Uttar Pradesh Journal of Zoology

Volume 45, Issue 21, Page 398-405, 2024; Article no.UPJOZ.4352
ISSN: 0256-971X (P)

Screening the Antibacterial Activity of Garlic (*Allium sativum*) Extract against Methicillin- Resistant *Staphylococcus aureus* (MRSA)

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i214649

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/4352

Original Research Article

Received: 23/09/2024
Accepted: 26/11/2024
Published: 30/11/2024

ABSTRACT

The growth of human skin pathogens or kill them with no toxicity to host cells are considered candidates for developing new antimicrobial drugs. Consequently, there is a critical need to research for new antimicrobial agents with promising natural activities to provide an alternative to common antibiotics. The isolation of *Staphylococcus aureus* pus samples from wounds was used. Totally 52 pus samples were collected from Government Hospital in Virudhunagar. Fluid thioglycolate medium was used as an enriched medium for *S. aureus*. The methanol extract of *Allium satiuvm* were screened for antibacterial activity against isolated *Staphylococcus aureus*. The maximum zone of inhibition in methanol extract is 11 mm/30 ul (SA5) and in methanol extract is 7 mm/30 ul (SA10). These strains SA5 and SA10 denotes isolated *S.aureus* strains numbers. Some of the strains are not responded to the plant extracts so, as the concentration of the extract

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Cite as: *Wahid, Jamith Basha Abdul. 2024. "Screening the Antibacterial Activity of Garlic (Allium Sativum) Extract Against Methicillin- Resistant Staphylococcus Aureus (MRSA)". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (21):398-405. https://doi.org/10.56557/upjoz/2024/v45i214649.*

increases the zone of inhibition can also be increased. The minimum inhibitory concentrations of the methanol extract of *Allium satiuvm* and were determined. But, 40 mg/ml concentration inhibited the growth.

Keywords: Staphylococcus aureus; pus samples; MRSA strains and Allium satiuvm.

1. INTRODUCTION

"Control of the spread of antibiotic resistant bacteria and the treatment of infections caused by them is a major problem worldwide. In particular, methicillin-resistant *Staphylococcus aureus* (MRSA) presents major infection control problems for patients and hospital staff, as its incidence in Europe has risen from 3% in 1992 to 37% in 1999" (Roth et al., 2002).

"Healthcare associated MRSA strains are resistant to many antibiotics" (Chang et al., 2000). "MRSA infection markedly increases the morbidity and mortality in hospitalized patients. Thus there is a need to develop novel agents with greater inhibitory activity against MRSA. Our previous animal study revealed that garlic extract and its two diallyl sulphides (DAS) and diallyl disulphide (DADS) could effectively decrease MRSA viability in blood and organs and reduce the plasma levels of fibronectin and interleukin (IL)-6 in non-diabetic mice" (Tsao et al., 2003).

"Medicinal herbs are worldwide and heavily used in folk medicine; therefore, screening of such herbs may result in the discovery of novel effective compounds capable of working against pathogenic microorganisms. For many decades, garlic has been known for its medicinal activities. Its antibacterial potency is well known against many bacterial species including gram-positive bacteria such as *S. aureus"* (Zhang et al., 2016).

"Garlic (*Allium sativum* L.) and garlic extracts have been previously demonstrated as effective in inhibiting the growth of different bacterial pathogens, including Staphylococci" (Houshmand et al., 2013). "Chemical analysis of garlic oil extract showed that 54.5% of the total sulphides comprise diallyl monosulphide, diallyl disulphide, diallyl trisulphide and diallyl tetrasulphide, and the minimum inhibitory concentration of whole garlic oil extract 32 /ml" (Ratthawongjirakul et al*.,*2016).

"Allicin, the active compound in fresh garlic extract, also inhibited the proliferation of MRSA in a dose-dependent manner. Recently, garlic extract has been shown to diminish the biofilm formation of some microbes, such as *Staphylococcus epidermidis, Pseudomonas aeruginosa*, and *Candida* albicans. Allicin (the name being derived from that of the garlic species *Allium sativum*) is considered to be the main biologically active antimicrobial phytochemical produced in garlic extracts" (Zhu et al*.,*2022).

"Plant extracts formulations have therefore become a better alternative, especially in developing nations. Moreover, most antimicrobial agents that are currently in use have been rendered ineffective by the wide occurrence of multiple drug resistant strains of microbes" (Alsalihy, 2011).

2. MATERIALS AND METHODS

2.1 Specimen Collection and Bacterial Isolates

This study was conducted from December 2018 to April 2019 on 52 untreated patients with clinical symptoms of wounds for the isolation of *S.aureus* pus samples from wounds were used. The specimens of pus samples were collected from Government Hospital in Virudhunagar. Fluid thioglycolate medium was used as an enriched medium for *S aureus*. The specimen was inoculated on Mannitol salt agar, plates. The plates were incubated aerobically at 37 °C overnight.

Cultures without any colony at the end of 24h incubation were further incubated for 48h. Samples with colony count equal or more than 10^5 Cfu/ml was considered positive. The isolates were identified and confirmed using standard microbiological methods including Gram staining, colonial morphology on media, growth on enriched medium and biochemical tests, lactose and mannitol fermentation, H_2S production, catalase, oxidase, coagulase, indole, and citrate utilization, and urease test. Detection of MRSA the Kirby-Bauer disk diffusion test was developed for this study.

2.2 Collection of Herbal Plants

The Preparation of plant extract for methanol extract were collected and then dried at room temperature for 5 days. They were subsequently grounded into fine powder. 10g of powdered plant materials were mixed with 100 ml of methanol and kept in a shaker for 24 hours. The Methanol extract was filtered by using whatman no: 1 filter paper.

2.3 Methanol Extraction

30 g of powdered plant material is mixed with 120ml of methanol solvent and was kept for 12h separately. The suspension was filtered through a whatman no.1 filter paper (Prem kumar et al*.*, 2016).

2.4 Phytochemical Analysis

2.4.1 Test for alkaloids

To the 1ml of extract and 1 ml of Dragen dorff's reagent. An orange red coloured precipitate indicates the presence of alkaloids.

2.4.2 Test for Carbohydrates

Molish Test: To the1ml of extract and 1ml of α-naphthol solution was added and con. H_2SO_4 was added along the sides of the test tube. Purple or reddish violet colour at the junction between the two liquids indicates the presence of carbohydrates.

2.4.3 Test for phenols

To the 5 ml of extract, 5% ferric chloride is added and the violet colouration indicates presence of pheols.

2.4.4 Test for steroids

The 1 ml of extract was dissolved in chloroform and equal volume of H_2SO_4 was added to it. Bluish red to cherry red colour was observed in chloroform layer; whereas acid layer assumes marked green fluorescence indicates presence of steroids.

2.4.5 Test for flavonoids

In a crucible, 5 ml of extract was taken and a few drops of dilute ferric chloride chloride solution were added. The color change to pale green or red brown color indicated the presence of flavonoids.

2.4.6 Test for tannins

Five ml of extract was taken in a tube and 1 ml of lead acetate solution was added. Formation of flocculants brown precipitated indicated the presence of tannins.

2.5 Antibacterial Assay

2.5.1 Standardization of inoculums

The nutrient broth was prepared and a well isolated colony of the same type the culture equals or exceeds the turbidity of a 0.5 McFarland standard. The time usually required was 2-8 hours.

2.5.2 Preparation of McFarland solution

The turbidity standard was prepared by adding 0.5 ml of 0.048M Bacl2 to 99.5 ml of 0.35N H_2SO_4 (1% v/v) and was agitated on a vortex mix before use. The turbidity standard gave an optical density of 0.08 to 0.10 at 625 nm when tested in spectrophotometer with a 1 cm light path. McFarland 0.5 turbidity corresponded to inoculums of 1 x 10^8 CFU/ml. The turbidity of actively growing broth culture was adjusted with sterile saline or broth to obtain turbidity comparable to the 0.5 McFarland turbidity standards (Gayathri et al., 2018).

2.5.3 Evaluation of antibacterial activity of ß-lactam antibiotics against MRSA strains

A sterile cotton swab was dipped in the inoculums and excess was removed by rotating the swab against the inside wall of the tube above the fluid level. The surface of the Muller Hinton agar plate was inoculated by streaking the swab over the surface. Streaking was repeated 3 times and for each time the plate was rotated 60°C (Elfadhi et al*.*,2023).

As soon as not later than 15 mins after the inoculation of plates the antibiotic discs vancomycin, teicoplanin, cloxacillin, ofloxacin, ticarcillin, cafatoxime, cefatazidime, methicillin was applied with forceps. To ensure complete contact of the disc to the agar surface, the disk was pressed down with slight pressure. Inoculated plates were incubated at 35°C for 18 hrs in an inverted position.

2.5.4 Determination of the degree of methicillin resistance

The Kirby-Bauer disk diffusion test was developed for this study. MRSA strains were individually tested for their degree methicillin resistance with methicillin (30 µg).

2.5.5 Evaluation of antibacterial activity of plant extracts against MRSA strains

Antibacterial activity was measured using disk diffusion method according to the clinical and laboratory standards institute (CLSI). Briefly petriplates containing 20 ml of Muller-Hinton agar medium were inoculated using a cotton swab with 4-6 fold culture of the bacteria. The Hi-media sterile disks were used for this study. About 30µl of plant extract was pipetted onto each disk. The loaded disk were placed into inoculated plates and then incubated at 37°C for 18-24 hours (Aquil et al.,2006).

2.5.6 Disc susceptibility test

The Kirby-Bauer disk diffusion test was developed for these studies as described above with the disks of plant extract. The sensitivity pattern of the test organism was recorded.

2.5.7 Minimal inhibitory concentration (MIC)

The minimal inhibitory concentration was determined by disc diffusion method. The methanolic extract of plant extract for MRSA strains were used to determine MIC. Muller Hinton agar plates were prepared and 100 µl of actively grown culture was inoculated. The sterile discs were dipped in methanolic extract of plant extract (5 mg/ml, 10 mg/ml, 20 mg/ml, 40 mg/ml, and 80 mg/ml) and placed on inoculated plates using sterile forceps. The plates were incubated at 37°C for 24 hrs and the zone of inhibition was measured in millimeter.

3. RESULTS

3.1 Isolation and Characterization of *Staphylococcus aureus*

Methicillin resistant *Staphylococcus aureus* (MRSA) strains were isolated from pus. Mannitol salt agar was used as a differential and selective medium for recovering strain from in the specimen. *Staphylococcus aureus* produced yellow colour colonies in mannitol salt agar (Fig. 1).

3.2 Biochemical Characterization of *Staphylococcus aureus*

Staphylococcus aureus were subjected to various biochemical tests. The results were tabulated (Table 1). Biochemical tests for the isolates were carried out and the results reveal that MRSA strain was negative to indole and positive to methyl red test, VP and citrate utilization, Catalase and Coagulase test.

3.3 Phytochemical Analysis of Plant Extract

The methanol extract *Allium sativum* was subjected to qualitative phytochemical analysis (Table 2). Saponins, Phenols, steroids, flavonoids, tannis in both methanol extract of Allium sativum with reference to (Garba et al., 2013).

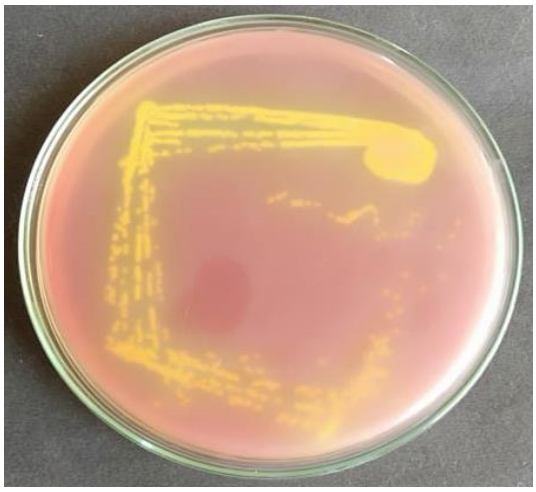

Fig. 1. Isolation of MRSA

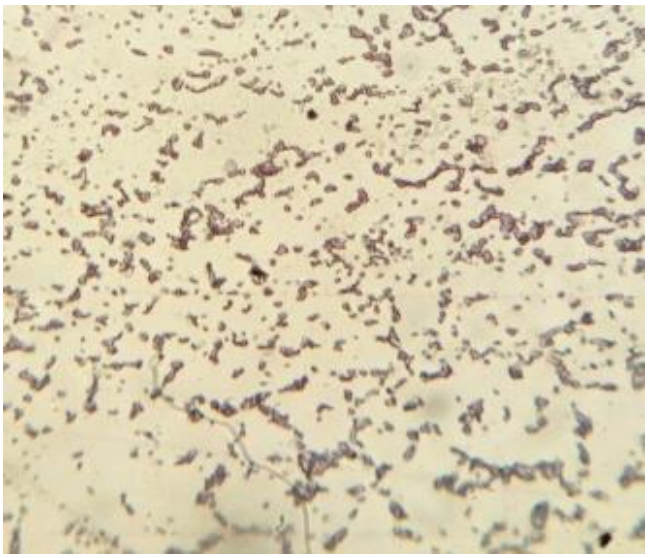

Fig. 2. Microscopic examinations of MRSA strain

Table 1. Biochemical characterization of *Staphylococcus aureus*

S.NO	Test	Result
1	Indole test	Negative
2	Methyl red test	Positive
3	Voges proskauer test	Positive
4	Citrate utilization test	Positive
5	Catalase test	Positive
6	Coagulase test	Positive

Table 2. Phytochemical analysis of *Allium sativum*

Test	Methanol extract
Alkaloids	+
Carbohydrates	+
Tannins	+
Phenols	+
Flavonoids	+

3.4 Minimal Inhibitory Concentration (MIC)

Minimal inhibitory concentration of methanolic extract of *Allium sativum* was determined in (Table 3).

3.5 Antibiotic Sensitivity Pattern of Isolated Strains of *Staphylococcus aureus*

Antibiotic sensitivity pattern of isolated *staphylococcus aureus* strains against in methicillin disc in the concentration of 10%.

The methanol extract of plant were used. MIC and antibacterial activity of plant extract were checked. The results revealed that the methanol extract of *Allium sativum* were against the isolated MRSA strains. The minimum inhibitory concentrations of the methanol extract of *Allium satiuvm* and were determined. But, 40 mg/ml concentration inhibited the growth.

4. DISCUSSION

MRSA samples were isolated from the pus sample from wounds. About 10 strains of MRSA were used for this study and the strains were maintained in mannitol salt agar plates.

Table 3. Minimal inhibitory concentration of methanolic extract of plant against MRSA

Sample	Zone of inhibition in mm				
	Allium sativum (Methanolic extract)				
	5 mg/ ml	10 mg/ ml	20 mg/ ml	40 mg/ ml	80 mg/ ml
1	0	0	5	7	10
2	0	0	4	5	7

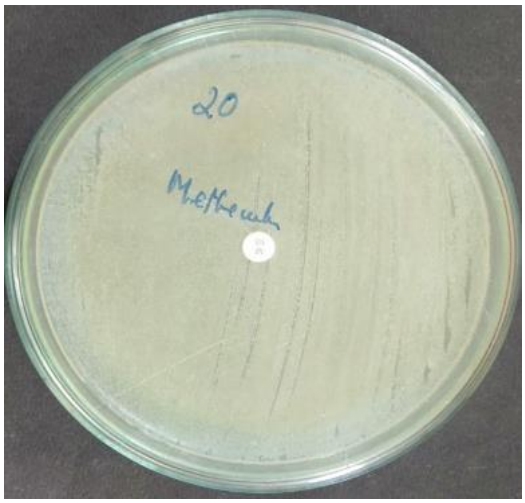

Fig. 3. MRSA resistances to methicillin antibiotic

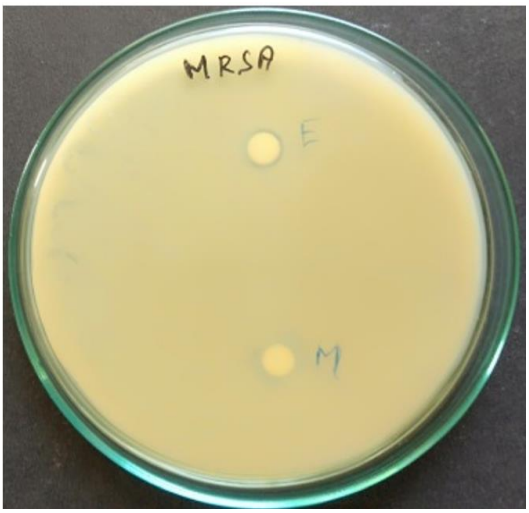

Fig. 4. Antibacterial activity of garlic extract against MRSA

The medicinal plant were collected from Virudhunagar district. The collected plant was individually extracted with methanol. Preliminary phytochemical screening of the plant extracts were carried out. It showed the presence of alkaloids and carbohydrates. Due to the presence of these phytochemical compounds in the four plants extract has efficient anti-bacterial activity against MRSA.

Screening medicinal plants for biologically active compounds offer clues to develop newer antimicrobial agents. Plants are commonly exploited in traditional medicine and their curative potentials. Plant derived medicines are widely used because they are safe than synthetic to determine the antibacterial activity of ethanol and methanol extracts antibiotics against MRSA isolates.

In recent years, multiple drugs, resistance has been developed in bacteria due to indiscriminate use of existing drugs in the treatment of infectious diseases. The major trust is to establish alternative antimicrobial agents in order to treat microbial infections with less or no toxicity and less or negligible side effects.

Medicinal plants are considered one of the most valuable resources for antibiotic development. Pathogenic strains of antibiotic resistant bacteria have emerged due to the, misuse of antibiotics. As a result, bacteria become resistant to antibiotics, which are in turn less effective after extended periods of use in medicinal plant of *Allium Sativum.*

The methanolic and water extract of plant were used against MRSA strains. The methanolic extract of Allium sativum (Poonkothai, 2006) were highly effective against the MRSA than the methanol. Zone of Methanolic extracts of Allium sativum (13mm/30) had a sensitivity towards bacterial isolates then the other extracts, Strika et al*.,* 2017 reported that the allicin present in Allium sativum has been shown that in pure form it displays: antibacterial activity against a broad spectrum of Gram positive and Gram-negative bacteria.

The methanol extract *Allium sativum* were effective because the phytochemical compounds are highly soluble in methanol solvent than the ethanol.

In our present investigation, one extracts prepared from leaf (ethanol and methanol) exhibited highest antibacterial activity against *S. aureus*.

The maximum zone of inhibition in methanol extract is 11 mm/30 ul (SA5) and in methanol extract is 7 mm/30 ul (SA10). Some of the strains are not responded to the plant extracts so, as the concentration of the extract increases the zone of inhibition can also be increased.

5. CONCLUSION

Medicinal plants can be valuable therapeutic resources without any side effects. MRSA infections are difficult to treat because of their resistance to many of the commonly used antibiotics. This research found to be methanolic extract of *Allium Sativum* showed the better antibacterial activity against MRSA. In further research carried out for purified phytocompound isolated from this plant for drug discovery.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ACKNOWLEDGEMENT

The authors extend their appreciation to the Deanship of Scientific Research at Northern Border University, Arar, KSA for funding this research work through the project number "NBU-FFR-2024-1329-07".

COMPETING INTERESTS

Author has declared that no competing interests exist.

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