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ORIGINAL ARTICLE

Evaluation of the Anti-Bacterial Potential of Allium Sativum Against some Resistant Human Pathogenic Isolates and its Synergy with Antibiotics

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ABSTRACT

Background: Allium Sativum has long been considered one of the most efficient medicinal plants for treating bacterial illness. Taking into consideration the current research study was conducted to assess the effect of Allium sativum extract on Multi-Drug Resistant (MDR) bacterial isolates and its association with antibiotics against MDR pathogens commonly found in the hospital environment.

Methods: The test organisms (Staph aureus, Staphylococcus, Yersinia, Micrococcus) which were clinically isolated from patients were collected from Lady Reading Hospital Peshawar. The clinical samples were cultured into their defined media. The disc diffusion method was used to test the antibiotic susceptibility of Meropenem, Ciprofloxacin, Cefixime, Ampicillin, and Erythromycin. Antibacterial activity was performed by using the agar well diffusion method. Four different solvents (methanol, n-hexane, ethyl acetate, and distilled water) were used to extract the bioactive compounds of Allium sativum. The synergistic activity of fresh garlic extracts and antibiotics was performed by disc diffusion assay using antibiotic and extract at the same time.

Results: Out of 25 samples, 7 samples (Yersinia, Staphylococcus, and Micrococcus species) were MDR to antibiotics. A high level of sensitivity of microbes was recorded for MEM. Depending on the susceptibility of fresh garlic extract, the aqueous extract showed a maximum zone of inhibition of 42mm against Yersinia compared to the conventional antibiotic Ciprofloxacin which showed resistance. The minimum zone of inhibition of 10mm showed against Micrococcus. Ethyl acetate extract showed a maximum zone of inhibition of 30mm against staphylococcus while a minimum zone of inhibition of 11mm against Micrococcus. Methanolic extract showed a maximum zone of inhibition of 21mm against staphylococcus while a minimum zone of inhibition of 12 against Micrococcus and other isolates. N-hexane extract showed the lowest activity among all extracts. Fresh garlic extract inhibited MDR isolates demonstrating a synergistic association with antibiotics.

Practical Implication: The potential use of such a combination may be beneficial especially in inhibiting drug-resistant pathogens. Based on the results of this study, taking garlic as a supplement during antibiotic therapy improves its efficiency.

Conclusion: Garlic extracts showed good activity against selected MDR isolates. Aqueous extract and ethyl acetate extract showed maximum activity against MDR bacterial isolates. This study also showed good synergistic activity of garlic extracts in combination with antibiotics while no antagonistic activity has been shown.

Keywords: Allium sativum**,** Antibacterial activity, MDR, Fresh Garlic Extract, Synergy

INTRODUCTION

The evolution of antibiotic resistance in pathogenic microbes or their hosts has necessitated the development of novel and different classes of antimicrobial chemicals and antimicrobial genes [1-2]. Antibiotics have tremendously helped humans; however, the world must now focus on resolving their consequences, such as drug resistance and negative impacts on human health and environmental safety [3]. Rising antibiotic resistance is a serious impediment to successful infectious disease treatment and microbial pathogenicity reduction. By adopting the mechanism of mutation, multiple-resistant 'superbug' bacteria can change targets and diminish antibiotic permeability [4]. Garlic (Allium sativum) is a member of the Alliaceae family [5]. It has long been used as an anti-infective agent in diet and medicine [6]. These applications are supported by in vitro evidence of the antibacterial activity of fresh and freeze-dried garlic extracts against human pathogenic bacteria [7] fungi [8] and viruses [9]. Garlic is a perennial member of the onion family. Garlic is one of those plants that has been widely known for many years and has been used for centuries to treat infections, improve, and maintain physical and mental health [10.] Garlic antimicrobial activity is attributed primarily to organosulfur compounds such as allicin and diallyl sulfide. Allicin has been shown to have antimicrobial properties and is responsible for the majority of garlic's pharmacological properties [11.] Garlic juices (Allium sativum) have a wide range of antibiotic action against Gram-positive and Gram-negative bacteria, as initially described by Louis Pasteur [12]. The emergence of bacterial resistance to modern medical antibiotics is currently a threat to the successful treatment of communicable diseases [13.] This study aimed to evaluate the in vitro antibacterial activity of garlic extracts against some resistant human pathogenic isolates and to check the synergistic effect with antibiotics.

MATERIALS AND METHODS

Study Design and Setting: This cross-sectional study was carried out in Microbiology Department, Abasyn University, Peshawar with collaboration of Lady Reading Hospital, Peshawar.

Study Duration: The study was conducted in duration of six month.

Study Inclusion and Exclusion Criteria: For this purpose, oral, nasal, tracheal, and pus samples were collected from different infectious parts of patients.

Data Collection Procedure: Nutrient agar media were prepared and sterilised for 15 minutes at 121 °C. The medium was autoclaved and then allowed to cool to room temperature. Petri plates were filled with LFH sterilised medium and kept at 37 °C for 24 hours of incubation. For identification, biochemical tests such gramme staining, catalase testing, oxidase, indole, etc. were performed. The disc diffusion method was used to evaluate the susceptibility of bacterial isolates to antibiotics. To assure sterility, nutrient agar media were made and incubated for 24 hours at 37°C. To make a bacterial lawn, the microbial inoculum from incubated nutrient broth was spread over ready nutrient agar plates using a cotton swab. The plates were topped with the chosen antibiotics, and they were then incubated for 24 hours at 37°C.

We bought market-purchased garlic and dried it by air. They were finely ground after drying. In order to extract the bioactive components, four extracts were created by mixing 4 grammes of powdered garlic with 200 ml of each solvent and storing them at

room temperature for seven days. The extract was filtered and then dried by air.

The antibacterial effectiveness of various garlic extracts was assessed against MDR isolates, including Staphylococcus, Yersinia, Streptococcus, Staphylococcus aureus, and Micrococcus species. Plates of nutrient agar media were made. The plates were incubated at 37°C for 24 hours to verify their sterility. To make a bacterial lawn, the microbial inoculum was taken from the nutrient broth that had been incubated and evenly spread out on prepared nutrient agar plates. Four wells were made in each of the media plates with the help of a sterile metallic borer. Different extracts (100µl each) were loaded in each well. The plates were incubated for 24hrs at 37°C.

The synergistic effect of crude Allium sativum extracts and antibiotics was investigated using the disc diffusion method, which involved simultaneously placing an antibiotic disc and an extract on a nutrient agar plate. The plates were incubated for 24 hrs at 37°C. After incubation zone of inhibition was measured.

All the data collected and recorded in Microsoft Excel 2007 and further process through Statistical Package for Social Science version 22. Data were presented in percentage in the tables.

RESULTS

25 samples were collected from different infectious parts of patients including pus, tracheal, mouth, and nasal samples. The results revealed that each sample had a varied percentage of bacterial species present. Seven different microbial species were identified as Multi-Drug Resistant spp. 80% of the tracheal samples were positive for microbial presence followed by 63%, 60%, and 50% (Table 1).

Table 1: Samples collected from different patients and percentages of positive samples

S. No	Source of sample	Total samples	Positive samples	Percentage %
1	Tracheal	5	4	80
2	Oral	11	7	63
3	Pus	5	3	60
4	Nasal	4	2	50

Antibiotic Susceptibility Assay: Meropenem, ciprofloxacin, erythromycin, cefixime, and ampicillin were checked against bacterial isolates (Staphylococcus, Yersinia, Streptococcus, Micrococcus). MEM showed maximum activity (40mm) against S. aureus while CFM showed minimum activity (15mm) against proteus. Six bacterial species were considered multi-drug resistant bacterial isolates. The sensitivity towards used antibiotics was determined based on the CLSI index 2016.

Table 2: Effect of different Allium Sativum extracts on selected MDR isolates

Bacterial Isolates	Distilled water extract	Ethyl acetate extract	Methanol extract	N. Hexane extract	Ciprofloxacin
	Zone of inhibition (mm)				
Staph aureus	30	12	21	10	R
Yersinia	42	16	19	12	R
Other species of Yersinia	16	12	16	17	R
Staphylococcus	15	30	21	16	R
Other species of Staphylococcus	16	17	14	10	R
Other species of Yersinia	22	21	13	10	R
Micrococcus	10	11	12	13	R

Distilled water extract showed maximum activity of (42mm) against Yersinia followed by methanol, ethyl acetate, and n-hexane extract.

Table 3: Synergistic effect of antibiotics and different extracts of Allium Sativum

Isolate No	Methanol extract					Distilled water extract					Ethyl acetate extract					N.Hexane extract				
	Mem	Cip	Ery	Cfm	Am	Mem	Cip	Ery	Cfm	Amp	Mem	Cip	Ery	Cfm	Amp	Mem	Cip	Ery	Cfm	Amp
	Zone of inhibition (mm)					Zone of inhibition (mm)					Zone of inhibition (mm)					Zone of inhibition (mm)				
1	30	30	07	11	07	30	10	07	07	07	10	30	07	10	10	10	35	09	7	35
2	50	38	07	07	15	33	10	07	07	07	30	10	07	16	08	22	08	07	10	07
3	37	40	12	30	10	45	30	10	25	25	10	10	10	07	19	25	08	10	07	07
4	20	20	18	07	10	20	10	12	12	12	30	10	07	11	08	10	10	07	10	07
5	15	22	12	20	15	12	11	18	20	20	15	11	07	07	07	10	15	11	07	15
6	30	15	10	10	07	27	07	07	11	11	27	10	11	07	10	13	10	12	07	15
7	27	30	10	12	10	29	10	08	30	30	15	15	18	22	25	23	25	12	22	12

Key: MEM= Meropenem; CIP= Ciprofloxacin; ERY= Erythromycin; CFM= Cefotaxime; AMP= Ampicillin; mm= millimeter

The synergistic effect of antibiotics and Allium sativum extracts were checked against MDR bacterial isolates to check their combined effects. The combination of MEM and distilled water extract had the highest zone of inhibition (50 mm) against Yersinia (isolate no 02), followed by CIP, CFM, AMP, and ERY, while some combinations had the lowest zone of inhibition (7 mm). The rest of the combination showed intermediate results (Table 2).

Figure 1: Growth of bacterial isolate on nutrient agar media.

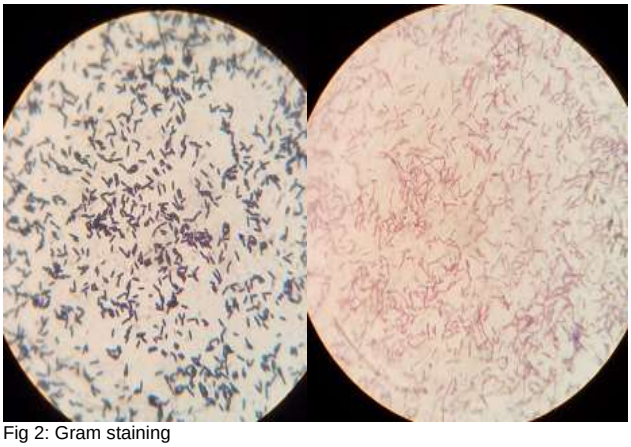

Fig 2: Gram staining


Fig 3: Biochemical tests.

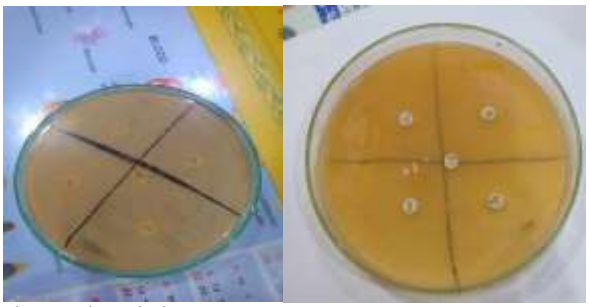
Fig 4: Resistant isolates.

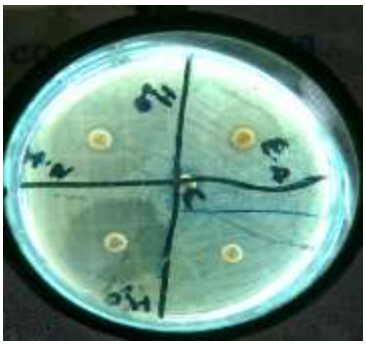
Fig 5: Synergistic effect

DISCUSSION

Garlic extracts have a wide range of antibacterial activity against both gram-positive and gram-negative bacteria. Garlic's various components reduce the risk of cardiovascular disease, as well as have antitumor and antimicrobial properties. Although the mechanism by which garlic acts on microbes is not fully understood, it has a significant effect on the growth reduction of bacterial species. This is because garlic compounds, particularly allicin, affect bacterial growth by inhabiting their DNA and protein synthesis. In this study different crude extracts of Allium Sativum including methanol, n-hexane, aqueous & ethyl acetate extracts were applied to different MDR isolates to assess the antibacterial effect of the extracts on MDR isolates. The maximum zone of inhibition (42 mm) was recorded for crude aqueous extracts of Allium Sativum while the minimum (10mm) was recorded for crude n-hexane extract. Methanol extract showed maximum activity (21mm) while ethyl acetate extract showed (a 30mm) zone of inhibition. Comparatively, methanol and n-hexane extract revealed lower activity. Prior research has shown that lipid polar solvents, particularly ethanol, ethyl acetate, and water, have a stronger inhibitory effect. This is because non-polar extracts have faster diffusion rates and lower viscosity[14]. The susceptibility of bacterial strains is determined by their structural composition. S aureus, for example, has just 2% lipid; nonetheless, the lipid concentration of the membrane influences the permeability of hydrophobic and volatile bioactive compounds in Allium Sativum. As a result, the effect might encourage the breakdown of S aureus genetic material and cell wall [15]. Extract with high viscosity have a slower diffusion rate, and they inhibit less bacterial population. Their results are comparable to our study. A similar study had also been reported where garlic aqueous extract showed a 30mm zone of inhibition against S aureus [16]. Their results are comparable to our study. E. coli and S. aureus demonstrated potential sensitivity to aqueous extracts of garlic, according to earlier studies carried out [17]. The disc diffusion method was used to test the synergistic effects of several Allium Sativum extracts and antibiotics on selected MDR isolates in this investigation. Different extracts and antibiotics revealed enhanced growth reduction activity against selected MDR isolates. The mechanism governing the combined action of plant extracts with other compounds is still unknown, though some researchers believe phytocompounds disrupt cell walls or increase cytoplasmic membrane permeability, allowing antibiotics to enter the cell, producing efflux pump inhibitors, or inhabiting Penicillin Binding protein [18]. Many investigations have revealed the ability of garlic extract and antibiotics to yield synergistic interaction in combination against MDR isolates [19].

CONCLUSION

Allium sativum extracts effectively inhibit the growth of different bacterial isolates which were initially MDR isolates. It can be used synergistically with different antibiotics and has the potential to increase the efficiency of antibiotics against different MDR isolates.

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