



## Exploring the bioprospecting potential of endophytic bacteria from *Tribulus terrestris*: Antibiotic sensitivity and resistance pattern

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### Abstract

Endophytes have a symbiotic relationship with their host plant. The collaborative function of this micro flora with their host, form a relationship that causes several changes in the plant, which leads to the synthesis of numerous chemicals that are advantageous for survival of the plant. In this study, endophytes from stem tissue of *Tribulus terrestris* were isolated, screened and then subjected to identification. Standardized surface sterilizing methods were applied to obtain diverse bacterial isolates. Using disc diffusion method, 14 distinct antibiotics were used to investigate the antibiotic sensitivity pattern of bacterial endophytes found in stem. Investigating the pattern of antibiotic susceptibility, about 15 different isolated bacterial endophytes in *Tribulus terrestris* screened against 14 different antibiotics. Out of all CIP, GEN, TE, AZM, PB, GAT were found to be highly sensitive, while CFM, AMP, TR, MET were found to be highly resistant. The discovery serves as a foundation for future research on the multidrug resistance and many other properties of endophytic bacteria present in this plant.

**Keywords:** Endophytic bacteria, antibiotic susceptibility, screening, surface sterilization, antimicrobial activities

### Introduction

Many studies have focused on medicinal plants as they are highly beneficial to human wellbeing as both ancient and contemporary medications (Abdelshafy et al., 2020)<sup>[1]</sup>. The genus *Tribulus* is also an important medicinal plant that belongs to family Zygophyllaceae (Sasikala et al., 2014)<sup>[17]</sup>. Endophytes are microorganisms that live inside plant tissues without causing any harm to the plant and they reside in cells in the vascular system (Wilson, 1995<sup>[20]</sup>; Zinniel et al., 2002)<sup>[22]</sup>. Endophytes and plants develop a symbiotic relationship in which the endophytes receive nourishment and shelter from the plants (Liarzi et al., 2016)<sup>[9]</sup>, whereas host are protected from herbivores and various pathogens (Qin et al., 2017)<sup>[15]</sup>. They have been used from a long time as they contain metabolites of therapeutic values and can be utilized in treating many human diseases (Sasikala et al., 2014<sup>[17]</sup>; Daniel et al., 2018<sup>[5]</sup>; Waheed et al., 2013<sup>[19]</sup>; Péterfi & Domokos, 2018<sup>[12]</sup>; Bungtongdee et al., 2019)<sup>[3]</sup>. Important significant bioactive metabolites such as steroids, terpenoids, alkaloids, lactones, phenols and other have been discovered to be produced by endophytic micro flora (Deshmukh et al., 2015)<sup>[6]</sup>. As this micro flora is essential in the discovery of natural metabolites, they are being investigated (Caruso et al., 2020)<sup>[4]</sup>

The selection of plants for endophytic exploration is entirely based on occurrence of natural compounds from plants which is a significant research for staying ahead of evolving resistant strains of bacteria, fungi and viruses (Praptiwi et al., 2018)<sup>[14]</sup>. These microbial endophytes are important and precious source of novel compounds not only useful in formation of drugs but also served as a source for various applications in a variety of industries, including food, medicine, pharmaceuticals, and cosmetics. Clarifying the genetic processes behind antibiotic resistance in endophytic bacteria and creating focused intervention plans to slow the emergence of resistance in clinical and environmental contexts should be the main goals of future research (Sarkar et al., 2024)<sup>[16]</sup>. Therefore, current study is focused upon

screening of antibiotic sensitivity and resistance pattern of endophytic bacteria from selected plant.

### Materials and Methods

#### 1. Sample collection and sterilization

Plant samples were collected and allow for washing processed for isolation of endophytic bacteria by cutting longitudinally with a sterile scalpel into pieces of 2-3 cm long size. These parts were surface sterilized using 70% alcohol and 0.1% sodium hypochlorite, followed by double-distilled water.

#### 2. Sterility check

To confirm that the surface of stem was effectively sterilized, 1 ml of the sterile distilled water that was used in final rinse of surface sterilization procedures were plated on to nutrient agar media and incubated at 37°C for 24 hrs. Bacterial growths were observed after 24 hrs.

#### 3. Isolation of endophytic bacteria

Nutrient agar medium was prepared and poured to different labelled petriplates for purification of endophytic bacteria. In order to isolate endophytic bacteria, the tissues were transfer to nutrient agar medium. Endophytic bacteria were isolated from *Tribulus terrestris* shoots and were cut longitudinally into 2-3 cm long pieces then surface sterilized with 90% ethanol followed by 3% sodium hypochlorite and finally rinsed with double distilled water. Such all sterilized shoots were aseptically sectioned into 1 cm sized, transferred to nutrient agar medium containing petriplates at 28 C for 2-3 days. Each plate was ensured with endophytic bacterial growth and pure colonies were obtained via regular sub culturing. All pure culture petriplates were prepared for further antibiotic sensitivity test.

**4. Antibiotic Sensitivity test**

The most frequently used disc diffusion method was used to assess endophytic bacterial strains for antibiotic susceptibility (Bauer, 1966) [2]. Using antibiotic impregnated discs containing Ciprofloxacin, Mithicillin, Vanomycin, Ceftriaxone, Cefixime, Gentamicin, Tetracycline, Azithromycin, Polymixin B, Streptomycin, Trimethoprim, Gatifloxacin and Nalidixic acid, an antibiotic sensitivity test was conducted. For every isolate, bacterial lawn culture was carried out on 14 different agar plates that had already been prepared. After positioning the antibiotic discs over the inoculum, they were incubated for 48 hours at 37°C. After incubation, zone of inhibition around each disc was calculated in diameter and organisms were categorized as resistant and sensitive based on measurement.

**Result**

Approx. fifteen different *Tribulus terrestris* bacterial endophytes that are TS1 to TS15 were screened against fourteen antibiotics like CIP, MET, VA, CTR, CFM, GEN, AMP, TE, AZM, PB, S, TR, GAT and NA. Out of different 14 antibiotics, all endophytic strains exhibit that they are resistant to CFM followed MET, TR, AMP, CTR, VA and S, while sensitive to the CIP, GEN, TE, AZM, PB followed by GAT, NA.(Table 1). Findings evaluated on the basis of Zone of inhibition (ZOI), indicates that strain TS2 and TS5 have highest ZOI (30 mm) against CIP and CTR, lowest ZOI (1 mm) reported in TS11 and TS15 plate against MET and CFM respectively.

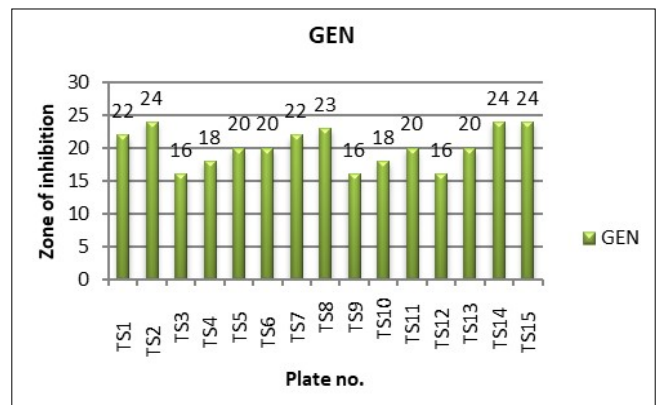
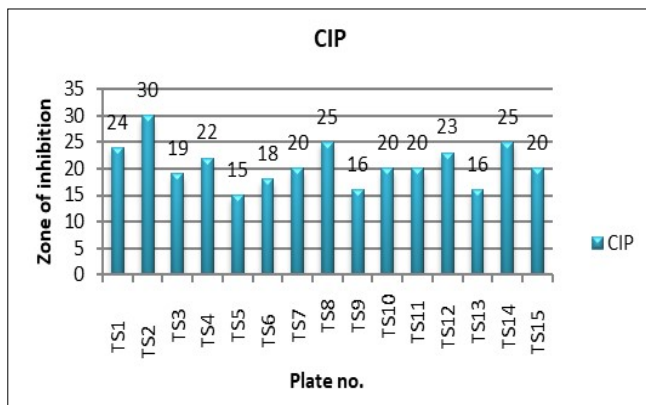
**Table 1:** Antibiotic sensitivity of the Bacterial endophytes from stem of genus *Tribulus terrestris*

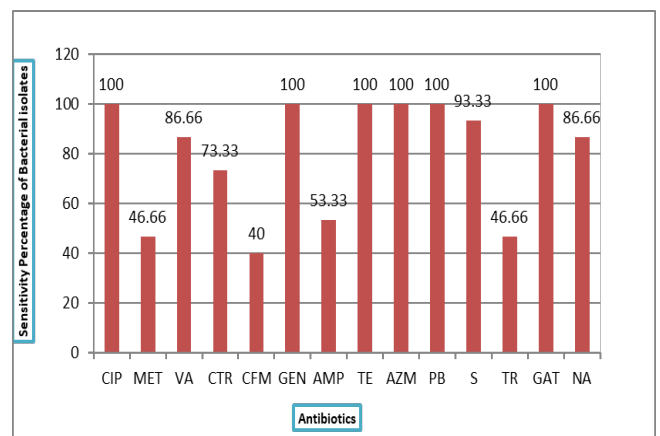
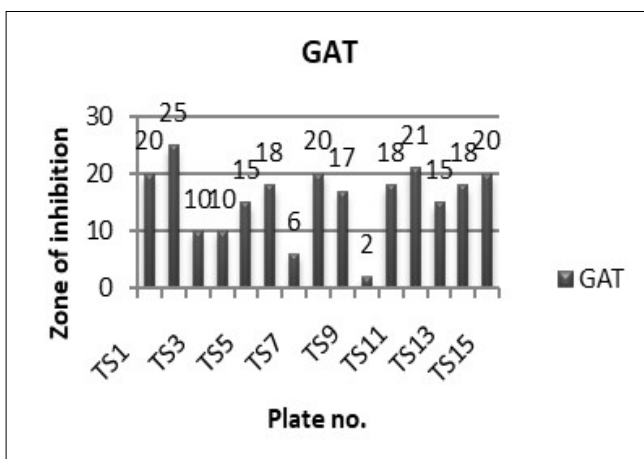
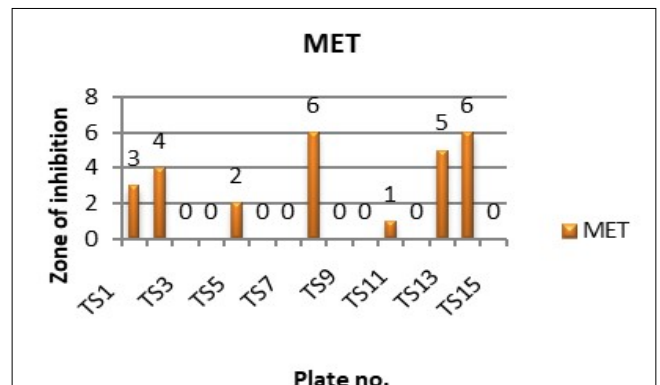
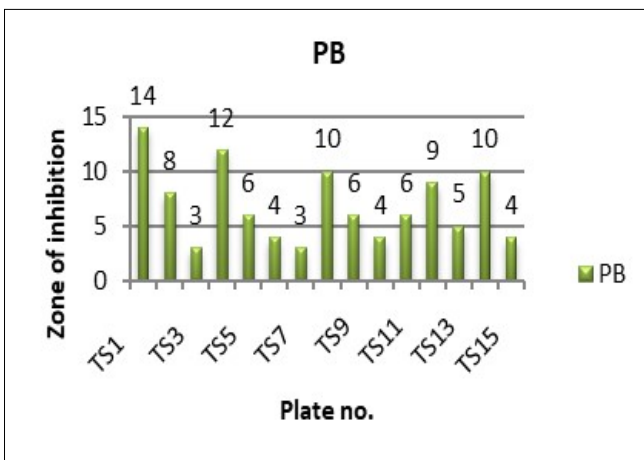
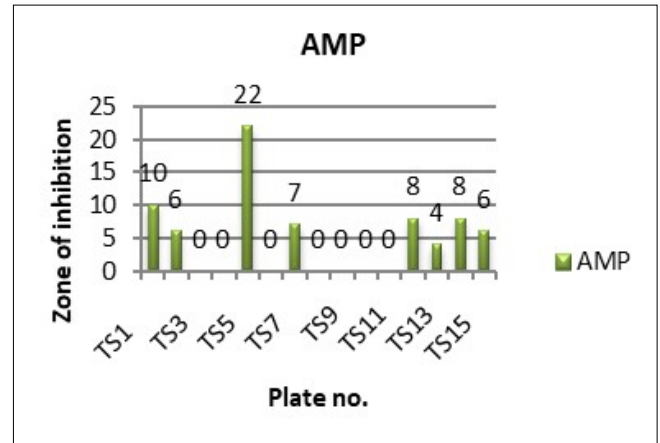
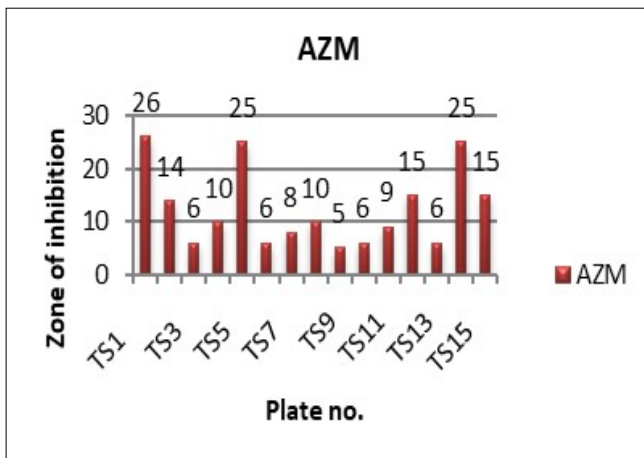
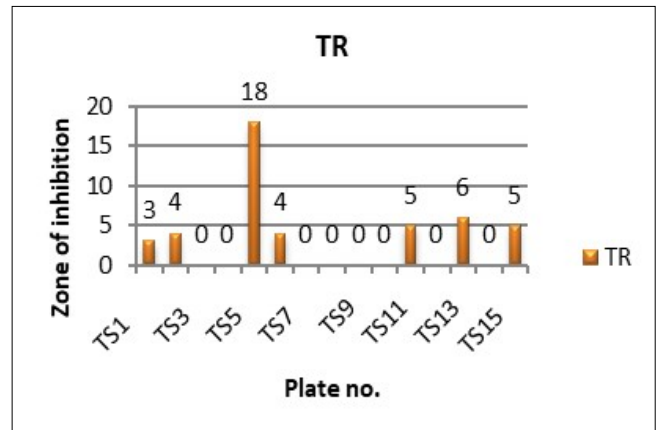
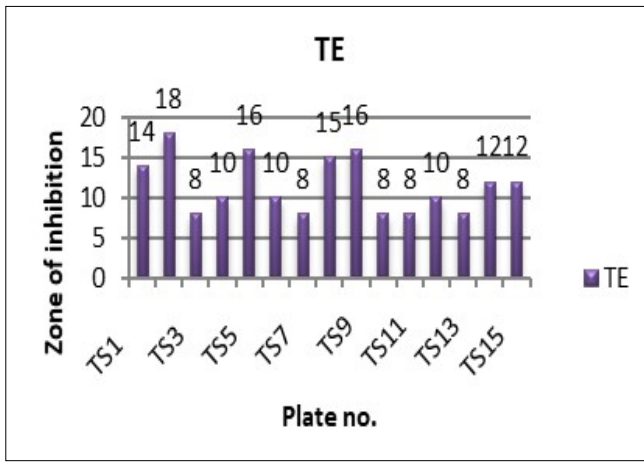
Plate No	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	CIP	MET	VA	CTR	CFM	GEN	AMP	TE	AZM	PB	S	TR	GAT	NA
TS1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TS2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TS3	+	-	+	+	-	+	-	+	+	+	+	-	+	+
TS4	+	-	-	-	-	+	-	+	+	+	+	-	+	+
TS5	+	+	+	+	+	+	+	+	+	+	+	+	+	-
TS6	+	-	+	+	-	+	-	+	+	+	+	+	+	+
TS7	+	-	-	-	-	+	+	+	+	+	+	-	+	-
TS8	+	+	+	+	-	+	-	+	+	+	+	-	+	+
TS9	+	-	+	+	-	+	-	+	+	+	+	-	+	+
TS10	+	-	+	+	-	+	-	+	+	+	+	-	+	+
TS11	+	+	+	-	-	+	-	+	+	+	+	+	+	+
TS12	+	-	+	+	+	+	+	+	+	+	+	-	+	+
TS13	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TS14	+	+	+	-	-	+	+	+	+	+	+	-	+	+
TS15	+	-	+	+	+	+	+	+	+	+	+	+	+	+

Plus, sign (+) means Sensitive; Zone of inhibition more and minus sign (-) means Resistant; Zone of inhibition less. The Abbreviations: CIP-Ciprofloxacin, MET- Mithicillin, VA- Vanomycin, CTR- Ceftriaxone, CFM- Cefixime, GEN- Gentamicin, AMP- Ampicillin, TE- Tetracycline, AZM- Azithromycin, PB- Polymixin B, S- Streptomycin, TR- Trimethoprim, GAT- Gatifloxacin, NA- Nalidixic acid

**Table 2:** Zone diameter (in mm.) of Antibiotic sensitivity of the Bacterial endophytes from stem of genus *Tribulus terrestris*

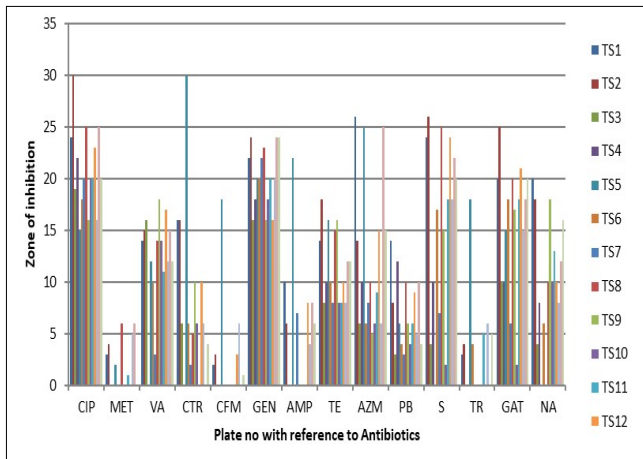
Plate No.	CIP	MET	VA	CTR	CFM	GEN	AMP	TE	AZM	PB	S	TR	GAT	NA
TS1	24	3	14	16	2	22	10	14	26	14	24	3	20	20
TS2	30	4	15	16	3	24	6	18	14	8	26	4	25	18
TS3	19	-	16	6	-	16	-	8	6	3	4	-	10	4
TS4	22	-	-	-	-	18	-	10	10	12	10	-	10	8
TS5	15	2	12	30	18	20	22	16	25	6	-	18	15	-
TS6	18	-	10	6	-	20	-	10	6	4	17	4	18	6
TS7	20	-	3	2	-	22	7	8	8	3	7	-	6	-
TS8	25	6	14	5	-	23	-	15	10	10	25	-	20	10
TS9	16	-	18	10	-	16	-	16	5	6	15	-	17	18
TS10	20	-	14	6	-	18	-	8	6	4	2	-	2	10
TS11	20	1	11	-	-	20	-	8	9	6	18	5	18	13
TS12	23	-	17	10	3	16	8	10	15	9	24	-	21	10
TS13	16	5	12	6	6	20	4	8	6	5	18	6	15	8
TS14	25	6	15	-	-	24	8	12	25	10	22	-	18	12
TS15	20	-	12	4	1	24	6	12	15	4	20	5	20	16





**Graph 1:** Antibiotic sensitivity or Resistance pattern of the all 15 bacterial endophytes from stem of genus *Tribulus terrestris*

**Graph 2:** Graph showing Sensitivity Percentage of different bacterial isolates for selected antibiotics



**Graph 3:** Antibiotic sensitivity pattern of bacterial endophytes from stem of genus *Tribulus terrestris*

### discussion

In present study, endophytic bacteria (EB) isolated from the plant *Tribulus terrestris* were analysed for antibiotic resistance profile. The antibiotic resistance of EB may vary from species to species and it allows them to survive in diverse environments, ensuring continuous protection (Bauer *et al.*, 1966<sup>[2]</sup>; Nair and Padmavathy, 2014<sup>[11]</sup>; Praptiwi *et al.*, 2020<sup>[13]</sup>). According to an evaluation of the antibiotic susceptibility profile of the chosen endophytic bacteria in different sections of *Cordia dichotoma*, the isolates were resistant to Rifampicin, Amoxicillin, and Bacitracin but primarily sensitive to Erythromycin and Streptomycin (Sharma and Mallubhotla, 2022<sup>[18]</sup>). Results are supported by previous research upon 20 EB isolated from Grapewine and 5 EB from *Biophytum sensitivum* exhibits resistance against ampicillin and oxacillin (Holkar *et al.*, 2024<sup>[8]</sup> and George *et al.*, 2021<sup>[7]</sup>). Recently Sarkar *et al.*, 2024<sup>[16]</sup>, isolated 113 endophytic bacteria from five distinct medicinal plants like Thankuni, Neem, Aparajita, Joba, and Snake plant and screened antibiotic test against 14 antibiotics, concluded not only significant prevalence of multidrug-resistant (MDR) bacteria but also analyzing the development of antibiotic resistance in plant-microbe ecosystems. Antibiogram investigations in bacterium *Marinobacter* have revealed that the strain showed considerable resistance against broad-spectrum antibiotics such as ceftazidime, cefotaxime, colistin and neomycin. This shows strain's ability for multidrug resistance, which plays significantly of its prospective medical application (Yaradoddi *et al.*, 2024<sup>[21]</sup>).

### Conclusion

According to present investigations, all of the isolates demonstrated antibiotic properties against the different antibiotics which reflect that bacterial endophytes could be used as a novel source of antibiotics. Present investigation intends to examine the endophytic bacterial communities in stem of *Tribulus terrestris* and analyse their antibiotic sensitivity pattern which play a significant role in plant defence. This article's goal is to raise awareness of the present state of research on the link between *Tribulus terrestris* and mutualistic bacterial endophytes.

### Acknowledgement

The authors are grateful to head, department of Botany, University of Rajasthan, Jaipur for providing facilities to conduct research work.

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