eV ionization voltage. Temperatures of 200 and 300°C were selected as the ion source and transfer lines, respectively. The components were identified by comparing their retention durations and mass spectra with those in the NIST 05 mass spectral database.[15]

RESULTS

The AEESA inhibits the growth of *S. aureus*

The effects of various AEESA concentrations on *S. aureus* proliferation are presented in Figure 1. The percentage of surviving microbes decreased as the concentration of the plant extract increased. At 5 µg/mL of the plant extract, robust growth was observed in comparison to the untreated control, whereas at 500 µg/mL, a substantial suppression (78%) was observed. With an MIC of 31.6µg/mL, plant extract exhibited significant antibacterial activity against *S. aureus*. (Figure 1).

The inhibitory effects of the AEESA against *S. aureus*

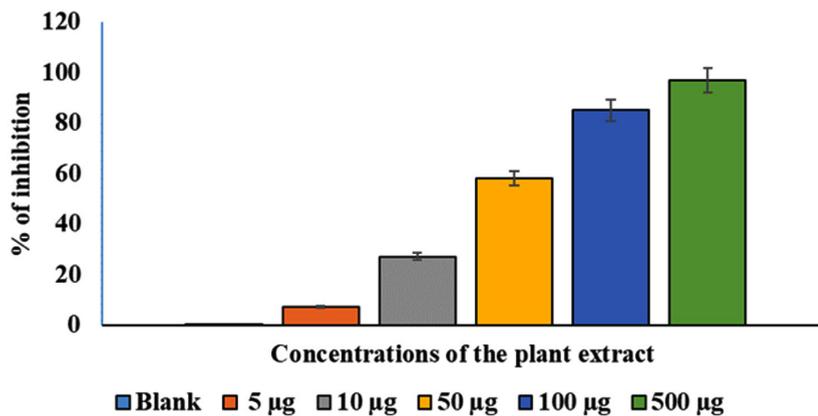The effects of various AEESA concentrations on the growth of *S. aureus* are

Figure 1. Effects of various AEESA concentrations (µg/mL) on *S. aureus* growth. The AEESA concentrations used were 5 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, and 500µg/mL
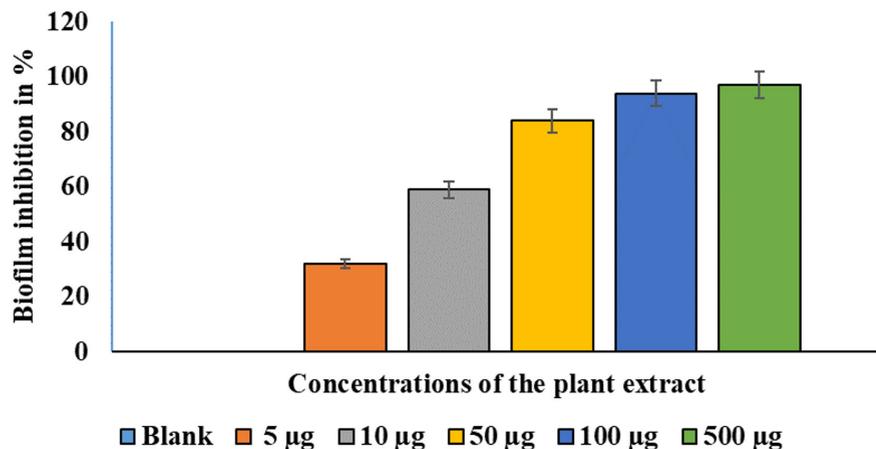

Figure 2. Effects of various AEESA concentrations (µg/mL) on the biofilm formation (percent) of S. aureus. The following AEESA concentrations were utilised: 5 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, and 500 µg/mL

shown in Figure 1. The survival percentage of the bacterial strains decreased as the plant extract concentration increased. In comparison to the untreated control, good growth was detected at 5 µg/mL of the plant extract, whereas a substantial suppression (78%) was observed at 500 µg/mL. Plant extract exhibited substantial antibacterial activity against *S. aureus*, with the MIC being 31.6 µg/mL (Figure 1).

A crystal violet test was used to measure biofilm production.[14] The effects of different EESB

Figure 3. Effect of the EESB at the MIC (31.6 µg/mL) on the DNA integrity of *S. aureus*

concentrations (5 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, and 500 µg/mL) were evaluated, and the results are presented in Figure 2. As depicted in Figure 2, the treated cells exhibited substantially less biofilm formation than the control cells. At higher concentrations of AEESA, the proportion of biofilm formed was proportionally lower in the treated cells compared with the control cells. The amount of biofilm formation following treatment with AEESA at 10 µg/mL, 50 µg/mL, 100 µg/mL, and 500 µg/mL was significantly reduced by 50%, 80%, 92%, and 94%, respectively, when compared to the control. AEESA exhibited a comparable antibacterial effect against the existing *S. aureus*-generated biofilms (24 h old). Biofilm formation of 100% in the control corresponds to an OD530 of 0.48 0.05 in the absence of AEESA (0 µg/mL) and uninhibited biofilm formation by the bacterial strain. Significant biofilm inactivation was observed at 31.6 µg/mL, which was generally consistent with the MIC value.

Using the standard "DNA ladder" technique, DNA is extracted from apoptotic cells and separated on an agarose gel to study DNA fragmentation. As shown in Figure 3, treatment of *S. aureus* genomic DNA with AEESA at the MIC (31.6 µg/mL) resulted in the fragmentation of chromosomal DNA into small internucleosomal fragments compared to untreated DNA.
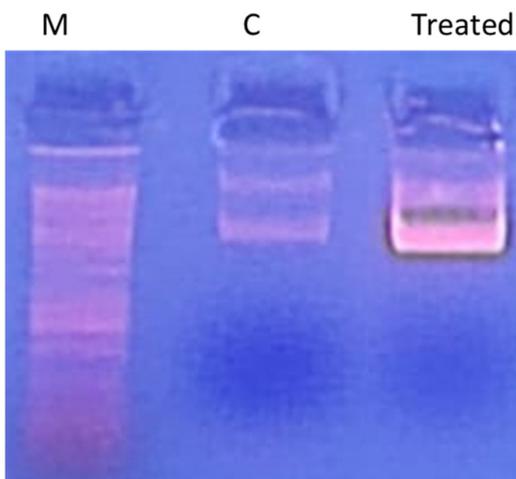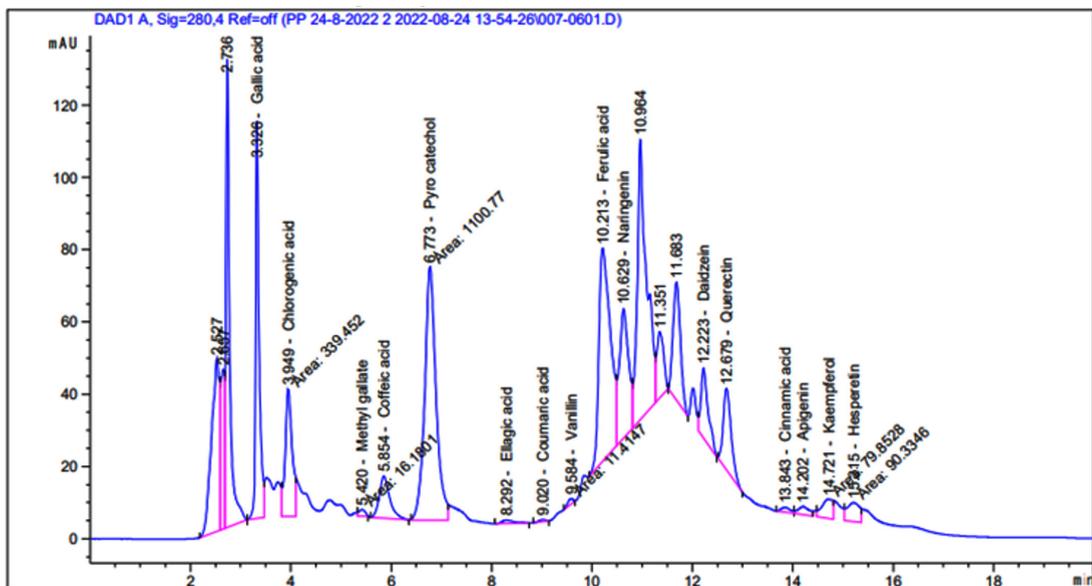

Figure 4. HPLC of the phenolic compounds in AEESA

Phenolic compounds in AEESA using HPLC

According to the findings in Figure 4 and Table 1, the phenolic components in the 70% aqueous ethanol extract of *S. argel* had notable concentrations of phenolic compounds, with pyrocatechol (9519.95 µg/g), ferulic acid (3221.41 µg/g), chlorogenic acid (3221.41 µg/g), and gallic acid (2730.85 µg/g), being the most abundant. Naringenin (2262.80µg/g) and quercetin (1750.25µg/g) were the major flavonoids observed.

Table 1. Phenolic compounds in AEESA using HPLC

Retention time (min.)	Compounds	Conc. (µg/g)	Compounds nature
3.326	Gallic acid	2730.85	Phenolic acid
3.949	Chlorogenic acid	2934.62	
4.522	Catechin	0.00	Flavonol
5.420	Methyl gallate	70.03	Phenolic acid
5.854	Coffeic acid	803.38	
6.479	Syringic acid	0.00	
6.773	Pyrocatechol	9519.95	Phenolic compound
7.795	Rutin	0.00	Flavonoid glycoside
8.292	Ellagic acid	208.52	Phenolic acid
9.020	Coumaric acid	7.10	phenolic derivative of cinnamic acid
9.584	Vanillin	23.25	phenolic aldehyde
10.213	Ferulic acid	3221.41	Phenolic acid
10.629	Naringenin	2262.80	Flavanone
12.223	Daidzein	654.07	Hydroxyisoflavone
12.679	Quercetin	1750.25	Flavonol
13.843	Cinnamic acid	25.01	Phenolic acid
14.202	Apigenin	139.33	Flavone
14.721	Kaempferol	399.74	Flavonol
15.215	Hesperetin	305.38	Flavanone

Figure 5. GC-MS spectral analysis of the AEESA

Table 2. GC-MS spectral analysis of AEESA

Peak No	RT	Area %	Compounds
1.	13.037	5.46	Butylated Hydroxytoluene
2.	13.483	1.01	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-
3.	14.344	0.73	18-Methyl-nonadecanol, trimethylsilyl ether
4.	16.277	1.81	5-Caranol, trans,trans-(+)-
5.	16.849	4.77	2-Pentadecanone, 6,10,14-trimethyl-
6.	17.632	2.11	Cyclodecasiloxane, eicosamethyl-
7.	18.158	7.22	Ascorbic acid 2,6-dihexadecanoate
8.	18.361	2.02	Docosanoic acid, ethyl ester
9.	19.019	2.16	1,3-dioxolane,4-methyl-2-pentadecyl
10.	19.257	0.67	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione
11.	20.264	2.18	Cyclononasiloxane, octadecamethyl-
12.	20.387	1.75	N-(2-Methylbutyl)undeca-(2E,4E)-diene-8,10-diynamide
13.	20.777	1.04	2-Heptanone, 6-methyl-, O-methyloxime
14.	21.41	2.2	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-
15.	21.519	1.28	E-11-Hexadecenal
16.	21.596	0.81	2H-Pyran-2-one, tetrahydro-6-tridecyl-
17.	21.631	1.48	Eicosanoic acid, 2,3-bis(acetyloxy)propyl ester
18.	22.134	33.44	9,12-octadecadienoic acid (z,z)-, methyl ester
19.	22.49	3.58	linalool
20.	22.733	2.76	Hexanoic acid, octadecyl ester
21.	22.788	1.01	Estra-1,3,5(10)-trien-17.beta.-ol
22.	23.057	2.1	di(2- ethylhexyl) phthalate
23.	23.233	1.04	1,4-Piperazinediethanol, .alpha.,.alpha.'-bis(phenoxymethyl)-
24.	23.33	0.72	cis-13-Eicosenoic acid
25.	23.503	2.08	Hexadecanoic acid, methyl ester
26.	24.479	2.64	Nonanoic acid, 9-oxo-, methyl ester
27.	25.1	3.35	14-Methyl-pentadecane-1,2-diol, isopropylidene derivative
28.	25.585	3.33	10-octadecenoic acid, methyl ester
29.	26.189	1.9	Lupeol
30.	28.384	3.35	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-

Identification of volatile compounds in AEESA using the GC-MS analysis

The results of the GC-MS analysis of *S. argel* extract are presented in Figure 5 and Table 2. The data revealed a high percentage of 9,12-octadecadienoic acid (z,z)- methyl ester (33.44%), ascorbic acid 2,6-dihexadecanoate (7.22%), and butylated hydroxytoluene (5.46%), followed by 2-Pentadecanone, 6,10,14-trimethyl (4.77%), 2-pentadecanone, 6,10,14-trimethyl (3.35%),stigmasta-5,22-dien-3-ol, and acetate, (3.Beta; 3.35%).

DISCUSSION

In this study, multiple investigations indicated that the aerial parts of *S. argel* ethanolic extract are rich in bioactive compounds, and the major phenolic compounds(pyrocatechol, ferulic acid, chlorogenic acid, and gallic acid) and flavonoids (naringenin and quercetin) were identified. These results were in line with the findings of Soliman et al.,[3] which revealed that gallic acid was the most abundant phenolic acid, followed by syringic, p-coumaric, caffeic, and ferulic acids.[3] Catechin, quercetin, luteolin, and rutin were detected in the methanolic extract. *Staphylococci* are common constituents of the human skin microbiome and are among the most common bacteria responsible for serious infections worldwide.[8] Overuse of antibiotics may result in the spread of antibiotic resistance. Alternatively, natural compounds derived from wild heterotrophic plants may be used to fight pathogenic bacteria without negatively affecting health or the environment. AEESA was tested

against *S. aureus* using disc diffusion, MIC determination, biofilm development, and DNA fragmentation tests. HPLC and GC-MS were used to identify the main components of AEESA. The diameter of the zone of inhibition and MIC are inversely related. When bacteria become increasingly sensitive to antimicrobial agents, their MIC decreases and the zone of inhibition increases. In contrast, as the bacteria become more resistant to antibiotics, the MIC increases, and the zone of inhibition decreases.

The MIC of AEESB against *S. aureus* was 31.6 µg/mL. This MIC value was less than those for *S. villosa* (6.25 mg/mL) and *S. persia* (1.56 mg/mL).[16] Oueslati[17] reported similar findings, revealing that an ethyl acetate extract from the roots of *S. imbricata* demonstrated possible antibacterial action against *S. aureus*, as shown by a relatively low MIC value (16 µg/mL).[17] However, it has recently been reported that the ethanolic extract of *S. argel* displayed no inhibitory effects against several bacterial strains, including *S. aureus*.[18] The differences in MIC values may be related to various variables, including the plant species used, plant portion extracted, extraction procedures, type of solvent used, and the bacterial strain. As a result, the chemical makeup, including the active components, changed, as did the possible mechanisms of action against microbial cells. Furthermore, flavonoids have been proposed to exhibit antibacterial activities that are mediated by the inhibition of nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, attachment and biofilm formation, porin inhibition on the cell membrane, and membrane alteration.[19,20]

Some chemicals in *S. argel* extract, such as pyrocatechol, may have antibacterial activity against *S. aureus*. A possible explanation for this is the toxic effects of the reactive oxygen species (ROS) generated during catechol oxidation. ROS are highly reactive radicals that freely attack vital molecules within cells, including DNA and RNA, and cause undesired redox reactions.[21] Similar results have been recently reported for *Uncaria Gambir* leaf extract and ester derivatives of catechol against peri-implantitis-related bacteria.[22] In addition, 9,12-octadecadienoic acid (z,z)-methyl ester interacts with the cell membrane, destroying its integrity because of its lipophilic

nature. Bacterial cell-selective permeability, efflux pumping systems, ATPase machinery activity, and RNA synthesis are compromised, resulting in bacterial mortality.[21] All of these processes contribute to the high antibacterial activity of the chemicals. The methanolic extracts of aerial parts of *S. argel* have been found to be high in 2-(5-methyl-5 vinyl tetrahydro-2-furanyl)-2-propanol (11.63%), hexanoic acid methyl ester (10.93%), 3-dioxolane,4-methyl-2-pentadecyl (9.69%), and phenol, 2-(1,1-dimethylethyl) (8.50%).[18] The variability in the GC-Mass profile could be attributed to multiple factors, including differences in the chemical properties of the solvent used, part of the plant, and the growing phase of the plant. It was recently reported that the ethyl acetate extract of *S. argel* possesses antifungal, antioxidant, and wound-healing properties.[23]

Bacterial biofilms are formed when cell aggregates attach to surfaces or interfaces and are embedded in a self-produced extracellular matrix composed of proteins and polysaccharides.[24] Biofilm production is critical for bacterial survival, particularly in challenging environments containing antimicrobial substances.[24] According to our findings, AEESA displayed an antibiofilm-forming activity comparable to its antibacterial activity. Antibiotic resistance in bacteria is associated with their propensity to form biofilms rather than their presence as free bacteria. Antimicrobial agents cannot reach bacterial cells in the presence of biofilms, because biofilms render them ineffective.[24] Biofilm development is a well-documented multi-step process that includes adhesion, maturation/proliferation, and separation. Nonspecific antibiotic resistance is also involved in biofilm-associated *S. aureus* infection.[8] The use of AEESA to reduce biofilm development may minimize antibiotic resistance.

DNA fragmentation is a fundamental mechanism of microbial mortality. The release of the intercellular component DNA suggests a ruptured membrane. After 72 h of combined treatment with bacterial cells, our findings showed distinct smear production in contrast to the untreated cells. Previous studies have shown that herbal components exert their antibacterial effects through DNA fragmentation. Treatment with Caesalpinia coriaria glycosides

and flavonoids, for example, causes the apparent synthesis of damaged DNA in pathogenic bacteria such as *S. aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. The antibacterial activity of *Syzygium cumini* ethanolic leaf extract has also been demonstrated through an underlying method of action involving DNA breakage. DNA fragmentation activity was used to determine the antibacterial activity of *S. cumini* methanolic seed extract.[25] Certain carbohydrates such as chitosan have been shown to be efficient against *S. aureus* through DNA fragmentation.[26] Genomic DNA fragmentation is fatal and irreversible. Endonucleases cleave linker DNA selectively into mono- and oligonucleosomal DNA pieces. Our results indicate that AEESA contains major phenolic compounds (pyrocatechol, ferulic acid, chlorogenic acid, and gallic acid) and flavonoids (naringenin and quercetin), as reported by Elsanhoty et al.,[18] who found that gallic acid, synergic acid, and p-coumaric acid are the primary phenolic acids, and catechin, quercetin, luteolin, and kaempferol are the common flavonoids present in the methanolic extract of *S. argel*. A GC-MS study also revealed that the high percentages of volatile components in AEESA were 9,12-octadecadienoic acid (z,z), methyl ester, ascorbic acid 2,6-dihexadecanoate (7.22%), and butylated hydroxytoluene, as reported by Elsanhoty et al.[18]

CONCLUSION

AEESA shows promising antibacterial activity against *S. aureus* in disc diffusion, biofilm formation, and DNA fragmentation tests. The MIC was 31.6 g/mL. The ability of AEESA to inhibit *S. aureus*. The polyphenolic and volatile compounds in AEESB act as antibacterial agents. The use of AEESA to inhibit biofilm formation would thereby reduce antibiotic resistance and pave the way for the future deployment of AEESA components for potentially useful applications. The use of AEESA to inhibit biofilm formation would prevent antibiotic resistance and promote the future applications of AEESA components in therapeutics.

ACKNOWLEDGMENTS

FUNDING

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Not applicable.

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