

# Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

## Original article

### The antibacterial activity of *Hibiscus rosa-sinensis* extract on methicillin-resistant *Staphylococcus aureus* and multi-drug resistant *Acinetobacter baumannii* isolates

Safaa Mohammed Abdel-Rahman<sup>1</sup>, Reham Aly khalifa<sup>1</sup>, Nashwa Almaabady<sup>2</sup>, Amany Raafat Maher\*<sup>1</sup>

1- Medical Microbiology and Immunology - Faculty of Medicine - Ain Shams University Cairo-Egypt.

2- National Bank of Egypt Hospital

#### ARTICLE INFO

##### Article history:

Received 29 September 2024

Received in revised form 7 October 2024

Accepted 8 October 2024

##### Keywords:

*Hibiscus rosa-sinensis*

Methicillin-resistant

*Staphylococcus aureus*

*Acinetobacter baumannii* isolates

#### ABSTRACT

**Background:** Increasing untreatable infections caused by multi-drug resistant (MDR) bacteria necessitate the development of new antibacterial agents. Natural plant extracts are considered an appropriate alternative to antibiotics. Studies have shown that *Hibiscus rosa-sinensis* (*H. rosa-sinensis*) extract has a wide antibacterial, antipyretic, anti-inflammatory and antioxidant effects. **Aim:** To detect the antibacterial activity and potential synergistic effect of *H. rosa-sinensis* extract on methicillin-resistant *Staphylococcus aureus* (MRSA) and MDR *Acinetobacter baumannii* (*A. baumannii*) isolates. **Method:** The present study was done on 30 MRSA and 30 MDR *A. baumannii* isolates. For each bacterial isolate antimicrobial susceptibility was done before and after addition of *H. rosa-sinensis* extract to the tested antibiotics. The synergistic effect of *H. rosa-sinensis* addition was detected by the percentage of change in the zone of inhibition for the combined *H. rosa-sinensis* extract/antibiotic disc in comparison to the antibiotic disc alone. **Results:** There was a statistically significant increase in zone of inhibition of all used antibiotics against tested bacterial isolates after *H. rosa-sinensis* extract addition. For MRSA the synergistic effect of *H. rosa-sinensis* extract was detected in 53.3% of the tested MRSA isolates for oxacillin and 3.3 % for vancomycin. For MDR *A. baumannii*, the synergistic effect of *H. rosa-sinensis* was detected in 86.7% of the tested MDR *A. baumannii* isolates in cefoxitin and 80% in cefotaxime, ceftazidime, cefepime and ciprofloxacin. **Conclusion:** *H. rosa-sinensis* is a natural extract that showed marked antibacterial and synergistic effect on MRSA and MDR *A. baumannii* isolates. *H. rosa-sinensis* extract/antibiotic combination is a potential alternative to the conventional antibiotics with less side effects.

#### Introduction

The empiric treatment of COVID-19 cases during the pandemic with broad spectrum antibiotics increased the incidence of infections caused by multi-drug resistant (MDR) organisms including

extended spectrum beta lactamase producing *Klebsiella pneumoniae*, *Acinetobacter baumannii* (*A. baumannii*) and methicillin-resistant *Staphylococcus aureus* (MRSA) [1].

Infection with MRSA is one of the leading causes of many hospitals acquired infections such as

DOI: 10.21608/MID.2024.324805.2257

\* Corresponding author: Amany Raafat Maher

E-mail address: [amanyboody2@gmail.com](mailto:amanyboody2@gmail.com)

© 2020 The author (s). Published by Zagazig University. This is an open access article under the CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

skin infection, meningitis, pneumonia, lung abscess and empyema with significantly increased morbidity and mortality rates [2]

*Acinetobacter baumannii* is responsible for most nosocomial infections that show extensive resistance to antibiotics. Prolonged hospital stay, immune suppression, advanced age, previous antibiotics use, invasive procedures and indwelling catheter are the critical risk factors for acquiring *Acinetobacter* species(spp) infection [3].

World Health Organization (WHO) documented that 80% of population still use traditional medicine including many plant –based medications with helpful therapeutic effects on human health [4].

Natural extracts have become an important source of medicinal agents with a well-known antimicrobial and antioxidant activities against different microorganisms. *Hibiscus rosa-sinensis* is one of the effective natural plants, as extracts from its leaves and flowers possess a potent antibacterial activity[5]. Many active chemical compounds with antimicrobial properties present in this plant include cyanidin, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acids [6]. Those compounds show different patterns of antimicrobial activity like forming complex compounds against extracellular protein of bacterial cell disrupting the integrity of bacterial cell membrane, denaturation of proteins and nucleic acid and inhibition of bacterial enzymes production [7].

The study aimed to detect the potential antibacterial effect of hibiscus extract on MRSA and MDR *A.baumannii* isolates.

## Materials and Methods

The present study was done at Medical Microbiology and immunology Department-Faculty of Medicine-Ain shams University on 30 MRSA and 30 MDR *A. baumannii*, isolates obtained from the central microbiology lab, Ain Shams University Hospitals. For each isolate included in the study, the antimicrobial susceptibility test results were compared between antibiotic alone and *H. rosa-sinensis* extract/antibiotic combination.

Isolates were isolated from different sources and identified as MRSA and MDR *A.baumannii* by conventional microbiological methods according to **Bailey et al.** 2022 [8].

Antimicrobial susceptibility testing was done for each isolate using disc diffusion method. The used antibiotic discs (Himedia, India) are listed

in **table (1)** and the results interpretation was done according to **CLSI** 2023 [9]

Methicillin-resistant *Staphylococcus aureus* were identified to be ceftazidime resistant isolates with inhibition zone > 22 mm [9], MDR *A. baumannii* was defined as resistant isolates to at least one agent in three or more of the following five antimicrobial group according to **Magiorakos et al.** [10]: Cephalosporins (ceftazidime or cefepime), Carbapenems (imipenem or meropenem), Ampicillin-sulbactam, Fluoroquinolones (ciprofloxacin or levofloxacin) and Aminoglycosides (gentamicin, tobramycin, amikacin, netilmicin).

*Hibiscus rosa-sinensis* extract was prepared according to **Farasayu et al.** [7] as the following: The Hibiscus flowers were washed, cut into small pieces, then put in the dryer for 48 hours at 45°C. The dried Hibiscus flowers are made into powder using The grinder, then a total of 100 g of flower air dried powder was weighed and was placed in 1000 mL of ethanol 70% in a conical flask then kept in a rotary shaker at 190-220 rpm for 24 h, then it was filtered with the help of muslin cloth and centrifuged at 10 000 rpm for 5 min. The supernatant was collected and evaporated by solvent distillation apparatus, giving an extract concentration of 100 mg/mL.

The antibacterial effect of *H. rosa-sinensis* extract on the tested bacterial isolates was detected by both disc diffusion method, and well diffusion methods according to **Hemeg et al.** 2020 [11] and **Saqib et al.** 2021 [12].

For disc diffusion method, 50 µL of the prepared *H. rosa-sinensis* extract was introduced into sterile blank filter paper discs 0.5 mm (himedia, India) and then dried at 100 °C for two hours in hot air oven to obtain completely saturated discs with the extract. After saturation, discs were placed directly on the swabbed Muller Hinton agar plates, and the plates were incubated at 37 °C for 24 h and zone of inhibition was measured and recorded **Hemeg et al.** [11]

For well diffusion method, wells measuring 6 mm diameter were formed in the swabbed Muller Hinton agar plates by the cap of sterile syringe, 50 µL of the extract was transferred in each well, the plate incubated for 24 hours at 37 °C and the diameter of the inhibition zone of bacterial growth around each well was also

measured and recorded according to **Saquib et al.** 2021 [12].

The combined *H. rosa-sinensis* extract/antibiotic discs were prepared by adding 50 µL of the *H. rosa-sinensis* extract to the antibiotic till saturation, the combined discs were employed on the inoculated agar plates and the zones of inhibition were measured & recorded after overnight incubation. The antimicrobial effect of the added *H. rosa-sinensis* extract was interpreted as the following according to **Saquib et al.** [12]:

- The synergistic effect: combined *H. rosa-sinensis* extract/antibiotic zone of inhibition >(zone of *H. rosa-sinensis* extract +used antibiotic zone of inhibition).
- The additive effect: combined *H. rosa-sinensis* extract/antibiotic zone of inhibition =(zone of *H. rosa-sinensis* extract + used antibiotic zone of inhibition).
- The antagonist effect: combined *H. rosa-sinensis* extract/antibiotic zone of inhibition <(zone of *H. rosa-sinensis* extract + used antibiotic zone of inhibition).

### Statistical analysis

Data was collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 26. The quantitative data were presented as mean, standard deviations and ranges when parametric and median, inter-quartile range (IQR) when data found non-parametric.

The comparison between two paired groups with quantitative data and parametric distribution, was done by using **Paired t-test**.

The confidence interval was set to 95%. So, the p-value was considered significant as the following:

- $p > 0.05$ : Non-significant (NS)
- $p < 0.05$ : Significant (S)
- $P < 0.01$ : Highly significant (HS).

### Results

The study involved 60 clinical isolates that were confirmed to be 30 MRSA and 30 MDR *A. baumannii*, obtained from Ain Shams University Hospital.

Most of MRSA isolates were collected from wound swabs [50 % (15/30 isolates)], followed by blood [43.33 % (13/30)], fewer isolates were collected from pleural fluid [6.66 % (2/30)]. Most of the MDR *A. baumannii* isolates were obtained from wound swabs [43.33 % (13/30)], followed by blood [40% (12/30)], fewer isolates were collected from sputum [13.33% (4/30)] and drain [3.33% (1/30)].

All the thirty tested MRSA isolates were resistant to oxacillin, out of 30 MRSA isolates 90% (27/30) were resistant to vancomycin, for MDR *A.baumannii* all used antibiotics showed resistance 100% (30/30).

On comparison between the disc diffusion & well diffusion method for detection of antimicrobial effect of hibiscus extract, there was statistically highly significant (HS) increase in zone of inhibition of extract with well diffusion method than disc diffusion method (**Table 2**)(**Figure 1**).

For MRSA isolates, there was statistically highly significant increase in zone of inhibition of combined *H. rosa-sinensis* extract/antibiotic than antibiotic alone, oxacillin showed a higher percent of change in zone of inhibition than vancomycin (**Table 3**) (**Figure2**).

For MDR *A.baumannii*, there was statistically highly significant increase in zone of inhibition of combined *H. rosa-sinensis* extract/antibiotic than used antibiotic alone for all antibiotics (**Table 4**).

Cefoxitin showed the highest rate of change in zone of inhibition, followed by cefotaxime and ciprofloxacin, The least rate of change was detected for gentamycin followed by amikacin.

As shown in **table (5)**, the added *H.rosa-sinensis* extract /oxacillin had a synergistic effect on most MRSA isolates while addition to vancomycin had antagonistic effect on most MRSA isolates.

As shown in **table(6)**, the addition of hibiscus extract to cefoxitin had a synergistic effect on most of *A.baumannii* isolates followed by cefotaxime, cefazidime, cefepime and ciprofloxacin, the least effect was on meropenem.

**Table1.** concentration of antibiotic discs used for antimicrobial susceptibility testing.

| Bacterial isolates     | Antibiotic discs                                                                                                                                                                                                                                                                      |
|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| MRSA                   | <ul style="list-style-type: none"> <li>▪ Oxacillin (cefoxitin 30 µg)</li> <li>▪ Vancomycin (3 µg)</li> </ul>                                                                                                                                                                          |
| MDR <i>A.baumannii</i> | <ul style="list-style-type: none"> <li>▪ Ciprofloxacin (5 µg)</li> <li>▪ Gentamycin (10 µg)</li> <li>▪ Amikacin (30 µg)</li> <li>▪ Ceftazidime (30 µg)</li> <li>▪ Cefepime (30 µg)</li> <li>▪ Cefotaxime (30 µg)</li> <li>▪ Cefoxitin (30 µg)</li> <li>▪ Meropenem (10 µg)</li> </ul> |

MRSA : Methicillin resistant *Staphylococcus aureus*MDR *A. baumannii* :Multi-drug resistant *Acinetobacter baumannii***Table 2.** comparison between results of susceptibility testing of hibiscus extract on MRSA and MDR *A. baumannii* isolates by well and disc diffusion methods.

| Bacterial isolates                 | Zone of inhibition (mm)<br>(Mean±SD) |              | Percent change | Test value | P-value | Sig. |
|------------------------------------|--------------------------------------|--------------|----------------|------------|---------|------|
|                                    | Disc method                          | Well method  |                |            |         |      |
| MRSA                               | 8.90 ± 0.96                          | 12.13 ± 0.73 | 37.45 ± 12.96  | 20.632     | <0.001  | HS*  |
| MDR <i>Acinetobacter baumannii</i> | 6.7 ± 0.47                           | 12.53 ± 0.51 | 87.94 ± 15.02  | 49.328     |         |      |

HS \*: highly significance

**Table 3.** Zone of inhibition for tested MRSA isolates.

| Antibiotics | Zone of inhibition (mm)<br>Mean±SD |                                       | Percent change | Test value | p-value | Sig. |
|-------------|------------------------------------|---------------------------------------|----------------|------------|---------|------|
|             | Antibiotic                         | <i>H.rosa-sinensis</i><br>/antibiotic |                |            |         |      |
| Oxacillin   | 6.63 ± 5.80                        | 16.53 ± 2.97                          | 73.68 ± 47.59  | 11.095     | <0.001  | HS*  |
| Vancomycin  | 18.27 ± 1.26                       | 22.40 ± 1.52                          | 23.40 ± 14.26  | 9.778      |         |      |

HS \*: highly significance

**Table 4.** zone of inhibition for tested MDR *A. baumannii*.

| Antibiotics   | Zone of inhibition (mm)<br>Mean±SD |                                       | Percent change | Test value | p-value | Sig. |
|---------------|------------------------------------|---------------------------------------|----------------|------------|---------|------|
|               | Antibiotic                         | <i>H.rosa-sinensis</i><br>/antibiotic |                |            |         |      |
| Amikacin      | 1.13 ± 2.1                         | 8.67 ± 0.48                           | 80.71 ± 21.68  | 19.379     | <0.001  | HS*  |
| Gentamycin    | 0.67 ± 1.73                        | 8.5 ± 0.68                            | 80 ± 0         | 25.805     |         |      |
| Ceftazidime   | 0.93 ± 1.91                        | 9.67 ± 0.48                           | 110 ± 32.25    | 24.14      |         |      |
| Cefepime      | 0.77 ± 1.61                        | 8.63 ± 0.49                           | 143.33 ± 62.5  | 27.061     |         |      |
| Ciprofloxacin | 0.53 ± 1.22                        | 7.73 ± 0.45                           | 153.33 ± 29.81 | 33.248     |         |      |
| Cefoxitin     | 0.43 ± 1.14                        | 9.33 ± 0.66                           | 212.5 ± 41.67  | 45.907     |         |      |
| Meropenem     | 1.83 ± 2.45                        | 9.73 ± 0.45                           | 96.36 ± 8.09   | 17.835     |         |      |
| Cefotaxime    | 0.7 ± 1.47                         | 8.73 ± 0.45                           | 161.94 ± 9.89  | 29.861     |         |      |

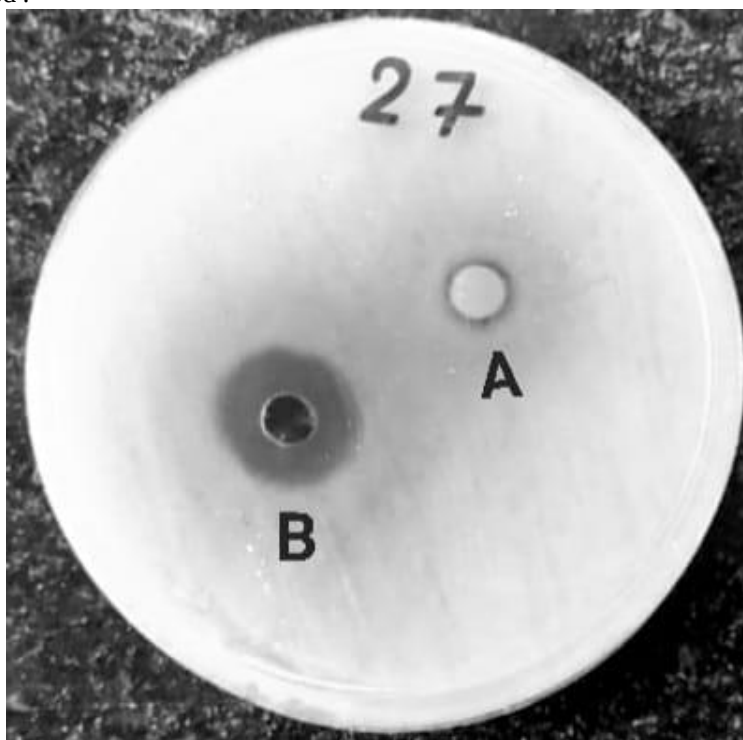
HS \*: highly significance

**Table 5.** The antimicrobial effect of *H. rosa-sinensis* extract on MRSA isolates.

| Antibiotic | Synergistic    | additive      | Antagonist     |
|------------|----------------|---------------|----------------|
| Oxacillin  | 53.3 % (16/30) | 23.3 % (7/30) | 23.3 % (7/30)  |
| Vancomycin | 3.3 % (1/30)   | 20% (6/30)    | 76.7 % (23/30) |

**Table 6.** The antimicrobial effect of *H.rosa-sinensis* on MDR *A.baumannii*

| Antibiotic    | Synergistic    | Additive      | Antagonist    |
|---------------|----------------|---------------|---------------|
| Meropenem     | 63.3% (19/30)  | 0             | 36.7%(11/30)  |
| Cefazidime    | 80% (24/30)    | 3.3% (1/30)   | 16.7% (5/30)  |
| Cefoxitin     | 86.7 % (26/30) | 13.3% (4/30)  | 0             |
| Cefotaxime    | 80% (24/30)    | 3.3% (1/30)   | 16.7% (5/30)  |
| Amikacin      | 76.7 % (23/30) | 0             | 23.3% (7/30)  |
| Cefepime      | 80% (24/30)    | 0             | 20% (6/30)    |
| Gentamycin    | 76.7 % (23/30) | 10%(3/30)     | 13.3 % (4/30) |
| Ciprofloxacin | 80 % (24/30)   | 6.66 % (2/30) | 13.3% (4/30)  |

**Figure 1.** *H. rosa-sinensis* extract susceptibility testing for MRSA isolates by both disc diffusion method and well diffusion method .

**A:** zone of inhibition of *H. rosa-sinensis* extract saturated disc(50µl).

**B:** zone of inhibition around the added *H. rosa-sinensis* extract (50µl) to the 6mm well.

**Figure 2.** susceptibility testing of oxacillin , vancomycin and combination with *H. rosa-sinensis* for MRSA isolates.



A: zone of inhibition for Oxacillin

B: zone of inhibition for Vancomycin

C: zone of inhibition for combined *H. rosa-sinensis* extract/oxacillin

D: zone of inhibition for combined *H. rosa-sinensis* extract/vancomycin

## Discussion

Antibiotic resistance has emerged as a substantial and triggering phenomenon with increasing costs for healthcare systems worldwide, and it has been related to significant morbidity, mortality, and increased cost due to both prolonged length of hospitalization and treatment [13].

Plants have evolved the ability to synthesize chemical compounds, that were used to treat human diseases [14]. Medicinal properties of plants have been preferred throughout the world due to their potent pharmacological activities, low toxicity and economic viability when compared with synthetic drugs [15]. Combination between antibiotics and active compound derived from plants increase the effectiveness of these antibiotics [12].

*Hibiscus rosa-sinensis* is a tropical evergreen shrub with red flowers, traditionally used for the treatment of flu and cough, bronchitis, stomach pain, dysentery and diarrhea, and also for regulation of menstruation and stimulation of blood circulation [16]. Its flower is a rich source of bioactive compounds that exhibit potent antioxidant and anti-inflammatory, antitumour, anti-asthmatic, antipyretic, analgesic, antimicrobial and antifungal effects [17].

Therefore, this study aimed to detect the antibacterial activity and potential synergistic effect of *H.rosa-sinensis* extract on MRSA and MDR *A.baumannii* isolates that will provide a new natural extract/ conventional antibiotic combination with less side effects.

Atef et al. [15] study reported that ethanolic extraction of *Moringa oleifera* (leaves) and *Matricaria recutita* (flowers) produced an inhibitory effect against *Pseudomonas aeruginosa*, *Staphylococcus* spp., *Escherichia coli* (*E.coli*), and *Proteus mirabilis* and confirmed its potential value as antibacterial agents against resistant and susceptible bacteria, supporting the significant use of plant extracts in treating wound infections.

This cross-sectional study was carried on 30 MRSA isolates and 30 MDR *A. baumannii* isolates. In our study most of MRSA isolates were collected from wound swabs [15/30 (50%)], followed by blood [13/30 (43.33 %)], fewer isolates were collected from pleural fluid [2/30 (6.66 %)]. Wound swabs are a major source of MRSA infection because *Staphylococcus aureus* (*S. aureus*) is carried on the skin and spread through contact with infected people and touch infected skin [18], in accordance with the study conducted by Alkharsah et al. [19] who analysed MRSA isolates from both infection sites and colonization sites and reported same results

as most MRSA isolates [55/106 (51.9%)] were isolated from infection sites, mostly wound swabs.

In our study most of the MDR *A.baumannii* isolates were obtained from wound swabs [13/30 (43.33%)], followed by blood [12/30 (40%)], fewer isolates were collected from sputum [4/30 (13.33%)] and drain [1/30 (3.33%)]. Wound swabs and blood are major source because *acinetobacter* frequently causes colonization especially among patients with prolonged hospitalization and need invasive devices [20], however in **Araya et al.** study that was done on 893 *A. baumannii* isolates, Blood was the major source of the isolates [535/893 (60 %)] followed by urine [294/893 (33 %)] [21].

In this study, all the thirty tested MRSA isolates were resistant to oxacillin, out of 30 MRSA isolates 90% (27/30) were resistant to vancomycin.

In another study to assess antimicrobial susceptibility of MRSA, vancomycin and oxacillin were tested against 69 isolates, all isolates were resistant to oxacillin but vancomycin retained full efficacy against all isolates [22]

In our study all the tested MDR *A.baumannii* 100% (30/30) showed resistance to all used antibiotics (amikacin, gentamycin, ceftazidime, cefepime, ciprofloxacin, ceftazidime, meropenem and cefotaxime).

In another study conducted on 847 *A.baumannii* isolates, the overall resistance rate of *A. baumannii* was high (>80%) for multiple classes of antibiotics, including penicillins, cephalosporins, carbapenems, quinolones and aminoglycosides, except for colistin and tigecycline (1.1% and 4.3%, respectively) [23].

The discrepancy of the result is attributed to the small sample size (30 MRSA isolates and 30 MDR *A. baumannii* isolates in our study compared to other studies and the different distribution of MDR strains among different hospital department that was affected by the degree of commitment to the provided antibiotic policy in this health care setting.

In this study when testing the effect of *H.rosa-sinensis* on the tested bacterial isolates, there was statistically highly significant increase in zone of inhibition of extract with well diffusion method than disc diffusion method when testing against both MRSA and MDR *A. baumannii* isolates.

In the same line with our study, when evaluated the antibacterial activity of essential oils against *S. aureus* and *E. coli*, **Ngamsurash et al.**

showed that usage of well agar diffusion method demonstrated better sensitivity than disc diffusion method [24].

Also, in a study that done to assess the effect of *H.rosa-sinensis* flower extract on *S. aureus*, *E. coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, the extract was proved to have potential antibacterial activity [25]

Interestingly, **Karnwal et al.** [26] carried out a study to assess efficacy of extracts from 3 different flowers (*H.rosa sinensis*, *Chrysanthemum indicum*, and *Calendula officinalis*) on four Gram-positive strains (*S. aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Listeria monocytogenes*), and three Gram-negative strains (*E. coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*) using well diffusion assay and confirmed the antimicrobial efficiency of those extracts against tested bacterial strains.

In this study, ethanolic extraction of *H.rosa-sinensis* flowers showed antibacterial effect against MRSA isolates with inhibition zone [ $12.13 \pm 0.73$ ] by well diffusion method and [ $8.90 \pm 0.96$ ] by disc diffusion method using 50 µg, similar results were detected by **Khan et al.** [27] when studied the antibacterial effect of *H.rosa-sinensis* extract on *staphylococcus sp.*, *Bacillus sp.* and *Escherichia coli*, showed growth inhibitory effect in the range of  $12.75 \pm 1.17$  to  $16.75 \pm 2.10$  mm.

While using methanolic extraction from *H.rosa-sinensis* flowers in concentrations ranging from 4 mg/disc to 0.017 mg/disc against (MRSA), **Al-Snafi** showed similar inhibition zones with diameters > 12 mm [5]

According to our findings, there was statistically highly significant increase in zone of inhibition of combined antibiotic & extract against MRSA than antibiotic alone with percent of change of [ $73.68 \pm 47.59$ ] in oxacillin and [ $23.40 \pm 14.26$ ] in vancomycin.

**Fan et al.** [28] carried out a study on MRSA to evaluate antibacterial effect of *Sanchen* powder (Chinese herb seeds) when used alone and after vancomycin addition and found that, this powder has weak antibacterial effect while combination with vancomycin has strong synergistic effect that can inhibit bacteria in mature biofilms.

In their study, **Sehim et al.** [29] reported that methanol extraction of *Hibiscus Sabdariffa* (other type of hibiscus) exhibited antibacterial effect

against *S.aureus* and *E.coli* and as well as synergistic action when combined with antibiotics.

**Kuok et al.** [30] found that herbal extracts called *Magnolia officinalis*, *Verbena officinalis*, *Momordica charantia*, and *Daphne genkwa* had strong inhibitory effect on MRSA isolates and also had partial synergistic effect in combination with antibiotics like oxacillin and gentamycin.

In the current study, antibacterial effect of *H.rosa-sinesis* flowers extract against MDR *A.baumannii* was evaluated by zone of inhibition with well method [ $12.53 \pm 0.51$ ] and with disc method [ $6.7 \pm 0.47$ ].

In **Abdallah et al.** [31] the methanolic extraction of *Hibiscus sabdariffa* exhibited significant antibacterial properties against the non-MDR *A. baumannii* as well as the MDR *A. baumannii* strains with a zone of inhibition ranging from ( $11.3 \pm 0.3$ ) to ( $13.6 \pm 0.3$ ) mm.

In the present work on MDR *A.baumannii* isolates, there was statistically highly significant increase in zone of inhibition of extract in combination with antibiotic than used antibiotic alone, that was in accordance with **Tiwari et al.** [32] study which reported that phenolic compounds of plant extracts enhance the potential effect of synthetic antibiotics against *A.baumannii* in vitro and also with **Saeloh et al.** [33] study which reported that Combination therapy between plant extract called *Myrtaceae* extract and meropenem showed significant reduction of bacterial growth compared to meropenem alone against *A.baumannii*.

Also, the combination between berberine (plant extract) and ciprofloxacin decreased the minimal inhibitory concentration (MIC) levels of ciprofloxacin in *A. baumannii* isolates [34].

In our work, the addition of *H.rosa-sinensis* to cefoxitin had a synergistic effect on most of *A.baumannii* isolates [86.7% (26/30)] and the least effect on meropenem [63.3% (19/30)]. In the same line with this result **Ildiz et al.** [35] reported that combination between coriander seed extract and cefoxitin has a synergistic interaction against Extended Spectrum Beta-lactamase (ESBL) and MRSA and the combination had stronger effect than cefoxitin alone on each resistant strain

Additionally, combination between amikacin and methanolic extraction of herbal plants (*Salvia chorassanica* and *Artemisia khorassanica*)

exhibited synergistic activity against MDR *acinetobacter* isolates [36].

In agreement with our study, **Al-Alak et al.** [37] studied the antibacterial activity of *H.rosa-sinensis* on *P.aeruginosa*, *Enterobacter*, *Micrococcus* and *Salmonella* and reported the synergistic effect of combination between it and amoxycillin compared to effect of each one of them alone.

A study conducted on *E. coli* isolates to evaluate antibacterial effect of *H.rosa-sinensis* extract with antibiotics reported enhancement effect in zone size with many resistant antibiotics in combination with the extract. Extract showed equal enhancement in zone size of cefotaxime and amikacin and no effect of extracts was seen on levofloxacin [38].

Various in vitro experiments have established the fact that a combination of plant extracts and antibiotics possess a synergistic effect, which results in a significant decrease in levels of MIC for the antibiotics [39].

Herbal extracts combined with antibiotics, such as  $\beta$ -lactams, quinolones, aminoglycosides, tetracyclines and glycopeptides, could greatly enhance the antibacterial effects of the antibiotics, reduce the dosage and toxic side effects, and reverse bacterial resistance [40].

## Conclusion

*Hibiscus rosa-sinensis* extract is a natural extract that showed antibacterial activity against MRSA and MDR *A.baumannii* by increasing zone of inhibition with significant percent of change and recorded synergistic effect, *H.rosa-sinensis* extract/antibiotics combination has a potential role in treatment of infection caused by MRSA and MDR *A.baumannii*.

## Limitation of the study

The small sample size (only 30 isolates each for MRSA and MDR *A. baumannii*) and the potential variability in the quality of the *H. rosa-sinensis* extract may impact the generalizability of our findings.

## Conflict of interest

There is no conflict of interest stated by the author.

## Funding:

None.

**Data availability**

All data generated or analyzed during this study are included in this published article.

**Author's contribution**

All of the listed authors participated in the work and approved it for publication

**References**

- 1- **Lai CC, Chen SY, Ko WC, Hsueh PR.** Increased antimicrobial resistance during the COVID-19 pandemic. *Int J Antimicrob Agents.* 2021; 57(4).
- 2- **Siddiqui AH, Koirala J.** Methicillin-Resistant *Staphylococcus aureus*. Treasure Island (FL): In: StatPearls Publishing, 2023; Available at: <https://www.ncbi.nlm.nih.gov/books/NBK482221>.
- 3- **Ibrahim S, Al-Saryi N, Al-Kadmy IMS, Aziz SN.** Multidrug-resistant *Acinetobacter baumannii* as an emerging concern in hospitals. *Mol Biol Rep.* 2021; 48(10): 6987-6998.
- 4- **Zaidi SF, Saeed SA, Khan MA, Khan A, Hazazi Y, Otayn M, et al.** Public knowledge, attitudes, and practices towards herbal medicines; a cross-sectional study in Western Saudi Arabia. *BMC Complementary Medicine and Therapies.* 2022; 22(1): 326-337.
- 5- **Al-Snafi AE.** Chemical constituents, pharmacological effects and therapeutic importance of *Hibiscus rosa-sinensis*-A review. *IOSR Journal of Pharmacy,* 2018; 8(7): 101-119.
- 6- **Portillo-Torres LA, Bernardino-Nicanor A, Gómez-Aldapa CA, González-Montiel S, Rangel-Vargas E, Villagómez-Ibarra JR, et al.** . *Hibiscus* acid and chromatographic fractions from *Hibiscus sabdariffa* calyces: Antimicrobial activity against multidrug-resistant pathogenic bacteria. *Antibiotics.* 2019; 8(4): 218-229.
- 7- **Farasayu R, Rachmawati MW, Ana I, Syaify A, Listyarifah D.** The Effect of Hibiscus Flower Extract (*Hibiscus rosa-sinensis* L.) on the Growth of *Streptococcus sanguinis* Bacteria. *EDP Sciences.* 2021; 41: 7006.
- 8- **Bailey SL, Buckingham C, Maxwell-Scott H, Rashidghamat E, Ferguson FJ, Malhomme de la Roche H, et al.** Microbial Isolates and Association with Disease Severity and Quality of Life in Individuals with Hidradenitis Suppurativa: An Observational Study. *Skin Appendage Disorders.* 2022; 8(3): 211-220.
- 9- **Clinical & Laboratory Standards Institute (CLSI).** Performance Standards for Antimicrobial Susceptibility Testing ACLSI supplement for global application, 32nd Edition M100. Wayne, PA: Clinical and Laboratory Standards Institute, 2023; [Online]. Available at: [www.clsi.org](http://www.clsi.org).
- 10- **Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al.** Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection.* 2012; 18(3): 268-81.
- 11- **Hemeg HA, Moussa IM, Ibrahim S, Dawoud TM, Alhaji JH, Mubarak AS, et al.** Antimicrobial effect of different herbal plant extracts against different microbial population. *Saudi Journal of Biological Sciences,* 2020; 27(12): 3221-7.
- 12- **Saquiab SA, AlQahtani NA, Ahmad I, Arora S, Asif SM, Javali MA, et al.** Synergistic antibacterial activity of herbal extracts with antibiotics on bacteria responsible for periodontitis. *The Journal of Infection in Developing Countries.* 2021; 15(11): 1685-93.

- 13-**Vrancianu CO, Gheorghe I, Czobor IB, Chifiriuc MC.** Antibiotic resistance profiles, molecular mechanisms and innovative treatment strategies of *Acinetobacter baumannii*. *Microorganisms*. 2020; 8(6): 935-941.
- 14-**Chopra B, Dhingra AK.** Natural products: A lead for drug discovery and development. *Phytotherapy Research*. 2021; 35(9): 4660-4702.
- 15-**Atef NM, Shanab SM, Negm SI, Abbas YA.** Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causing wound infection. *Bulletin of the National Research Centre*. 2019; 43(1): 1-11.
- 16-**Ngan LTM, Tan MT, Hoang NVM, Thanh DT, Linh NTT, Hoa TTH, et al.** Antibacterial activity of *Hibiscus rosa-sinensis* L. red flower against antibiotic-resistant strains of *Helicobacter pylori* and identification of the flower constituents. *Brazilian Journal of Medical and Biological Research*. 2021; 54(7): e10889.
- 17-**Geeganage JR, Gunathilaka MDTL.** Mechanistic Insight Into Anti-inflammatory Potential of *Hibiscus rosa-sinensis* Flower Extract as a Herbal Remedy: A Systematic Review. *Journal of Herbal Medicine*. 2024; 45: 100884.
- 18-**Tamanna S, Zareen A, Islam KR.** Methicillin-Resistant *Staphylococcus aureus* (MRSA) prevalence in clinical and hospital environmental samples: a review (Doctoral dissertation, Brac University), 2023. Available at: <http://hdl.handle.net/10361/22133>.
- 19-**Alkharsah KR, Rehman S, Alkhamis F, Alnimr A, Diab A, Al-Ali AK.** Comparative and molecular analysis of MRSA isolates from infection sites and carrier colonization sites. *Annals of clinical microbiology and antimicrobials*. 2018; 17: 1-11.
- 20-**Bartal C, Rolston KV, Nesher L.** Carbapenem-resistant *Acinetobacter baumannii*: colonization, infection and current treatment options. *Infectious diseases and therapy*, 2022; 11(2), 683-694.
- 21-**Araya S, Gebreyohannes Z, Tadlo G, Gessew GT, Negesso AE.** Epidemiology and multidrug resistance of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from clinical samples in Ethiopia. *Infection and Drug Resistance*. 2023; 2765-2773.
- 22-**Almutairi H, Albahadel H, Alhifany AA, Aldalbahi H, Anezary FS, Alqusi I, et al.** Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) at a maternity and children hospital in Saudi Arabia: A cross-sectional study. *Saudi Pharmaceutical Journal*. 2024; 32(4): 102001.
- 23-**Chen CH, Wu PH, Lu MC, Ho MW, Hsueh PR.** Geographic patterns of carbapenem-resistant, multi-drug-resistant and difficult-to-treat *Acinetobacter baumannii* in the Asia-Pacific region: results from the Antimicrobial Testing Leadership and Surveillance (ATLAS) program, 2020. *International journal of antimicrobial agents*. 2023; 61(2): 106707.
- 24-**Ngamsurach P, Praipipat P.** Antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* of extracted Piper betle leaf materials by disc diffusion assay and batch experiments. *RSC advances*. 2022; 12(40): 26435-26454.
- 25-**Ruban P, Gajalakshmi K.** In vitro antibacterial activity of *Hibiscus rosa-sinensis* flower extract against human pathogens. *Asian*

- pacific journal of tropical biomedicine. 2012; 2(5): 399-403.
- 26-**Karnwal A.** In vitro antibacterial activity of Hibiscus rosa sinensis, Chrysanthemum indicum, and Calendula officinalis flower extracts against Gram negative and Gram positive food poisoning bacteria. *Advances in Traditional Medicine*. 2022; 22(3): 607-619.
  - 27-**Khan ZA, Naqvi SA, Mukhtar A, Hussain Z, Shahzad SA, Mansha A, et al.** Antioxidant and antibacterial activities of Hibiscus Rosa-sinensis Linn flower extracts. *Pak J Pharm Sci*. 2014; 27(3): 469-474.
  - 28-**Fan J, Sun H, Liu Y, Li X, Wu H, Ren X.** Sanchen powder extract combined with vancomycin against methicillin-resistant Staphylococcus aureus. *Journal of Traditional Chinese Medical Sciences*. 2022; 9(2): 181-187. d
  - 29-**Sehim AE, Amin BH, Yosri M, Salama HM, Alkhalifah DH, Alwaili MA, et al.** GC-MS Analysis, Antibacterial, and Anticancer Activities of Hibiscus sabdariffa L. Methanolic Extract: In Vitro and In Silico Studies. *Microorganisms*. 2023; 11(6): 1601-1615.
  - 30-**Kuok CF, Hoi SO, Hoi CF, Chan CH, Fong IH, Ngok CK, et al.** Synergistic antibacterial effects of herbal extracts and antibiotics on methicillin-resistant Staphylococcus aureus: A computational and experimental study. *Experimental Biology and Medicine*. 2017; 242(7): 731-743.
  - 31-**Abdallah EM.** Antibacterial activity of Hibiscus sabdariffa L. calyces against hospital isolates of multidrug resistant Acinetobacter baumannii. *Journal of Acute Disease*. 2016; 5(6): 512-516.
  - 32-**Tiwari V, Roy R, Tiwari M.** Antimicrobial active herbal compounds against Acinetobacter baumannii and other pathogens. *Frontiers in microbiology*. 2015; 6: 618-627.
  - 33-**Saeloh D, Visutthi M, Leeha M, Limsuwan S, Voravuthikunchai SP.** Enhanced antibacterial activity of Meropenem against extensively drug-resistant Acinetobacter baumannii by Myrtaceae plant extracts. *Walailak Journal of Science and Technology (WJST)*. 2020; 17(11): 1168-1176.
  - 34-**Mahmoudi H, Zare Fahim N, Alikhani MY, Shokoohezadeh L.** Investigation of antimicrobial effect of berberine on ciprofloxacin and imipenem resistance Acinetobacter baumannii isolated from Hamadan Hospitals. *Iranian Journal of Medical Microbiology*. 2020; 14(1): 44-54.
  - 35-**Ildiz N, Baldemir A, Ince U, Ilgun S, Konca Y.** Synergistic effect of Coriandrum sativum L. extracts with cefoxitin against methicillin resistant Staphylococcus aureus, extended-spectrum beta-lactamase producing Escherichia coli and Klebsiella pneumoniae. *Medicine Science*. 2018; 7(4): 777-80.
  - 36-**Fatemi N, Sharifmoghdam MR, Bahreini M, Khameneh B, Shadifar H.** Antibacterial and synergistic effects of herbal extracts in combination with amikacin and imipenem against multidrug-resistant isolates of acinetobacter. *Current Microbiology*. 2020; 77: 1959-1967.
  - 37-**Al-Alak SK, Al-Oqaili RMS, Mohammed BB, Abd-Alkhalik N.** Antibacterial activity of Hibiscus rosa-sinensis extract and synergistic effect with amoxicillin against some human pathogens. *Am J Phytomed Clin Ther*. 2015; 3(10): 20-27.
  - 38-**Maraskolhe D, Chimurkar L, Kamble P, Deotale V.** Evaluation of an antibacterial effect of hibiscus rosa sinensis leaves and petals extract along with antibiotics on

escherichia coli: in vitro study. Int J Cur Res Rev. 2020; 12(06): 18-24.

39-**Saquib SA, AlQahtani NA, Ahmad I, Kader MA, Al Shahrani SS, Asiri EA.** Evaluation and comparison of antibacterial efficacy of herbal extracts in combination with antibiotics on periodontal pathobionts: an in vitro microbiological study. Antibiotics. 2019; 8(3): 89-97.

40-**Bao M, Zhang L, Liu B, Li L, Zhang Y, Zhao H, et al.** Synergistic effects of anti-MRSA herbal extracts combined with antibiotics. Future Microbiology. 2020; 15(13): 1265-1276.

Abdel-Rahman SM, Khalifa RA, Almaabady N, Maher AR. The antibacterial activity of *Hibiscus rosa-sinensis* extract on methicillin-resistant *Staphylococcus aureus* and multi-drug resistant *Acinetobacter baumannii* isolates. Microbes Infect Dis 2025; 6(4): 6329-6340.