

Antimicrobial Activity of Fenugreek (*Trigonella foenum graecum*) and Marigold (*Tagetes erectus*) on Isolated Ampicillin Resistant *S. aureus*

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ABSTRACT

This study focuses on the isolation of ampicillin resistant *S.aureus* from soil collected from dumping site located in Boisar, Palghar, Maharashtra. Ampicillin resistant *S.aureus* was isolated on St. Salt mannitol agar containing 40 mg/ml of ampicillin. The MIC and MLC of the isolated culture was found to be 40 mg/ml and 50 mg/ml respectively. Characterization of isolated ampicillin resistant *S.aureus* was done by biochemical test. Antimicrobial activity of methanolic and aqueous extract of fenugreek and marigold leaves was studied on the isolated culture by agar cup and disc diffusion method respectively. Methanolic extract of fenugreek leaves, seeds and marigold leaves showed promising results.

Key words : Antibiotic resistant, Ampicillin, Marigold, Fenugreek, *S. aureus*.

Introduction

Antibiotic resistant organisms also referred as superbugs in today's world is a major threat for humans. Due to the frequent use, misuse of antibiotics in humans and animals there is the development of antibiotic resistant bacteria. These resistant bacteria when infects human and animals prevail in the body for the longer time and hence it becomes difficult to treat as compared to those infection caused by susceptible bacteria. *Staphylococcus aureus* are causative agent of bacteremia, infective endocarditis, skin infection, etc. Various antibiotics had been used to treat *S. aureus* infection in the past, however gradually the organism has developed resistance against ampicillin, methicillin, Vancomycin, etc.

Natural products have been used in traditional medicine in all over medicines which contains various compounds of known therapeutic and un-

known therapeutic uses. According to WHO, medicinal plants can be used to produce variety of drugs (Santos et al). Fenugreek (*Trigonella foenum graecum*) is one of the oldest medicinal plant from *Fabaceae* family. Fenugreek seeds has wide range of properties such as antibacterial activity, antioxidant activity, carminative characteristics, etc (Bouhenii et al., 2019). *Tagetes erectus* is a small shrub that is traditionally used in medicine for curing many bronchitis, cold, etc (Verma et al., 2012).

As the emergence of resistance bacteria is growing one can study the variety of medicinal plants and its pharmacological characteristics so as to develop an alternate therapy for treating the infection caused by the resistant bacteria.

Materials and Methods

Sample Collection

The soil sample was collected from dumping site

located in Boisar, Palghar [19°47'18.0"N 72°45'36.8"E] Maharashtra, in clean, sanitized tube and was brought in laboratory in a clean plastic bag.

Isolation and Screening of Ampicillin resistant colonies

1g of sample collected was dissolved in 10 ml of St. Saline followed by serial dilution (10 fold) and then last five dilution were spread on St. Salt mannitol agar containing different concentration of ampicillin from 10 mg/ml to 50 mg/ml by spread plate technique and incubated at 37°C for 24 hrs. After incubation, yellow colour colonies which showed growth on Salt mannitol media was chosen as ampicillin resistant bacteria.

MIC and MLC

For MIC, 1 ml of the last dilution of the soil was inoculated in St. Salt mannitol broth containing ampicillin concentration ranging from 10mg/ml to 50 mg/ml with positive and negative control. After inoculation the tubes were incubated at 37 °C for 24 hrs.

Further, the tubes after incubation which do not show visible growth was subcultured on St. Salt mannitol agar for determining the MLC.

Morphological and Biochemical identification

The isolated colonies was studied for their morphological characteristics by performing gram staining. Biochemical characteristics were studied by performing various tests like sugar fermentation, catalase test, coagulase test, IMViC test,etc.

Determination of Antimicrobial activity

Crude extract of fenugreek seeds, leaves and marigold leaves was prepared in methanol and water. The antimicrobial activity of prepared crude extract against ampicillin resistant isolate was studied by agar cup method.

For marigold leaves, disc was prepared using whatmann filter paper and antimicrobial activity was performed by disc diffusion method.

Results and Discussion

Screening and isolation of ampicillin resistant *S.aureus*

After incubation at 37 °C for 24 hrs on St. Salt mannitol agar containing ampicillin, growth was ob-

served and that colony was chosen as ampicillin resistant *S.aureus*. Colonies were yellow, smooth, opaque, small (1mm) and gram positive cocci.

MIC and MLC

After incubation, the MIC was found to be 40 mg/ml whereas MLC was found to be 50 mg/ml.

Morphological and Biochemical identification

The isolated colonies was gram stained and the results for biochemical test is as shown in below Table.

Table 1. Biochemical test result for isolated resistant colonies

Test	Results
1% glucose	+
1% lactose	+
1% mannitol	+
1% sucrose	+
Indole test	-
Methyl red test	+
Voges proskauer	+
Simmon citrate test	+
Triple sugar test	+
Urease	+
Catalase	+
Coagulase	+
Gram nature	Gram positive cocci

Antimicrobial activity of fenugreek seeds

The methanolic extract of fenugreek seeds has shown antibacterial activity on *S.aureus* (Bouhenii *et al.*, 2019; Jahan *et al.*, 2011). The antimicrobial activity of aqueous and methanolic crude extract of both was studied on ampicillin resistant colonies by agar cup and disc diffusion method on St. Mueller Hinton agar. Methanol was used as a control. The plates were incubated at 37 °C for 24 hrs after which the plates were examined for a zone of inhibition.

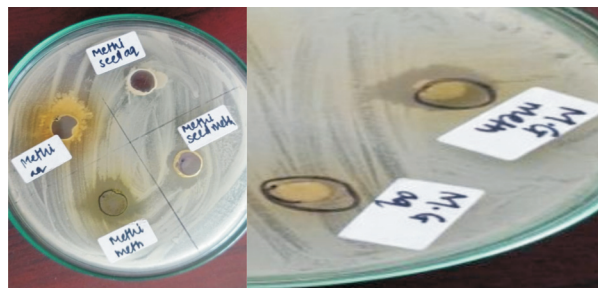


Fig. 2. Plates showing antimicrobial activity against resistant colony

Table 2. Antimicrobial activity of crude extract.

Extract	Zone of inhibition
Methanol	-
Aq. fenugreek leaf extract	-
Aq. fenugreek seed extract	-
Aq. Marigold leaf extract	-
Methanolic Fenugreek leaf extract	13 mm
Methanolic fenugreek seed extract	9 mm
Methanolic marigold leaf extract	8 mm

Conclusion

Antibiotic resistant *S.aureus* at concentration of 40 mg/ml of ampicillin was isolated from the soil sample collected. The morphological and Biochemical identification of the isolate was done. Antimicrobial activity of both plant by agar cup and disc diffusion method was studied on the isolated resistant colony. In which the methanolic extract of both plants showed promising results against resistant *S.aureus*.

Further, more exploration can be done by finding out which particular phytochemical has antibacterial activity, also many more other medicinal plants can be studied on the bacteria which has developed resistance to chemically synthesized drug.

Conflict of Interest : None

References

- Al-Timimi, L. A. N. 2019. Antibacterial and anticancer activities of fenugreek seed extract. *Asian Pacific Journal of Cancer Prevention: APJCP*. 20(12): 3771.
- Bouhenni, H., Doukani, K., Bekerođlu, N., Gezici, S. and Tabak, S. 2019. Comparative study on chemical composition and antibacterial activity of fenugreek (*Trigonella foenum graecum* L.) and cumin (*Cuminum cyminum* L.) seeds. *Ukrainian Food Journal*. 8(4): 755-767.
- Jahan, F., Lawrence, R., Kumar, V. and Junaid, M. 2011. Evaluation of antimicrobial activity of plant extracts on antibiotic susceptible and resistant *Staphylococcus aureus* strains. *J Chem Pharm Res*. 3(4): 777-89.
- Peechakara, B. V., Basit, H. and Gupta, M. 2021. Ampicillin. Stat Pearls [Internet].
- PRV Santos, ACX Olivera, TCB Tomassini. *Rev. Farm Bioquim*. 1995. 31: 35-38.
- Reddy, B.S., Kumari, K.N. and Sivajothi, S. 2016. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from dogs with recurrent pyoderma. *J Dairy Vet Anim Res*. 3(2) : 62-65. DOI: 10.15406/jdvar.2016.03.00073.
- Verma, P. and Verma, A. 2012. Evaluation of antibacterial activity of different parts of *Tagetes erecta*. *International Journal of Pharmacy and Life Sciences*. 3(6): 1766-1768.