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Evaluation of The Antibiotic Potency of Trigonella Foenum-Graecum Extracts Against Methicillin-Resistant Staphylococcus aureus

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ABSTRACT
The goal of this study was to evaluate the effectiveness of Trigonella foenum-graecum extracts against Methicillin-Resistant Staphylococcus aureus (MRSA) when coupled with antibiotics. Alkaloids, phenols, tannins, flavonoids, and saponins were all found, according to the phytochemical qualitative analysis. The colony suspension method was used to standardize the MRSA strain for the study of the antibiotic susceptibility. The suspensions had a concentration of 1.5 106 CFU/mL because they matched the 0.5 McFarland criteria. Under the guidelines of the Kirby-Bauer test, antimicrobial susceptibility tests were performed on Mueller Hinton agar using both the disc and well diffusion methods. The bacterial sample was injected into Mueller Hinton agar plates at a volume of 50 L. A higher level of antibacterial activity against methicillin-resistant Staphylococcus aureus was found in methanol-trigonella foenum-graecum extract. When the methanol plant extract and Ciprofloxacin (5mcg) were combined, the diameter of the inhibitory zone increased significantly to 2 0.4 mm. Tetracycline (30 mcg) raised Methanol T. foenum-graecum by 2 mm. Ciprofloxacin (5 mcg) boosted the water plant extract to 1 0.3 mm and Tetracycline (30 mcg) increased it to 1 0.7 mm. Only an increase of 1 0.4 mm in the diameter of the inhibitory zone with Ciprofloxacin (5mcg) was seen with the isopropanol plant extract. There was no increase in the diameter of the inhibitory zone in hexane solvent extract. This research has significant implications for expanding the effectiveness of antibiotics against pathogenic, multidrug-resistant microbes.

Keywords: *Methicillin-Resistant Staphylococcus aureus (MRSA); phytochemicals; antibiotics; Trigonella foenum-graecum*

I. INTRODUCTION

Due to its extraordinary success, Staphylococcus aureus (S. aureus) has evolved into a resilient and adaptable microbe. It contributes to several common infections. The altered penicillin-binding proteins of Methicillin- Resistant Staphylococcus aureus (MRSA) were first identified in 1961 [1]. MRSA can result in infections such as septicemia, pyemia, and bacteremia as well as mild suppurative infections [2]. MRSA-caused bacteremia makes the clinical course of organ transplantation more difficult and can be fatal [3].

From innocuous localized skin infections to fatal systemic illnesses, the bacteria can establish. Numerous toxins produced by staphylococci are known to cause illness [4]. These poisons represent a massive sequence of pathogenicity, causing diseases to be caused by bacteria [5].The organism has undergone evolutionary succession, as demonstrated by epidemiological and genomic data, leading to the dynamic loss of native genes and the gain of host-specific adaptation genes [6].

Methicillin-Resistant *Staphylococcus aureus* (MRSA)

MRSA is adaptable, powerful, and erratic. It poses a serious hazard because of their genetic variation and the sequential emergence of effective epidemic variants [7]. Their pathogenicity is connected to the spread of complicated diseases [8].The rise of MRSA has made therapy difficult. In hospitals, the strain colonizes healthy people and spreads dangerous illness [9]. Until recently, MRSA infections (also known as HA-MRSA or healthcare-associated MRSA) were only sometimes found in healthcare settings. Patients who had never been hospitalized before eventually contracted the same strains, giving rise to the name community-associated MRSA (CA-MRSA) [10]. Compared to infections caused by methicillin-sensitive S. aureus, hospitalization expenditures associated with MRSA infections are significantly greater [11,12].

Methicillin-Resistant *Staphylococcus aureus* in Hospital and Community Settings

For hundreds of years, hospital emergencies and illnesses acquired in the community have been considered significant issues [13]. Patients in both settings experience an increase in morbidity and mortality due to MRSA strains [14].

MRSA strains have spread and are now the main cause of bacterial infections in both environments [15]. The evolution of the pathogen population that lives in hospitals is significantly influenced by the clones [16].

The ability of MRSA to develop antibiotic resistance pathways increases their pathogenicity in the transmission of disease [17]. They differ genetically from other bacterial strains because they have changed genes [18]. The MRSA has an evolutionary history of its clone complex, as shown by the wild strain of Staphylococcus aureus [19].

Long-term hospitalization brought on by MRSA had increased the need for expensive drugs and specialized IV antibiotics [20]. MRSA has been made worse by the clinical usage of methicillin antibiotics [21].

The spread of germs that are multi-drug resistant is dangerous for world health. Staphylococcus aureus was found to be resistant to methicillin in 1961, just over a year after the antibiotic's introduction as a second-generation beta-lactam [22]. Antibiotic-resistant genes infiltrate populations of Staphylococcus aureus that are not already resistant, causing them to become resistant [23].

Their success as potentially fatal human infections has been facilitated by bacterial resistance to drugs [24]. A novel antimicrobial agent, more potent antibiotics, or an alternate therapy must be developed immediately due to the rise in MRSA cases [25].Many plants have shown the ability to suppress a variety of bacteria. Their combination may greatly improve the efficacy of the antibiotics currently being used against a variety of resistant microbes [26].

II. MATERIALS AND METHODS

Collection of Plant Material

Trigonella foenum-graecum plant leaves in good health were purchased in April at a market in Behat, Saharanpur, India. To remove dirt and dust, the leaves were washed under flowing water from the faucet. The leaves were then minced in a blender after being shade-dried for 20 days. For further analysis, minced samples were placed in a sterilized container that was then carefully closed with its cap and maintained in a dark location. The Botany Department at Global University in India was given credit for the authentication.

Solvents Used for Extraction

Water, methanol, isopropanol and hexane.

Plant Extraction Procedure

The stored dried ground leaves of *Trigonella foenum-graecum* (100 g) extracted using diverse solvents based on their differences in polarity indexes. Solvent polarity ranged from most polar organic solvent, mid-polar to less polar solvent. Water, methanol, isopropanol and hexane were the solvents used for extraction. The water solvent is the most polar with polarity index of 10.2, methanol polarity index (5.1), isopropanol polarity index (3.9) and hexane polarity index of (0.1).

The extraction procedure was done using Soxhlet extractor apparatus. 10 g of the minced plant sample were put on filter paper, folded and inserted into the thimble part of the Soxhlet apparatus; heat was adjusted to different boiling point based on the optimum boiling point of the used solvent. The water solvent was adjusted at 100.0°C, Methanol at 64.7°C, Isopropanol at 82.3°C and, hexane at 68.7°C. The extraction procedure repeated several cycles continuously for 8 hours until the solvent in the thimble appeared colorless. The extracted samples were filtered using Whatman No.1 filter paper. The solvents were evaporated using a rotary evaporator adjusted at their respective boiling points. The extracts were dried overnight in a vacuum oven at 37.0 °C and stored in a refrigerator for further use.

Methicillin-Resistant *Staphylococcus aureus*
Bacteria Sample

The bacteria were obtained from a IMTECH Chandigarh.

Identification of the Bacteria

Methicillin-Resistant *Staphylococcus aureus* bacteria were identified by growing on Mannitol Salt Agar (MSA) plate. The medium only select and enhance the growth of *Staphylococcus aureus* while hindering the growth of other bacteria. The samples were streaked on the media, and incubated at 37°C for 24 hours. There was an observation of yellow pigment colonies.

Media used on Susceptibility Test

Mueller Hinton agar.

Preparation of Mueller Hinton agar Media

38 grams of Mueller Hinton agar powder added to one liter of distilled water. It was stirred to dissolve. The sterilization was done by autoclaveat 121°C for 15 minutes. The liquid media was poured into the Petri dish and left to stand for 30 minutes to allow the media to solidify.

Antibiotics Used

The susceptibility test discs of Methicillin (10 mcg), Penicillin –G (10 mcg), Ciprofloxacin (5 mcg) and, tetracycline (30 mcg) were purchased from Himedia company.

Antibiotics Susceptibility Testing

The Methicillin-Resististant *Staphylococcus aureus* strain was standardized using the colony suspension method. The suspension was then matched with 0.5 McFarland standards ensuing resultant concentration of 1.5 $x10^6$ CFU/mL. Both disk and well agar diffusion methods were used for the antibacterial susceptibility test [39]. The Mueller Hinton agar plates were inoculated with 50 µL of the bacterial sample and spread on the surface using a sterilized glass spread. The plates were allowed to stand for half an hour.The sterilized 6 mm cork borer, was used to bore triplewells into each plate.

To the test the antibacterial susceptibility of the plan extract, each of the triple wells was loaded with 100 µL of *Trigonella foenum-graecum* extract. The antibacterial susceptibility test with antibiotics was done by disc agar diffusion method,and the combination of *Trigonella foenum- graecum* extract and antibiotics on well agar diffusion method. All the three categorized plates were allowed to settle for half an hour then later placed into the incubator. The plates were incubated at 37°C for 24 hours. Thereafter diameterzones of inhibitions were determined from the threeplates and interpreted using zone of inhibition diameter interpretative standards [40].

III. RESULTS

The diameter zone of inhibition on *T. foenum- graecum* water extract alone was 18± 1.2 mm. Methicillin (10 mcg) and Penicillin –G (10 mcg) showed no zone of inhibition. Ciprofloxacin(5mcg) had 28 ± 0.5 mm and Tetracycline (30 mcg), 25 ± 0.5 mm. The combination of the extract with Methicillin (10mcg) was 18 ± 0.8mm, *T. foenum- graecum* with Penicillin –G (10 mcg) 18 ± 0.8 mm,*T. foenum-graecum* with Ciprofloxacin (5mcg) 29

± 0.8 mm and *T. foenum- graecum* with Tetracycline (30 mcg) 26 ±1.2 mm (Table 2). There was a significant increase in the diameter zone of inhibition on the combination of the water plant extract with Ciprofloxacin (5mcg) of 1 ± 0.3 mm. The combination of *T. foenum- graecum* with Tetracycline (30 mcg) increased by 1 ± 0.7 mm**.**

Table 2. Antibacterial activity of *Trigonella foenum-graecium* water extract and their combinationwith antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA)

S.no	Anti-bacterial agent against MRSA	Diameter of zone of inhibition (mm) ±*SD*
1	Water Plant extract (100 µl) *Trigonella foenum-graecium*	18± 1.2
2	Antibiotics	
	Methicillin (10mcg)	-
	Penicillin-G (10 units)	-
	Ciprofloxacin (5 mcg)	28 ± 0.5
	Tetracycline (30 mcg)	25 ± 0.5
3	Combination: Plant extract (100µl/mg) + antibiotic	
	T. foenum-graecium+ Methicillin (10mcg)	18 ± 0.8
	T. foenum-graecium+ Penicillin-G (10 units)	18 ± 0.5
	T. foenum-graecium+ Ciprofloxacin (5 mcg)	29 ± 0.8
	T. foenum-graecium+ Tetracycline (30 mcg)	26 ± 1.2

+ [Combined]; **-** [No zone of inhibition]

Table 3. Antibacterial activity of *Trigonella foenum-graecium* methanol extract and theircombination with antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA)

S.no	Anti-bacterial agent against MRSA	Diameter of zone of inhibition (mm) ±*SD*
1	Methanol Plant extract (100 µl) *Trigonella foenum-graecium*	20±0.9
2	Antibiotics	
	Methicillin (10mcg)	-
	Penicillin-G (10 units)	-
	Ciprofloxacin (5 mcg)	28 ± 0.5
	Tetracycline (30 mcg)	25 ± 0.5
3	Combination: Plant extract (100µl/mg) + antibiotic	
	T.foenum-graecium+Methicillin (10mcg)	20 ± 0.5
	T.foenum-graecium+Penicillin-G (10 units)	20 ± 0.5
	T. foenum-graecium+Ciprofloxacin (5 mcg)	30 ± 0.9
	T. foenum-graecium+ Tetracycline (30 mcg)	27 ± 0.5

+ [Combined]; **-** [No zone of inhibition]

The diameter zone of inhibition on *T.foenum- graecium* methanol extract alone was 20±0.9 mm. Methicillin (10 mcg) and Penicillin –G (10 mcg) showed no zone of inhibition. Ciprofloxacin(5mcg) had 28 ± 0.5 mm and Tetracycline (30 mcg), 25 ± 0.5 mm. The combination of the extract with Methicillin (10 mcg) was 20 ± 0.5 mm, *T. foenum- graecum* with Penicillin –G (10 mcg) 20 ± 0.5 mm,*T. foenum-graecum* with Ciprofloxacin (5 mcg) 30 ± 0.9 mm and *T. foenum-graecum* withTetracycline (30 mcg) 27 ± 0.5 mm (Table 3). There was a significant increase in the diameter zone of inhibition on the combination of the methanol T.foenum-graecium extract with Ciprofloxacin (5 mcg) of 2 ± 0.4 mm. The combination of *T. foenum-graecum* with Tetracycline (30 mcg) increased by 2 mm**.**The diameter zone of inhibition on *T. foenum- graecium* isopropanol extract alone was 12 ± 0.5 mm. Methicillin (10 mcg) and Penicillin –G (10 mcg) showed no zone of inhibition. Ciprofloxacin (5mcg) had 28 ± 0.5 mm and Tetracycline (30 mcg), 25 ± 0.5 mm. The combination of the extractwith Methicillin (10 mcg) was 12 ± 0.5 mm, *T. foenum-graecum* with Penicillin –G (10 mcg) 12 ± 0.5 mm, *T. foenum-graecum* with Ciprofloxacin (5mcg) 29 ± 0.9 mm and *T. foenum-graecum* with Tetracycline (30 mcg) 25 ± 0.5 mm (Table 4). There was a significant increase in the diameter zone of inhibition on the combination of the *T. foenum-graecium* isopropanol extract with Ciprofloxacin (5 mcg) of 1 ± 0.4 mm.

Table 4. Antibacterial activity of *Trigonella foenum-graecium* isopropanol extract and theircombination with antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA)

S.no	Anti-bacterial agent against MRSA	Diameter of zone of inhibition (mm) ±*SD*
1	Isopropanol Plant extract (100 µl) *Trigonella foenum-graecium*	12 ± 0.5
2	Antibiotics	
	Methicillin (10mcg)	-
	Penicillin-G (10 units)	-
	Ciprofloxacin (5 mcg)	28 ± 0.5
	Tetracycline (30 mcg)	25 ± 0.5
3	Combination: Plant extract (100µl/mg) + antibiotic	
	T. foenum-graecium+ Methicillin (10mcg)	12 ± 0.5
	T. foenum-graecium+ Penicillin-G (10 units)	12 ± 0.5
	T. foenum-graecium+ Ciprofloxacin(5 mcg)	29 ± 0.9
	T. foenum-graecium+ Tetracycline(30 mcg)	25 ± 0.5

+ [Combined]; **-** [No zone of inhibition]

Table 5. Antibacterial activity of hexane plant extract (*Trigonella foenum-graecium*) and their combination with antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA)

S.no	Anti-bacterial agent against MRSA	Diameter of zone of inhibition (mm) ±*SD*
1	Hexane Plant Extract (100 µl) *Trigonella foenum-graecium*	08 ± 0.5
2	Antibiotics	
	Methicillin (10mcg)	-
	Penicillin-G (10 units)	-
	Ciprofloxacin (5 mcg)	28 ± 0.5
	Tetracycline (30 mcg)	25 ± 0.5
3	Combination: Plant extract (100µl/mg) + antibiotic	
	T. foenum-graecium+ Methicillin (10mcg)	08 ± 0.5
	T. foenum-graecium+ Penicillin-G (10 units)	08 ± 0.5
	T. foenum-graecium+ Ciprofloxacin (5 mcg)	28 ± 0.5
	T. foenum-graecium+ Tetracycline (30 mcg)	25 ± 0.5

+ [Combined]; **-** [No zone of inhibition]

The diameter zone of inhibition on *T. foenum- graecium* hexane extract alone was 08 ± 0.5 mm. Methicillin (10 mcg) and Penicillin –G (10 mcg) showed no zone of inhibition. Ciprofloxacin(5mcg) had 28 ± 0.5 mm and Tetracycline (30 mcg), 25 ± 0.5 mm. The combination of the extract with Methicillin (10mcg) was 08 ± 0.5mm, *T. foenum- graecum* with Penicillin –G (10 mcg) 08 ± 0.5 mm,*T. foenum-graecum* with Ciprofloxacin (5mcg) 28 ± 0.5 mm and *T. foenum- graecum* with Tetracycline (30 mcg) 28 ± 0.5 mm (Table 5). There was no significant increase in the diameter zone of inhibition on the combination of the *T. foenum-graecium* hexane extract on all the antibiotics.

IV. DISCUSSION

The effectiveness of this plant in treating a variety of human ailments has demonstrated that it can be used therapeutically [34]. Their antibacterial activity is caused by the presence of various important organic and inorganic bioactive minerals and chemicals [37]. The plant Trigonella foenum-graecum contains a number of phytochemicals, according to the current study. Trigonella foenum-graecum's potency was assessed, and the results showed that antibiotics were more effective when used in combination with it than when used alone. The antimicrobial susceptibility test results for the plant extraction using different solvents against Methicillin-Resistant Staphylococcus aureus (MRSA) revealed different diameter zones of inhibition. The solvents used were water solvent (10.2), methanol (5.1), isopropanol (3.9), and hexane (0.1). It is crucial to choose the right polarity solvent for extracting different bioactive components from plants.

V. CONCLUSION

Trigonella foenum-graecum extract contains alkaloids, flavonoids, phenols, tannins, and saponins, all of which have antibacterial effects against methicillin-resistant Staphylococcus aureus. The variance in antibacterial impact was influenced by the extractions made using various solvents.

In comparison to using the antibiotic or plant extract alone, the combination of Trigonella foenum-graecum extract with Ciprofloxacin (5 mcg) and Tetracycline (30 mcg) significantly increased the diameter of the Methicillin-Resistant Staphylococcus aureus bacteria's zone of inhibition. This research is important for expanding the range of harmful microorganisms against which antibiotics are effective.

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