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RESEARCH ARTICLE OPEN ACCESS

Synergistic Effect of *Artemisia vulgaris* L. with Antibiotics against Clinical Isolates of Multidrug-resistant *Staphylococcus aureus*

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Abstract

Multidrug-resistance in *Staphylococcus aureus* is a global health concern. Alternative strategies to battle antibiotic resistance with novel antimicrobials is of prime importance in the current times. Plant bioactive compounds in combination with antibiotics have proven effective for modulating the antibiotic resistance of various drug-resistant bacteria. *Artemisia vulgaris* L. is a common herbaceous plant used in traditional medicine. This study evaluated the synergistic activity of methanol extract of the leaves of *Artemisia vulgaris* L. with selected antibiotics directed against clinical isolates of multidrug-resistant *Staphylococcus aureus.* The plant extract concentration of 500 mg/ml exhibited the largest zone of inhibition of 23.33 ± 0.57 mm against the isolate SA 05. The minimum inhibitory concentration of the plant extract determined was 3.90 mg/ml and the minimum bactericidal concentration was 7.81 mg/ml against isolates SA 08 and SA 10, respectively. The MBC/MIC value of ≤4 exhibited a bactericidal effect of the extract against most of the tested clinical isolates. The methanol extract of *A. vulgaris* showed synergistic activity with oxacillin and clindamycin against all the clinical isolates of *S. aureus*. Synergistic activity was also exhibited with penicillin, gentamicin, and ciprofloxacin against most of the clinical isolates. Thirty phytocompounds were detected in the extract of *A. vulgaris* by Gas chromatography-Mass spectrometry analysis. Results have revealed potential antibiotic resistance modulatory property of *A. vulgaris* against multidrug-resistant *S. aureus* through synergistic action with antibiotics.

Keywords: *Artemisia vulgaris*, Multidrug-resistant *Staphylococcus aureus,* GC-MS, Synergy, Antibiotics

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INTRODUCTION

Staphylococcus aureus is an important clinical pathogen in both the hospital and community settings. The infections caused by the bacterium have revealed an escalated disease and death rates.[1] The "World Health Organisation Bacterial Priority Pathogen List 2024" has listed methicillin-resistant *Staphylococcus aureus* in the "high-priority pathogen category" for the research and development to control antibiotic resistance.[2] Antibiotic resistance has become a major bottleneck for effective treatment of bacterial infections.[3] The development of cost-effective and safer alternative antimicrobial drugs becomes an important requirement for treatment of infections.[4] Plants have been utilised in traditional medicine since ancient civilizations.[5] A large number of published research indicates that phytochemicals and antibiotics work synergistically, suggesting that this combination may be useful in treating drug-resistant illnesses.[6,7] *Artemisia vulgaris* L. is a common herbaceous plant belonging to the *Asteraceae* family. It is an annual herb and a component used in traditional medicine. The plant is commonly termed as mugwort and is found in different parts of Europe, Asia, and North America.[8] Infusions of plant stems and leaves have been used in traditional medicine to treat tumours, menopausal and menstrual symptoms, epilepsy, diabetes, dermatitis, bacterial infections, and insomnia.[8] The study examined the antibacterial potential of *A. vulgaris* leaves methanol extract and its synergistic effect in conjunction with specific antibiotics directed against multidrug-resistant *Staphylococcus aureus*.

MATERIALS AND METHODS

The research was conducted in the Department of Microbiology, School of Life Sciences, Sikkim University, India. The chemicals and consumables used in the study were procured from Merck, Germany, and HiMedia Laboratories Private Limited, India.

Collection and Identification of plant

The leaves of *Artemisia vulgaris* L. were collected from Geyzing, India (27°17'206'' N, 088°14'039'' E). The herbarium of the test plant was prepared and the plant was identified by the plant taxonomist at the Department of Botany, University of North Bengal, India. One copy of the herbarium with Accession No. 12633 was deposited at the herbarium of the Plant Taxonomy Division, University of North Bengal, India.

Plant extract preparation

The test plant leaves were collected, properly cleaned, and then left to dry in the shade for 20 days. Using a grinder, the dried leaves were chopped into small fragments and ground into a fine powder. The powder was again sieved to separate the bigger husks and stalks to get the fine powder (Figure 1). Methanol extract was prepared by Soxhlet extraction using 10 g of powdered material (1:10). Whatman No. 1 filter paper was used to filter the methanol extract, and a rotary vacuum evaporator was used to concentrate the mixture. The extract was dried and kept in vials at 4 °C.[9]

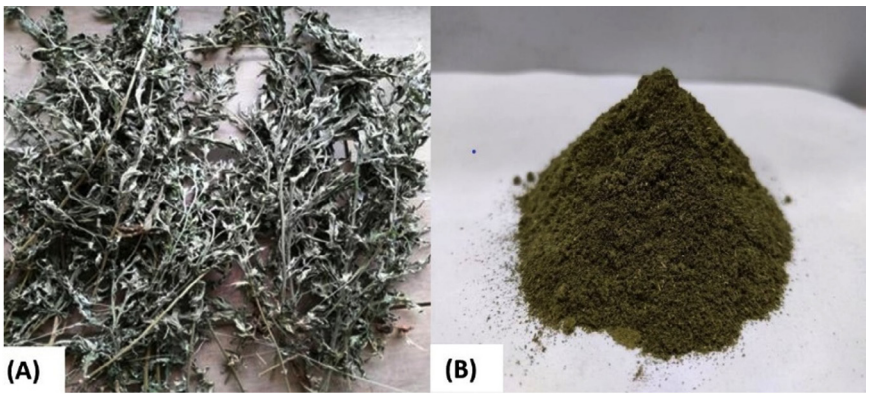

Figure 1. *A. vulgaris* (A) dried plant sample (B) powdered plant sample

Test bacteria

A total of twenty MDR *Staphylococcus aureus* were used for the present study. These isolates have been identified in our earlier published study[10] with respect to their multidrug-resistance antibiogram profile.

Determination of antibacterial activity

Using the agar-well diffusion method, the antibacterial activity of the extract of the leaves of *A. vulgaris* L. was determined.[11] The surface of the MHA plates was inoculated with an inoculum of the test bacteria at OD 0.1 at 600 nm, corresponding to the 0.5 McFarland Standard. Plant extract (500 mg/mL) was pipetted into the punctured 8 mm wells, followed by the addition of vancomycin (30 mg/mL) and DMSO (10%) as the positive and negative controls, respectively. The incubation of the plates was done at 37 °C for 24 hours. By measuring the zone of inhibition, including the well diameter, the antibacterial activity of the tested plant extract was determined. Each experiment was performed in three sets.

Determination of MIC and MBC

The broth microdilution technique was used to determine the minimum inhibitory concentration (MIC) using 96-well plates at various concentrations of the plant extract.[12] Two fold serial dilutions were made from column 1-10 to prepare the various concentrations (250 mg/ml to 0.48 mg/ml) of the plant extract in the 96 well microtiter plates. By immediately plating the contents of the 96 wells at concentrations greater than the MIC value in the MHA plates, the minimum bactericidal concentration (MBC) was ascertained. After incubating at 37 °C for 24 hours, the concentration at which no colony growth

Table 1. MIC and MBC of methanol extract of *Artemisia vulgaris* L. leaves against clinical isolates of MDR *S. aureus*

No.	Isolate	MIC (mg/ml)	MBC (mg/ml)	MBC/ MIC
1	SA 01	15.62	125	8
2	SA 02	31.25	62.5	2
3	SA 03	15.62	31.25	2
4	SA 04	15.62	31.25	2
5	SA 05	7.81	15.62	2
6	SA 06	7.81	15.62	2
7	SA 07	7.81	31.25	4
8	SA 08	3.90	7.81	2
9	SA 09	7.81	15.62	2
10	SA 10	3.90	7.81	2
11	SA 11	7.81	31.25	4
12	SA 12	7.81	31.25	4
13	SA 13	7.81	31.25	4
14	SA 14	7.81	31.25	4
15	SA 15	7.81	31.25	4
16	SA 16	7.81	31.25	4
17	SA 17	7.81	31.25	4
18	SA 18	7.81	31.25	4
19	SA 19	7.81	31.25	4
20	SA 20	7.81	31.25	4
21	MTCC 740 *S. aureus*	1.95	3.90	2

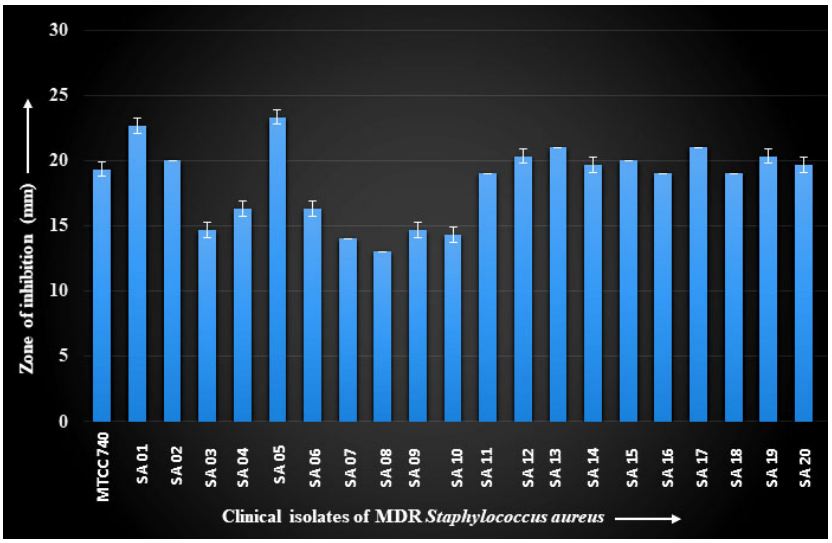

Figure 2. Zone of inhibition with methanol extract of *A. vulgaris* L. leaves against clinical isolates of MDR *S. aureus*

Table 2. Determination of synergistic activity of methanol extract of *A. vulgaris* L. leaves with clindamycin against clinical isolates of MDR *S. aureus*

No.	Clinical MDR *S. aureus*	Minimum Inhibitory Concen. (MIC)			Fractional Inhibitory Concen. Index (FICI)	Result
		Clindamycin (µg/mL)	*A. vulgaris* (mg/ml)	Clindamycin + *A. vulgaris*		
1	SA 01	0.48	15.62	0.03	0.12	Synergistic
2	SA 02	0.48	31.25	0.03	0.12	Synergistic
3	SA 03	0.48	15.62	0.03	0.12	Synergistic
4	SA 04	0.48	15.62	0.03	0.12	Synergistic
5	SA 05	0.48	7.81	0.03	0.12	Synergistic
6	SA 06	0.48	7.81	0.06	0.24	Synergistic
7	SA 07	0.48	7.81	0.03	0.12	Synergistic
8	SA 08	0.48	3.9	0.03	0.12	Synergistic
9	SA 09	0.48	7.81	0.03	0.12	Synergistic
10	SA 10	0.48	3.9	0.06	0.24	Synergistic
11	SA 11	0.12	7.81	0.01	0.2	Synergistic
12	SA 12	0.12	7.81	0.03	0.5	Synergistic
13	SA 13	0.12	7.81	0.01	0.2	Synergistic
14	SA 14	0.12	7.81	0.03	0.5	Synergistic
15	SA 15	0.12	7.81	0.01	0.2	Synergistic
16	SA 16	0.12	7.81	0.03	0.5	Synergistic
17	SA 17	0.12	7.81	0.03	0.5	Synergistic
18	SA 18	0.12	7.81	0.01	0.2	Synergistic
19	SA 19	250	7.81	31.25	0.24	Synergistic
20	SA 20	0.12	7.81	0.03	0.5	Synergistic
21	MTCC 740	0.12	1.95	0.03	0.5	Synergistic

Table 3. Determination of synergistic activity of methanol extract of *A. vulgaris* L. leaves with oxacillin against clinical isolates of MDR *S. aureus*

No.	Clinical MDR *S. aureus*	Minimum Inhibitory Concen. (MIC)			Fractional Inhibitory Concen. Index (FICI)	Result
		Oxacillin (µg/mL)	*A. vulgaris* (mg/ml)	Oxacillin + *A. vulgaris*		
1	SA 01	31.25	15.62	7.8	0.5	Synergistic
2	SA 02	31.25	31.25	3.9	0.24	Synergistic
3	SA 03	31.25	15.62	1.95	0.12	Synergistic
4	SA 04	31.25	15.62	1.95	0.12	Synergistic
5	SA 05	31.25	7.81	1.95	0.12	Synergistic
6	SA 06	31.25	7.81	1.95	0.12	Synergistic
7	SA 07	31.25	7.81	1.95	0.12	Synergistic
8	SA 08	31.25	3.9	1.95	0.12	Synergistic
9	SA 09	15.62	7.81	0.97	0.12	Synergistic
10	SA 10	31.25	3.9	3.9	0.24	Synergistic
11	SA 11	1000	7.81	250	0.5	Synergistic
12	SA 12	31.25	7.81	7.81	0.5	Synergistic
13	SA 13	31.25	7.81	3.9	0.24	Synergistic
14	SA 14	31.25	7.81	3.9	0.24	Synergistic
15	SA 15	15.62	7.81	0.97	0.12	Synergistic
16	SA 16	62.5	7.81	7.81	0.24	Synergistic
17	SA 17	62.5	7.81	15.62	0.5	Synergistic
18	SA 18	15.62	7.81	0.97	0.12	Synergistic
19	SA 19	15.62	7.81	3.9	0.5	Synergistic
20	SA 20	62.5	7.81	7.81	0.24	Synergistic
21	MTCC 740	3.9	1.95	0.48	0.24	Synergistic

Table 4. Determination of synergistic activity of methanol extract of *A. vulgaris* L. leaves with ciprofloxacin against clinical isolates of MDR *S. aureus*

No.	Clinical MDR *S. aureus*	Minimum Inhibitory Concen.			Fractional Inhibitory Concen. Index (FICI)	Result
		Ciprofloxacin (µg/mL)	*A. vulgaris* (mg/ml)	Ciprofloxacin + *A. vulgaris*		
1	SA 01	15.62	15.62	3.9	0.5	Synergistic
2	SA 02	3.9	31.25	0.48	0.24	Synergistic
3	SA 03	15.62	15.62	3.9	0.5	Synergistic
4	SA 04	15.62	15.62	3.9	0.5	Synergistic
5	SA 05	31.25	7.81	7.81	0.5	Synergistic
6	SA 06	31.25	7.81	7.81	0.5	Synergistic
7	SA 07	31.25	7.81	3.9	0.24	Synergistic
8	SA 08	15.62	3.9	3.9	0.24	Synergistic
9	SA 09	31.25	7.81	3.9	0.24	Synergistic
10	SA 10	31.25	3.9	3.9	0.24	Synergistic
11	SA 11	250	7.81	31.25	0.24	Synergistic
12	SA 12	125	7.81	31.25	0.5	Synergistic
13	SA 13	125	7.81	15.62	0.24	Synergistic
14	SA 14	125	7.81	62.5	1	Additive
15	SA 15	62.5	7.81	31.25	1	Additive
16	SA 16	125	7.81	31.25	0.5	Synergistic
17	SA 17	125	7.81	31.25	0.5	Synergistic
18	SA 18	125	7.81	31.25	0.5	Synergistic
19	SA 19	31.25	7.81	7.81	0.5	Synergistic
20	SA 20	62.5	7.81	15.62	0.5	Synergistic
21	MTCC 740	0.48	1.95	0.03	0.12	Synergistic

Table 5. Determination of synergistic activity of methanol extract of *A. vulgaris* L. leaves with penicillin against clinical isolates of MDR *S. aureus*

No.	Clinical MDR *S. aureus*	Minimum Inhibitory Concen. (MIC)			Fractional Inhibitory Concen. Index (FICI)	Result
		Penicillin (µg/mL)	*A. vulgaris* (mg/ml)	Penicillin + *A. vulgaris*		
1	SA 01	250	15.62	31.25	0.24	Synergistic
2	SA 02	15.62	31.25	1.95	0.24	Synergistic
3	SA 03	125	15.62	15.62	0.24	Synergistic
4	SA 04	62.5	15.62	7.81	0.24	Synergistic
5	SA 05	31.25	7.81	15.62	1	Additive
6	SA 06	250	7.81	62.5	0.5	Synergistic
7	SA 07	125	7.81	31.25	0.5	Synergistic
8	SA 08	125	3.9	31.25	0.5	Synergistic
9	SA 09	250	7.81	62.5	0.5	Synergistic
10	SA 10	250	3.9	62.5	0.5	Synergistic
11	SA 11	250	7.81	31.25	0.24	Synergistic
12	SA 12	125	7.81	31.25	0.5	Synergistic
13	SA 13	125	7.81	15.62	0.24	Synergistic
14	SA 14	125	7.81	62.5	1	Additive
15	SA 15	62.5	7.81	31.25	1	Additive
16	SA 16	125	7.81	31.25	0.5	Synergistic
17	SA 17	125	7.81	31.25	0.5	Synergistic
18	SA 18	125	7.81	31.25	0.5	Synergistic
19	SA 19	31.25	7.81	7.81	0.5	Synergistic
20	SA 20	62.5	7.81	15.62	0.5	Synergistic
21	MTCC 740	1.95	1.95	0.24	0.24	Synergistic

Table 6. Determination of synergistic activity of methanol extract of *A. vulgaris* L. leaves with gentamicin against clinical isolates of MDR *S. aureus*

No.	Clinical MDR *S. aureus* Concen.	Minimum Inhibitory Concen. (MIC)			Fractional Inhibitory Concen. Index (FICI)	Result
		Gentamicin (µg/mL)	*A. vulgaris* (mg/ml)	Gentamicin + *A. vulgaris*		
1	SA 01	15.62	15.62	1.95	0.24	Synergistic
2	SA 02	0.97	31.25	0.06	0.12	Synergistic
3	SA 03	7.81	15.62	0.97	0.24	Synergistic
4	SA 04	7.81	15.62	0.97	0.24	Synergistic
5	SA 05	31.25	7.81	15.62	1	Additive
6	SA 06	3.9	7.81	0.97	0.5	Synergistic
7	SA 07	15.62	7.81	3.9	0.5	Synergistic
8	SA 08	15.62	3.9	3.9	0.5	Synergistic
9	SA 09	62.5	7.81	15.62	0.5	Synergistic
10	SA 10	7.81	3.9	3.9	1	Additive
11	SA 11	62.5	7.81	15.62	0.5	Synergistic
12	SA 12	31.25	7.81	15.62	1	Additive
13	SA 13	15.62	7.81	3.9	0.5	Synergistic
14	SA 14	31.25	7.81	7.81	0.5	Synergistic
15	SA 15	0.97	7.81	0.12	0.24	Synergistic
16	SA 16	62.5	7.81	31.25	1	Additive
17	SA 17	62.5	7.81	31.25	1	Additive
18	SA 18	31.25	7.81	7.81	0.5	Synergistic
19	SA 19	125	7.81	31.25	0.5	Synergistic
20	SA 20	62.5	7.81	15.62	0.5	Synergistic
21	MTCC 740	0.01	1.95	0.002	0.45	Synergistic

occurred was determined as the MBC value. The experiment was done in three sets.

Determination of synergistic activity

Synergistic activity of the test plant extract in conjunction with selected antibiotics was determined by the checkerboard technique[13] with minor modifications. Prior to performance of the checkerboard assay, the MICs of four different antibiotics from selected classes were determined in triplicate. The antibiotics used were penicillin, oxacillin, gentamicin, ciprofloxacin, and clindamycin. After a 24-hour incubation period at 37 °C, 30 µl of 0.015% resazurin was added to each well and further incubated for an additional 2-4 hours to observe for any colour changes. The synergistic activity was determined by calculating the FIC index (FICI) of each combination.[14]

GC-MS analysis

Chemical characterization of the test plant extract was done using Gas Chromatography-Mass Spectrometry (GC-MS) instrument (QP 2010 Ultra SHIMADZU), at the Advanced Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi, India.[10]

RESULTS

Determination of antibacterial activity

The clinical isolates of MDR *S. aureus* showed a zone of inhibition ranging in diameter from 13.00 ± 0.00 mm to 23.33 ± 0.58 mm with the methanol extract of *A. vulgaris* L. leaves. The isolate SA 05 showed the largest zone of inhibition. The results are shown in Figure 2.

Determination of MIC and MBC

The isolates SA 08 and SA 10 exhibited the lowest MIC and MBC values of 3.90 mg/ml and 7.81 mg/ml, respectively. The results are presented in Table 1. When the MBC/MIC ratio is ≤4, the drug is regarded as bactericidal.[15] The test plant extract exhibited bactericidal activity against most of the clinical isolates of *S. aureus*.

Determination of synergistic activity

When combined with clindamycin and oxacillin, the methanol extract exhibited synergistic effect against all clinical isolates of multidrug-resistant *S. aureus* with FICI <0.5. The results are shown in Table 2 and Table 3. The extract showed a synergistic effect with ciprofloxacin against all isolates except SA 14 and SA 15, which exhibited an additive effect (FICI = 1) as shown in Table 4. With the exception of SA 05, SA 14, and SA 15, which had an additive effect in conjunction with the antibiotic, the plant extract exhibited synergistic effects with penicillin against the majority of isolates (Table 5). Similarly, gentamicin showed a synergistic effect against most of the isolates except SA 05, SA 10, SA 12, SA 16, and SA 17 which exhibited additive effect (Table 6). These findings suggested that the combination

Table 7. GC-MS analysis of methanol extract of *A. vulgaris* L. leaves

Peak	Retention Time	Peak area%	Compound Name	Molecular formula	Molecular weight
1	10.229	1.18	(3E,10Z)-Oxacyclotrideca-3,10-diene-2,7-dione	$C_{12}H_{16}O_3$	208
2	12.028	72.07	mome inositol	$C_7H_{14}O_6$	194
3	12.31	1.85	Neophytadiene	$C_{20}H_{38}$	278
4	12.563	0.69	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
5	12.765	0.49	Heptadecanal	$C_{17}H_{34}O$	254
6	13.046	0.5	10-12-Pentacosadiynoic acid	$C_{25}H_{42}O_2$	374
7	15.031	2.28	Phytol	$C_{20}H_{40}O$	296
8	15.56	0.46	bufa-20,22-dienolide, 14,15-epoxy-3,5,16-trihydroxy-	$C_{24}H_{32}O_6$	416
9	16.484	1.2	9-Acetyl-S-octahydrophenanthrene	$C_{16}H_{20}O$	228
10	16.911	1.46	5-Acetylimino-7-acetylamino-8-5H-quinolone	$C_{13}H_{11}N_3O_3$	257
11	17.097	0.13	cis-Arbusculone	$C_9H_{14}O_2$	154
12	17.442	0.27	2-Methyl-oct-2-enedial	$C_9H_{14}O_2$	154
13	17.83	0.11	1-cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	$C_{10}H_{16}O$	152
14	18.794	0.92	3-Buten-2-one, 4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl)-	$C_{13}H_{20}O_2$	208
15	19.068	0.58	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	$C_{25}H_{40}O_6$	436
16	19.809	2.46	Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-, [1R-(1.alpha.,3.beta.,4.alpha.,6.alpha.)]-	$C_{10}H_{18}O$	154
17	20.621	0.79	2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-	$C_{30}H_{50}$	410
18	21.226	0.73	4a(2H)-Naphthalenemethanol, octahydro-	$C_{11}H_{20}O$	168
19	21.462	0.72	N-hentriacontanol-1	$C_{31}H_{64}O$	452
20	23.09	0.16	2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	428
21	23.772	0.43	10-Methylundec-2-en-4-olide	$C_{12}H_{20}O_2$	196
22	24.189	0.4	Vitamin E	$C_{29}H_{50}O_2$	430
23	26.359	0.35	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	$C_{33}H_{54}O_3$	498
24	28.289	0.67	Olean-12-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	468
25	29.386	1.46	24-Noroleana-3,12-diene	$C_{29}H_{46}$	394
26	3.163	1.01	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$	424
27	31.066	0.45	D:B-Friedo-B':A'-Neogammacer-5-en-3-one	$C_{30}H_{48}O$	424
28	31.417	5.35	24-Norursa-3,12-diene	$C_{29}H_{46}$	394
29	33.066	0.33	cholest-20(22)-en-3-one, 4,5-epoxy-11-hydroxy-	$C_{27}H_{42}O_3$	414
30	33.469	0.51	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate	$C_{22}H_{42}O_2$	338

of methanol extract of *A. vulgaris* leaves and antibiotics synergistically inhibited growth of most of the clinical isolates of MDR *S. aureus.*

GC-MS analysis

The GC-MS analysis detected 30 phytocompounds as shown in Table 7. Some of the compounds detected were mome inositol (72.07%), 24-Norursa-3,12-diene (5.35%), Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-, [1R-(1.alpha.,3.beta.,4.alpha.,6.alpha.)]- (2.47%), Phytol (2.28%), Neophytadiene (1.85%), 5-Acetylimino-7-acetylamino-8-5H-quinolone (1.46%), 24-Noroleana-3,12-diene (1.46%), (3E,10Z)-Oxacyclotrideca-3,10-diene-2,7-dione (1.18%), and Vitamin E (0.4%).

DISCUSSION

Microorganisms resistant to antibiotics have emerged as a result of the widespread use of antibiotics. Drug-resistant pathogens have become a major hindrance in the treatment of infections. Many reviews have revealed phytochemicals from plants as possible substitutes for antibiotics against drug-resistant infections.[16] It has been observed that several solvent extracts of *Rhus chinensis* Mill exhibited antibacterial and cell envelope-damaging qualities against *Escherichia coli* and *S. aureus.*[17] The present study has examined the methanol extract of *A. vulgaris* L. leaves for its antibacterial activity and synergistic properties in conjunction with different test antibiotics against MDR *Staphylococcus aureus*.

It has been established that the phytochemicals have inhibitory effects on clinical isolates when administered as extracts.[7] The methanol extract of the leaves of *A. vulgaris* exhibited a zone of inhibition ranging from 13.00 ± 0.00 mm to 23.33 ± 0.57 mm against the tested MDR *Staphylococcus aureus.* Dahiya and Purkayastha[18] reported the antibacterial activity of various medicinal plants against MDR clinical isolates of both Gram-positive and Gram-negative bacteria. Recently, the MIC value of 2 mg/mL and MBC value of 5 mg/mL against *S. aureus* ATCC 25923 were reported using the ethanolic extracts of *Artemisia annua* L.[19] The lowest MIC value was 3.90 mg/mL, and the MBC value was 7.81 mg/mL against the isolate SA 10. Methanolic extracts of *A. vulgaris* have demonstrated comparable effectiveness against pathogenic bacteria, including *S. aureus* (ATCC:25923), with MIC and MBC values of 12.5 mg/mL and 25 mg/mL, respectively.[20] Methanol extract of *A. vulgaris* leaves was also shown to have a MIC value of 12.5 mg/ml against MRSA (ATCC 25923).[21] The primary components of the essential oils (caryophyllene, germacrene D, and humulene) were found to have antibacterial and antifungal properties against *S. aureus* and *Candida albicans*, respectively.[22,23] The antibacterial activity may be attributed to the synergistic interactions between the different phytoconstituents.[24] However, different solvent fractions of this plant should also be examined for antibacterial properties.

Our findings demonstrated synergistic interaction of the test plant extract in combination with gentamicin and penicillin, respectively against almost all the isolates studied. Previous synergistic studies using combination of penicillin and *Salvadora persica* stem ethanol extract against *S. aureus* have shown an increase in the zone of inhibition from 18 mm to 21 mm.[25] It has been reported that the flavonoids chrysosplenetin, penduletin, and chrysoeriol that were isolated from *Artemisia rupestris* L. have synergistic action with the fluoroquinolone-resistant strain SA1199B of *S. aureus*.[26] It has been discovered that dieckol, which was isolated from *Ecklonia stolonifera*, works in concert with ampicillin and penicillin to inhibit MRSA.[27] In our study, the reduction in MIC from 0.48 µg/ml to 0.03 µg/ml clearly showed a 16-fold reduction in MIC of clindamycin against nine test isolates. Combining oxacillin with the plant extract resulted in a similar 16-fold decrease in MIC against eight test isolates. MIC of oxacillin was decreased from 31.25 µg/ml to 1.95 µg/ml in the present study. According to reports, dieckol extracted from *E. stolonifera* and ampicillin together lowered the minimum inhibitory concentration against the MRSA from 512 µg/ml to 0.5 µg/ml.[27] Plant extract combined with clindamycin and oxacillin showed synergistic activity against all isolates (FIC ≤0.5). Antagonistic activity was not seen for the plant extract and antibiotic combination against the tested isolates. Synergistic activity of plant extracts with antibiotics has been reported, which has been attributed to various phytochemicals present in

the plant extracts.[10] The results of our study have shown the antibiotic-potentiating property of the extract of *A. vulgaris* leaves against the clinical isolates of MDR *S. aureus.*

GC-MS analysis has revealed 30 compounds. Among the compounds, mome inositol was detected, with a maximum peak area of 72.07%. Some of the compounds detected have also been found in different medicinal plants to have potent antimicrobial activities. *Mentha pulegium* methanolic leaf extract with a high neophytadiene content was reported to be effective against *Salmonella typhimurium* ATCC CCM 538 and *S. aureus* ATCC 6538/P, with a MIC of 8 mg/mL.[28] 3,7,11,15-tetramethyl-2-hexadecen-1-ol is one of the main bioactive components in *Solanum xanthocarpum* methanolic extracts, which have demonstrated antibacterial and antioxidant qualities against *Pseudomonas aeruginosa*, *Salmonella typhi*, *E. coli*, and *S. aureus.*[29] Phytol isolated from the aerial parts of *Aster yomena* had antibacterial properties by inducing oxidative stress in *P. aeruginosa.*[30] A very small amount of vitamin E (Peak area of 0.4%) was present in the tested extract. Vitamin E, both water-soluble and lipid-soluble forms, can act as adjuvants to make the bacterium susceptible to the effects of antibiotics. It binds to the bacterial lipocalin protein (BcnA), which is generated by bacteria at sub-lethal antibiotic concentrations.[31] Additional research could be done to isolate the pure bioactive components, elucidate their structure and screen for their potential pharmacological action.

CONCLUSION

We have demonstrated the antibacterial activity and antibiotic resistance modulatory activity of the extract of *A. vulgaris* L. leaves against clinical isolates of multidrug-resistant *S. aureus* through a synergistic interaction with the antibiotics. Therefore, the plant extract can be screened for the isolation of lead compounds for antibacterial medication development. Further exploration is required to know the mechanisms of synergistic action with resistant antibiotics against the clinical pathogen.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

BS designed and conceputualized the study. KHL performed the experiments. BS and KHL analysed the data. KHL wrote the manuscript. BS supervised, reviewed and edited the manuscript. Both authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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