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## Research Article

# Antimicrobial Activities of *Tribulus terrestris* L. on Selected Pathogenic Microorganisms

<sup>1</sup>T.Sasikala, <sup>2,\*</sup>R. Prabakaran and <sup>1</sup>S. Sabitha

<sup>1</sup>Department of Botany, Government Arts College, (Autonomous) Salem -7, India.

<sup>2,\*</sup>Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-641018, India.

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## Abstract

The antimicrobial effect of aqueous, acetone and chloroform extracts of leaf, stem and root of *Tribulus terrestris* on three bacteria namely *Escherichia coli*, *Lacto bacillus* and *Bacillus cereus* were determined using agar well diffusion and paper disk methods. In *Escherichia coli* the widest zone of inhibition (33.6±1.05mm) was demonstrated by the acetone leaf extract (80 µl). In *Bacillus cereus* the widest zone of inhibition (23.6±0.88mm) was demonstrated by the acetone leaf extract (80 µl) while the value dropped to 22.5±0.12 and 20.4±0.02 mm for chloroform stem and chloroform root extracts respectively when tested against the same organism. In *Lacto bacillus* the widest zone of inhibition (31.3±1.20mm) was demonstrated by the acetone leaf extract (80 µl) followed by 29.3±0.94mm and 28.5±1.33 mm for chloroform leaf and acetone root extracts respectively. There were no inhibitions in chloroform root extracts. This study revealed that the *Tribulus terrestris* demonstrated strong inhibitory effect on the test organisms than *T. terrestris*. The results therefore established a good support for the use of *T. terrestris* in traditional medicine

## 1. INTRODUCTION

India represented by rich culture, traditions and natural biodiversity, offers a unique opportunity for drug discovery researches<sup>1</sup>. Antimicrobials of plant origin have enormous therapeutic potential<sup>2</sup>. Plants are the main medicinal source to treat infectious diseases. Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value<sup>3</sup> plant extracts represent a continuous effort to find new compounds with the potential to act against multi resistant bacteria approximately 20% of the plants found in the world have been submitted to biological test and a substantial number of new antibodies introduced on the market are obtained from natural or semi synthetic resources<sup>4</sup>. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hyper sensitivity, immune suppression and allergic reaction<sup>5</sup>. They are effective in the treatment of infectious disease while simultaneously mitigating many of the side effect that are often associated with synthetic antimicrobial<sup>6</sup>.

The genus *Tribulus* (Zygophyllaceae) contains 25 species and many are regarded as noxious weeds. Because of the spiny nature of the fruits, they are a hazard to grazing animals. Also, sheep staggers is reported to be the result of beta-carboline alkaloids contained in the fruit<sup>7</sup>. *Tribulus terrestris* L. is widely distributed in Africa, Southern Europe, China, Japan, Korea and western parts of Asia<sup>8-9</sup>. *T. terrestris* grows well in light textured soils however it grows over a wide range of soil types. Generally, it can be found in cultivated crops, overgraze pastures, roadsides, lawns and neglected areas. *T. terrestris* commonly known as puncture vine or cathead because of the sharp spurs protruding from the fruit capsule, is a small prostrate annual herb bearing yellow flowers on

branches to 80cms<sup>10</sup>. The leaves of *Tribulus terrestris* are used traditionally in folklore for the treatment of various kinds of wounds. *T. terrestris* is found to be rich source of calcium<sup>11</sup>. Extracts from *T. terrestris* have immunostimulatory and antimicrobial effect<sup>12</sup>. According to the traditional Chinese medicine the plant is a strong aphrodisiac and may stimulate men sexual behavior, strengthen the heart muscle and reduce the level of Cholesterol<sup>13</sup>. Plant fruits are applied to treat eye diseases, abdominal diseases and vitiligo<sup>14</sup>.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant materials

The fresh Plants of *Tribulus terrestris* were collected from Mettur, Salem District Tamilnadu and being identified by Dr. Karmegam, Department of Botany, Government Arts College Salem. Voucher specimen were deposited in Department of Botany, Government Arts College Salem.

### 2.2 Preparation of extracts for antimicrobial activity

#### 2.2.1 Aqueous extract preparation

The *T. terrestris* leaves stem and roots were dried, powdered and 10gm was macerated in water (50 ml) at 70°C for 120 min. This process was repeated twice. The extract obtained was filtered and lyophilized. To perform the assays the extracts were solubilised in distilled water and sterilized by filtration through a 0.2µm membrane filter (Microclar).

#### 2.2.2 Preparation of different solvent extracts

The *T. terrestris* leaves stem and roots were carefully washed with tap water, rinsed with distilled water, and air dried for one hour. Then it was cut into small pieces, dried in room temperature for two weeks, grounded into powder with the help of hand mill and stored in room temperature. The powder was macerated solvents including acetone and chloroform in a 1:3 proportion at room temperature, undergoing mechanical shaking for 4 hours followed by filtration. The extracts obtained were concentrated in a rotary evaporator at 40°C and the residue was extracted twice again analogously, thereby obtaining the crude solvent extracts. The concentrated extracts were weighed and preserved for further use

### \*Corresponding Author:

R. Prabakaran

Plant Tissue Culture Division, PG and Research Department of Botany,  
Government Arts College (Autonomous),  
Coimbatore-641018, India.

Email: [raju.prabakaran@gmail.com](mailto:raju.prabakaran@gmail.com)

### 2.3 Test Microorganisms

The microorganisms used in this study include *Escherichia coli*, *Lacto bacillus* and *Bacillus cereus* obtained from the Department of Microbiology, Nandha Arts and Science College Erode. The bacterial strains were cultured on respective selective media and stored at 20±2°C.

### 2.4 Preparation of inoculum

Exactly 18 hour broth culture of the test bacteria isolates was suspended into sterile nutrient broth and were standardized according to National Committee for Clinical Laboratory Standards (NCCLS) by gradually adding normal saline to compare their turbidity to McFarland standard of 0.5 which is approximately  $1.0 \times 10^6$  CFU/ml.

### 2.5 Antimicrobial assay – Well diffusion method

The modified agar well diffusion method was employed to determine the antibacterial activities. About 0.2 ml of the standardized 24 hour old broth culture of the test organisms were spread onto sterile Muller Hinton Agar plates. These were then allowed to set. With the aid of a sterile cork borer, wells of about 6 mm in diameter were bored on the plates. Different concentrations (40µl, 60µl and 80µl) of aqueous, acetone and chloroform extracts were dispensed into the wells and then allowed to stand for about 15 minutes for pre diffusion of the extracts to occur. The plates were then incubated at 37°C for 24 hours. At the end of the incubation period, inhibition zones formed on the agar plates were observed, measured and tabulated for various bacterial strains used.

## 3. RESULT AND DISCUSSION

The results obtained showed that the leaf, stem and root of *T. terrestris* have bactericidal effects on pathogenic microorganisms. Table 1 showed that acetone was the best solvent for extracting antimicrobial substances from this plant compared to chloroform and water. In *Escherichia coli* the widest zone of inhibition (33.6±1.05mm) was demonstrated by the acetone leaf extract (80 µl, Plate 1 J) while the value dropped to 31.6±1.20mm and 30.8±1.10 mm for acetone stem and acetone root extracts respectively when tested against the same organism (Table 1, Plate 2 M, O) followed aqueous leaf extract by 80 µg/ml concentration (28.18±0.35mm, Plate 1 A). The result agrees<sup>16-17</sup>. Antimicrobial activities of *T. terrestris* are reported to vary with the origin of the plant and the part of the plant used. The ethanolic extracts of *T. terrestris* did not show antibacterial activity against bacteria tested whereas the methanolic/ethanolic extracts of different parts (fruits, roots and stems with leaves) of Iranian, Indian

or Turkish *T. terrestris* inhibited the growth of different microorganisms tested

In *Bacillus cereus* the widest zone of inhibition (23.6±0.88mm, Plate 1 K) was demonstrated by the acetone leaf extract (80 µl) while the value dropped to 22.5±0.12 and 20.4±0.02 mm for chloroform stem and chloroform root (Plate 1 V) extracts respectively when tested against the same organism (Table 1). However, the aqueous leaf extract was also much effective against *B. cereus* followed aqueous root extract by 80 µg/ml concentration (19.20±0.29mm and 18.8±0.79mm Plate 1B and H). According to<sup>18</sup>, antimicrobial activity of organic and aqueous extracts from fruits, leaves and roots of *T. terrestris* from Iraq was examined against 11 species of pathogenic and non-pathogenic microorganisms. All the extracts from the different parts of the plant showed antimicrobial activity against most tested microorganisms. The most active extract was ethanol extract from the fruits with a minimal inhibitory concentration of 0.15 mg/ml against different bacteria and fungi.

In *Lacto bacillus* the widest zone of inhibition (31.3±1.20mm) was demonstrated by the acetone leaf extract (Plate 1L) (80 µl) followed by 29.3±0.94mm and 28.5±1.33 mm for chloroform leaf (Plate 2 T) and acetone root extracts (Plate 2 Q) respectively when tested against the same organism (Table 1). However, the aqueous root extract was also much effective (27.8±1.19) against *Lacto bacillus* (Plate 1I) followed aqueous stem and acetone stem extract by 60 µg/ml concentration (26.0±0.93mm and 25.6±1.05mm Plate 1 F). Similar reports<sup>19</sup>, among the seven different saponins tested from *T. terrestris*, only the spirostanol saponins showed antifungal activity against *C. albicans* and *Cryptococcus neoformans*, while none of the furostanol derivatives exhibited inhibitory activity. Further, these compounds were not effective against *S. aureus*, *Aspergillus fumigatus*, *P. aeruginosa* and *Mycobacterium intracellulare*.

*T. terrestris* is of medicinal and pharmaceutical interest as it contains a number of secondary metabolites like steroidal saponins which may responsible for its use in muscle building, conditioning and treatment of certain ailments<sup>20-21</sup>. Similar reports that the *T. terrestris* might also active against oxidative stress and exhibits antitumor, cytotoxic, antifungal and anthelmintic properties<sup>22</sup>. The chloroform extracts of *T. terrestris* did not show much antibacterial activity against bacteria tested. Agar well diffusion method allows better diffusion of the extracts into the medium thus enhancing contact with the pathogenic organisms in plate. But in paper discs methods may act as a barrier between the extract and the microbes thus, preventing total diffusion of active components absorbed by the discs into the medium and may be responsible for the observed differences<sup>23</sup>.

**Table 1:** Antibacterial activity of aqueous acetone and chloroform extracts of *T. terrestris*.

S. No	Plant extract	Inhibition zone of in diameter (mm)								
		<i>Escherichia coli</i>			<i>Bacillus cereus</i>			<i>Lacto bacillus</i>		
		40µl	60µl	80µl	40µl	60µl	80µl	40µl	60µl	80µl
1	Aqueous leaf	18.30±0.37	23.65±0.25	28.18±0.35	8.98±0.24	12.21±0.17	19.20±0.29	16.26±0.28	20.63±0.23	24.33±0.44
2	Aqueous stem	8.56±0.38	13.2±0.82	23.0±0.70	7.60±0.43	11.5±0.76	14.0±0.57	10.5±0.76	22.5±0.76	26.0±0.93
3	Aqueous root	12.5±0.76	19.6±0.88	26.16±0.94	12.0±0.93	14.5±1.11	18.8±0.79	8.0±0.57	21.8±0.94	27.8±1.19
4	Acetone leaf	12.5±0.99	16.6±0.71	33.6±1.05	14.66±66	18.0±0.57	23.6±0.88	18.3±0.88	28.3±82	31.3±1.20
5	Acetone stem	18.5±0.76	23.16±1.57	31.6±1.20	8.5±0.76	10.5±1.17	11.9±0.58	19.3±1.05	23.4±1.23	25.6±1.05
6	Acetone root	17.8±1.07	22.3±1.38	30.8±1.10	10.8±0.79	15.3±0.66	18.16±0.94	8.0±0.57	10.3±1.14	28.5±1.33
7	Chloroform leaf	0.43±0.08	0.60±0.10	21.0±0.85	11.8±1.02	17.2±0.86	22.5±0.12	11.5±0.76	18±0.57	29.3±0.94
8	Chloroform stem	-	0.38±0.04	21.8±0.87	0.3.6±0.04	0.58±0.09	20.4±0.02	-	-	22.0±1.15
9	Chloroform root	-	-	22.3±1.11	-	-	18.6±0.88	-	-	19.5±0.76

PLATE – 1. Antibacterial activity of different extracts of *Tribulus terrestris*

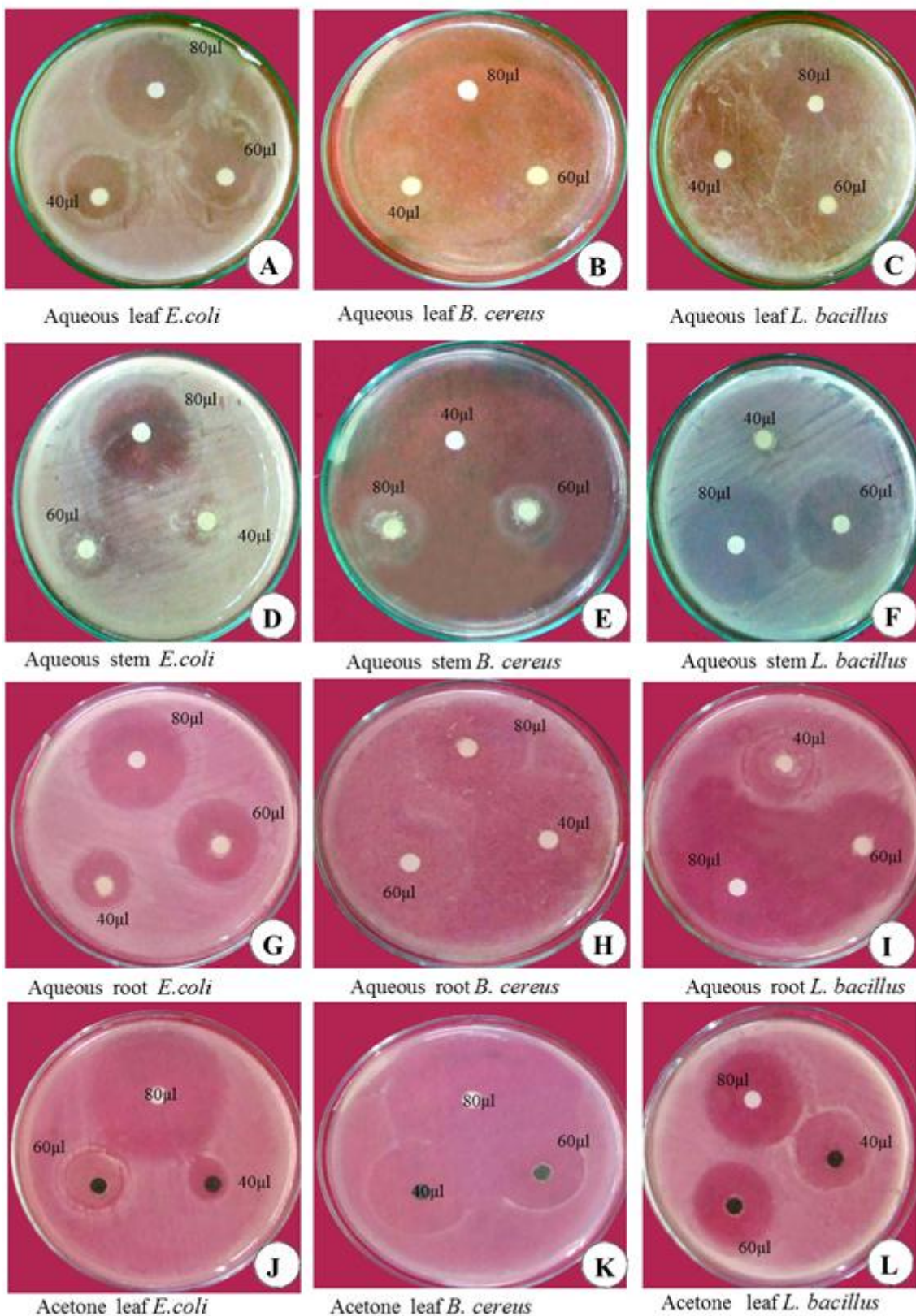
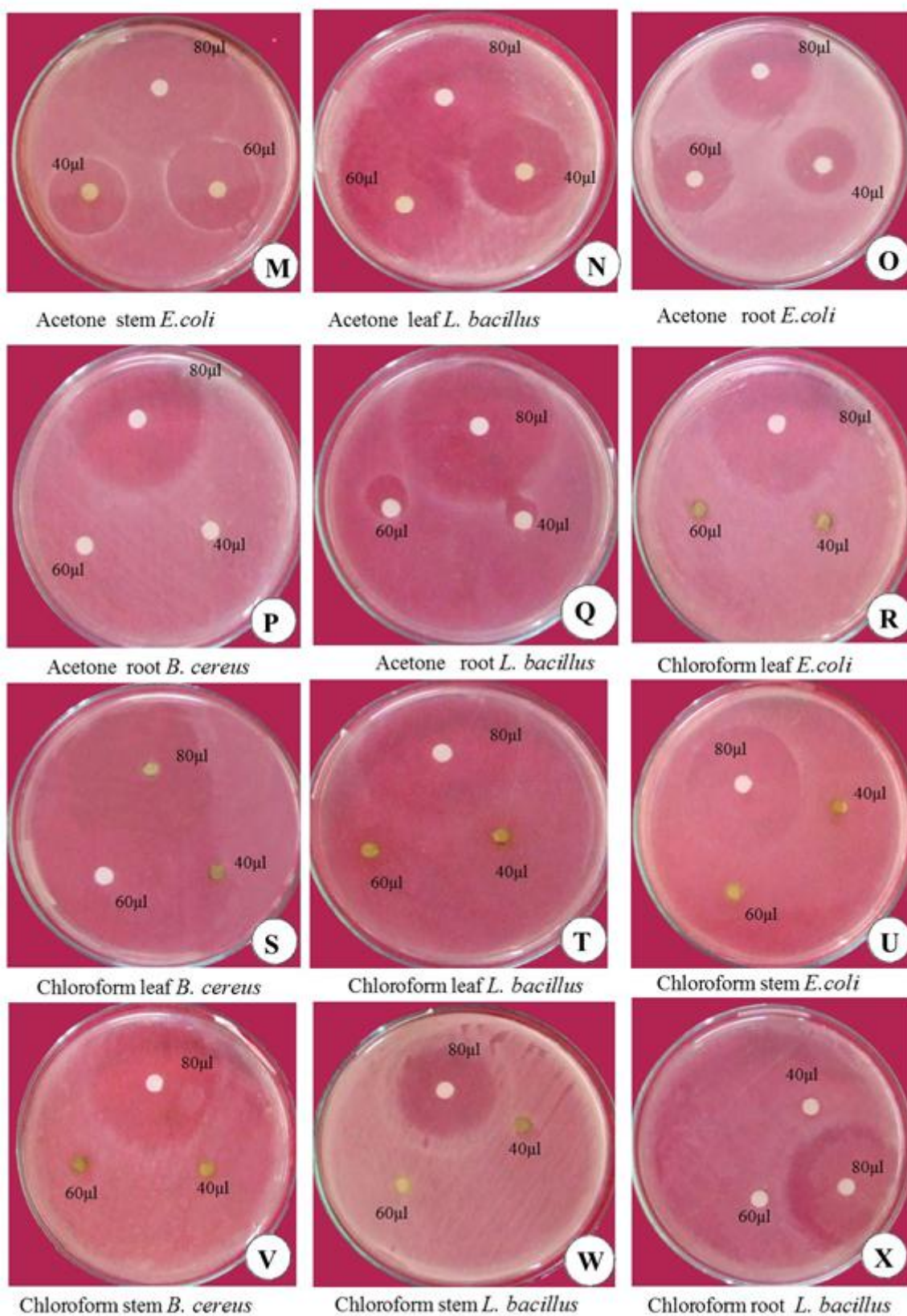




PLATE – 2 Antibacterial activity of different extracts of *Tribulus terrestris*



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