



## Homoeopathic Insights into the Antimicrobial Potential of Tribulus Terrestris for Urinary Tract Infection and Male Infertility

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### ABSTRACT:

Urinary tract infections (UTIs) pose a significant health concern, affecting millions globally and impacting male fertility. This study investigates the antimicrobial potential of *Tribulus terrestris*, a natural plant compound, in addressing UTIs and associated male infertility. UTIs, prevalent in both genders, contribute to complications such as kidney damage and reproductive organ issues. *Tribulus terrestris*, known for its traditional medicinal use, contains antimicrobial compounds like steroids and saponins. Previous studies have shown its efficacy against various microorganisms causing UTIs by inhibiting bacterial growth. Given the prevalence of UTIs in India and their strong association with male infertility, alternative efficacious treatments are crucial. This study introduces *Tribulus terrestris* in different potencies (MT, 30 CH, 200 CH, and 1M) and evaluates its antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Results demonstrate significant zones of inhibition, supporting the plant's potential as a broad-spectrum antibiotic. Considering the rising antibiotic resistance, *Tribulus terrestris* emerges as a promising homoeopathic remedy for UTIs and male fertility. In conclusion, the research underscores *Tribulus terrestris* as a valuable treatment option for UTIs and male infertility, providing an alternative to conventional antibiotics. Further exploration is needed to understand its therapeutic value and establish its role in managing and preventing UTIs, particularly in the context of chronic and recurrent genitourinary tract infections influencing male reproductive health.

### INTRODUCTION:

According to the World Health Organization, urinary tract infections affect tens of millions of people worldwide each year <sup>[1][2][3]</sup>. In India, urinary tract infections are a significant public health concern, affecting many individuals each year. A study conducted in India revealed that urinary tract infections are particularly common among the male population, with a high prevalence rate documented in various regions of the country <sup>[4]</sup>. The study found that a staggering 65% of men in India diagnosed with infertility also had urinary tract infections <sup>[5]</sup>.

In a study conducted in Tamil Nadu, India, the prevalence of urinary tract infections among infertile men was investigated. The study found that 53% of infertile men in the region had urinary tract infections <sup>[6-8]</sup>. This high prevalence indicates a strong correlation between urinary tract infections and male infertility in this population. These findings underscore the urgent need for alternative methods to prevent and treat urinary tract infections to improve male fertility outcomes <sup>[9]</sup>. Urinary tract infection is a common condition that affects both men and women. It is estimated that approximately 50% of women and 12% of men will experience at least



one urinary tract infection in their lifetime. These infections can cause various complications, including kidney damage and infertility in males. Many studies have shown that infections majorly cause male infertility. Fifteen percentage of male infertility are caused by the genitourinary tract infection<sup>[10]</sup>.

Urinary tract infections can also lead to other adverse effects, such as epididymo-orchitis<sup>[11]</sup> which causes severe pain, swelling, and potential damage to the reproductive organs further impacting fertility. Furthermore, the presence of bacteria in semen, particularly in men with urinary tract infections, has been associated with impaired sperm motility and vitality<sup>[12]</sup>. While the mechanism of infertility caused by urinary tract infections is not fully understood, it is clear that there is a correlation between these infections and male infertility<sup>[10][13]</sup>. In recent years, there has been growing interest in the potential antimicrobial properties of natural plant compounds. *Tribulus terrestris* has emerged as a notable remedy in recent times. It is also known as puncture vine or devil's thorn and is a plant that has been used in traditional medicine for centuries<sup>[14]</sup>. It is commonly used to treat a variety of conditions, including urinary tract infections. *Tribulus terrestris* contains several chemical compositions, including steroids and saponins, which have been found to exhibit antimicrobial properties. The antimicrobial potential of *Tribulus terrestris* against urinary tract infections has been the subject of several studies<sup>[15]</sup>. These studies have shown promising results, suggesting that *Tribulus terrestris* may indeed be effective in inhibiting the growth of bacteria responsible for urinary tract infections. One study conducted by researchers aimed to explore the potential antimicrobial activity of *Tribulus terrestris* against urinary tract infections<sup>[16]</sup>.

The researchers conducted in vitro experiments using extracts of *Tribulus terrestris* on isolated microorganisms from the genital tract of infertile cows. The microorganisms tested included *Escherichia coli*, *Pseudomonas aeruginosa*, *Citrobacter braakii*, *Providencia rettgeri*. The results of the study showed that the extracts of *Tribulus terrestris* at different concentrations were able to inhibit the growth of these microorganisms<sup>[16]</sup>. The antimicrobial activity of *Tribulus terrestris* against both Gram-positive and Gram-negative bacteria suggests its potential as a broad-spectrum antibiotic. In addition to its antimicrobial

properties, *Tribulus terrestris* has also been found to possess other pharmacological activities that may be beneficial in the treatment of urinary tract infections and male infertility. One of the pharmacological activities that *Tribulus terrestris* possesses is its antioxidant property. Antioxidants are known to help neutralize harmful free radicals in the body, which can contribute to the development of infections and infertility. Moreover, *Tribulus terrestris* has been reported to have anti-inflammatory effects. These anti-inflammatory effects can help reduce inflammation in the urinary tract, which is often associated with urinary tract infections<sup>[17]</sup>. Another pharmacological activity of *Tribulus terrestris* is its ability to promote urine flow and mitigate urinary tract membrane irritation<sup>[18]</sup>. This can be beneficial in the treatment of urinary tract infections, as it helps to flush out bacteria and relieve irritation in the urinary tract. Furthermore, *Tribulus terrestris* has been shown to have immunomodulatory effects<sup>[7]</sup>. These effects can enhance the immune response of the body, making it more effective in fighting off infections, including urinary tract infections.

UTIs are caused by several microorganisms like *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella* etc., amongst which *Escherichia coli* is the common cause of UTIs. Several studies have demonstrated the ability of *Tribulus terrestris* to inhibit the growth of bacteria including *Escherichia coli* and *Staphylococcus aureus*<sup>[15][16]</sup>. The antimicrobial potential of *Tribulus terrestris*, combined with its low toxicity and minimal side effects, make it a promising candidate for the prevention and treatment of urinary tract infections in men. By effectively eradicating bacterial infections in the urinary tract, by reducing the impact of urinary tract infections on reproductive health *Tribulus terrestris* may help in improving male fertility, especially sperm parameters<sup>[14][19][20][21]</sup>. An East Indian drug *Tribulus terrestris* useful in urinary affections especially dysuria and in debilitated states of the sexual organs as expressed in seminal weakness, ready emissions, and impoverished semen<sup>[22]</sup>. Also, this medicine has great utilization in case of partial impotence caused by overindulgence of advancing age, or when accompanied by urinary symptoms, incontinence, painful micturition, etc<sup>[22]</sup>. Genitourinary tract infections act as a nidus of infection for the seminal tract leading to chronicity due to the reservoir of



infection<sup>[14]</sup> [19][20][21]. The research papers so far reviewed, show the effect of antimicrobial activity of crude *Tribulus terrestris* extract only. This study further aims to evaluate the potential of antimicrobial activity of *Tribulus terrestris* in various potencies (MT, 30 CH, 200 CH and 1M) against *Escherichia coli* and *Staphylococcus aureus*. The antimicrobial potential of *Tribulus terrestris* against urinary tract infections provides a promising alternative method for the prevention and treatment of these infections, which can ultimately help improve male fertility outcomes<sup>[14][19][20][21]</sup>.

## MATERIALS AND METHODS:

### Medicines:

As per Homoeopathic pharmacopoeia of India, standardized homoeopathic Medicines *Tribulus terrestris* in different potencies like MT, 30 CH, 200 CH and 1M were procured from GMP certified Drug Manufacturing unit of Dr. Willmar Schwabe, India.

### Organisms:

The test microorganisms used for antibacterial analysis, bacteria *Escherichia coli* and *Staphylococcus aureus*, were purchased from Microbial Type Culture Collection and Gene Bank (*MTCC*) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA).

### Nutrient Broth Preparation

Pure culture from the plate was inoculated into a Nutrient Agar plate and sub-cultured at 37°C for 24 h. Inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/L saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of  $1.5 \times 10^8$  cfu/ml. Standardized inoculum is used for antimicrobial tests.

### Antibacterial Test:

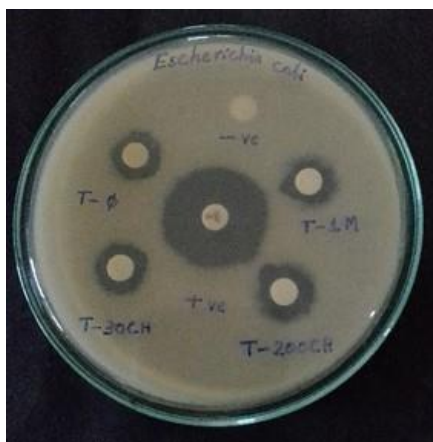
Antibiotic susceptibility tests were determined by the agar disc diffusion (Kirby-Bauer)<sup>[23]</sup> method. The medium was prepared by dissolving 38 g of Mueller-Hinton Agar Medium (Hi-Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121°C for 15 min (pH 7.3). The autoclaved medium was cooled and poured into Petri plates (25 ml/plate) the plates were swabbed with pathogenic bacterial cultures *Escherichia coli* and *Staphylococcus aureus*. Finally, The Sample or Sample loaded disc was then placed on the surface of the Mueller-Hinton Agar medium. The standard drug Ciprofloxacin 5 mcg and Cefazolin 30 mcg concentration disc was used as positive control depending on the sensitivity of bacterial culture and an empty sterile disc was used as negative control. The plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with a transparent ruler in millimeters. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity<sup>[24-26]</sup>.

## RESULTS:

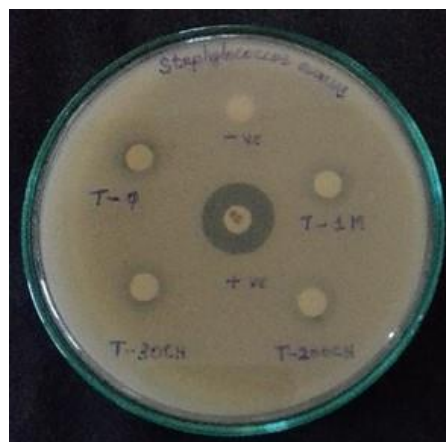
The present study revealed that *Tribulus terrestris*, in different potencies, developed a marked zone of inhibition against *Escherichia coli* and *Staphylococcus aureus*. Among these, *Tribulus terrestris* 30 CH and 200 CH exhibited a 13 mm zone of inhibition, whereas *Tribulus terrestris* MT and 1M exhibited a 12 mm zone of inhibition, respectively, against *Escherichia coli* (Table 1 & Figure 1). *Tribulus terrestris* 1M exhibited an 11 mm zone of inhibition against *Staphylococcus aureus*, while *Tribulus terrestris* 30 CH and 200 CH exhibited a 10 mm zone, and *Tribulus terrestris* MT exhibited a 9 mm zone of inhibition, respectively, against *Staphylococcus aureus* (Table 1 & Figure 2).

**Table 1: Zone of inhibition *Tribulus terrestris* in various potencies**

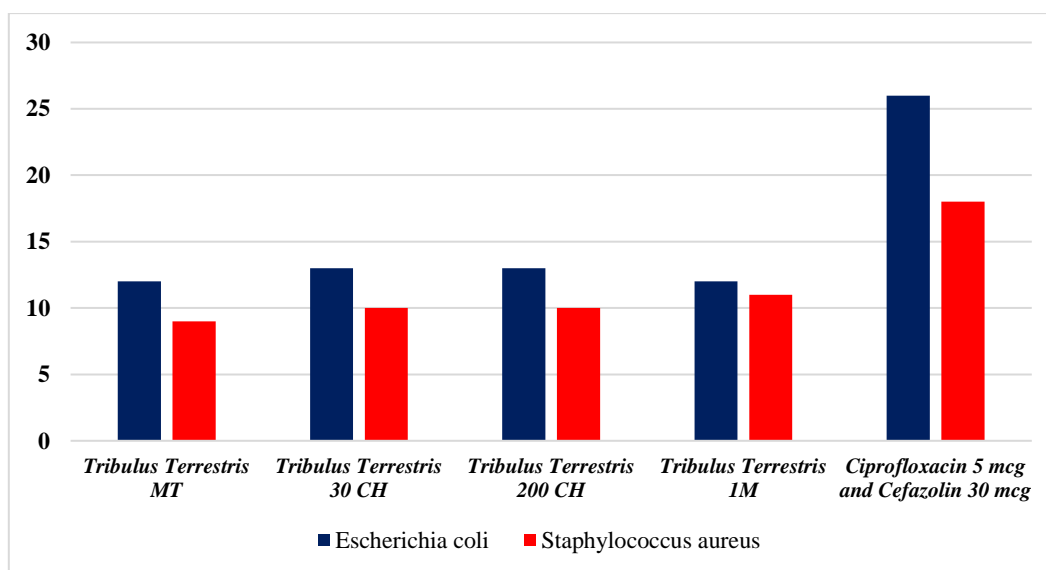
Bacteria	T-MT	T-30CH	T-200CH	T-1M	+ve control	-ve control
<i>Escherichia coli</i>	12 mm	13 mm	13 mm	12 mm	26 mm	No zone
<i>Staphylococcus aureus</i>	9 mm	10 mm	10 mm	11 mm	18 mm	No zone



**Figure 1: Zone of Inhibition of Tribulus terrestris in different potencies against *Escherichia coli***



**Figure 2: Zone of Inhibition of Tribulus terrestris in different potencies against *Staphylococcus aureus***



**Figure 3: Comparison of Tribulus terrestris in different potencies based on the zone of inhibition against *Escherichia coli* and *Staphylococcus aureus***

## DISCUSSION:

The antimicrobial potential of *Tribulus terrestris* against urinary tract infections was evaluated in this study. The results demonstrated that different potencies of *Tribulus terrestris*, including MT, 30 CH, 200 CH and 1M were able to inhibit the growth of isolated microorganisms, including *Escherichia coli* and *Staphylococcus aureus* commonly found in the genital tract<sup>[16]</sup>.

Various studies have shown that *Tribulus terrestris* exhibits significant antimicrobial activity against both *Escherichia coli* and *Staphylococcus aureus*, two common bacterial pathogens that are responsible for a

range of infections in humans<sup>[16]</sup>. For instance, the fruit extract of *Tribulus terrestris* synthesized silver nanoparticles exhibited excellent antibacterial activity against multi-drug resistant human pathogens, including *Escherichia coli* and *Staphylococcus aureus*<sup>[27]</sup>. Furthermore, the results showed that the maximum antibacterial activity was observed against *E. coli*. This is in line with a separate study which reported the antimicrobial activity of methanolic extracts of *Tribulus terrestris* against *Escherichia coli* and *Staphylococcus aureus*<sup>[15]</sup>. The broad spectrum antibacterial activity of *Tribulus terrestris* against both Gram-positive and



Gram-negative bacteria is significant and suggests that it may contain compounds with broad spectrum antibiotic properties<sup>[16]</sup>. The presence of pharmacological active metabolites, such as saponins, in *Tribulus terrestris* may contribute to its antimicrobial activity. In addition to its antibacterial properties, *Tribulus terrestris* also exhibits other biological activities that can further contribute to its effectiveness as a natural remedy.

Furthermore, the geographic sources of the *Tribulus terrestris* plant may also contribute to the variations in antibacterial activity<sup>[28]</sup>. The variations in antibacterial activity of *Tribulus terrestris* observed in different studies could be attributed to factors such as the use of different strains of bacteria, variations in the concentrations of *Tribulus terrestris* extract, and differences in the methodologies used for antimicrobial testing. For example, one study found that the methanolic extract of *Tribulus terrestris* fruits exhibited the most effective antibacterial activity against both gram-positive and gram-negative bacteria<sup>[29][30][31]</sup>. Another study found that the ethanol extract of *Tribulus terrestris* had a stronger antimicrobial effect against gram-positive bacteria compared to gram-negative bacteria<sup>[16]</sup>. These variations in antibacterial activity could be due to differences in the chemical composition of the extracts, as different solvents may extract different bioactive compounds from the plant.

However, further research is needed to fully understand the mechanisms through which *Tribulus terrestris* exhibits its antibacterial activity and to identify the specific bioactive compounds responsible for this activity<sup>[28]</sup>. Furthermore, it would also be valuable to conduct comparative studies using standardized strains of bacteria and consistent methods for antimicrobial testing in order to accurately compare the antibacterial activity of different *Tribulus terrestris* extracts.

All the homoeopathic medicines are prepared uniformly based on the standard pharmacological methods prescribed by the Homoeopathic Pharmacopoeia across the world. Hence the variations in antibacterial activity which is evident in the extract preparation is not applicable to the homoeopathic medicines, however the biological compounds remain the same<sup>[32][33]</sup>.

The findings in our study suggests that *Tribulus terrestris* may possess broad-spectrum antibiotic properties, which could be attributed to the presence of active metabolites such as flavonoids, alkaloids, and saponins in the plant

extract. The inhibition zones observed in the present study indicate that *Tribulus terrestris* has significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, one of the most common causative agents of urinary tract infections. The marked zones of inhibition observed in this study suggest that *Tribulus terrestris* has the potential to be used as an effective treatment for urinary tract infections. This is particularly important considering the increasing problem of antibiotic resistance and the need for new antimicrobial agents. The results of this study are consistent with previous research that has demonstrated the antimicrobial activity of *Tribulus terrestris*.

The mechanism by which homoeopathic medicine *Tribulus terrestris* exerts its antimicrobial activity is still unclear. However, it has been suggested that the active metabolites present in *Tribulus terrestris* may disrupt bacterial cell membranes, inhibit protein synthesis, or interfere with other essential cellular processes. In another research, it is clarified that due to the recurrence and chronicity of urinary tract infections, seminal tract infections occur, leading to changes in the motility of spermatozoa causing male infertility<sup>[14]</sup>. [19-22]. Strong evidence of the antimicrobial potential of *Tribulus Terrestris* is evident from the homoeopathic literature which states that partial impotence caused by or when accompanied by urinary symptoms, incontinence, painful micturition leading to impoverished semen causing Male Infertility<sup>[22]</sup>. Furthermore, the results of this study highlight the potential of *Tribulus terrestris* as a homoeopathic remedy for urinary tract infections that can lead to male infertility, providing a promising alternative to conventional antibiotics.

## CONCLUSION:

In conclusion, the antimicrobial potential of *Tribulus terrestris* against urinary tract infections leading to male infertility is evident. This is supported by the significant zones of inhibition observed against *Escherichia coli* and *Staphylococcus aureus*. The results suggest that *Tribulus terrestris* could be a valuable and effective treatment option for urinary tract infections, particularly in the face of increasing antibiotic resistance. Chronic and recurrent genitourinary tract infection is the major cause of male infertility which affects the sperm count, motility, morphology and vitality. Further clinical research is needed to evaluate the efficacy of *Tribulus terrestris* to



explore its potential as a therapeutic agent for the management and prevention of genitourinary tract infections and male infertility.

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#### CONFLICT OF INTEREST:

Nil

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## Author Contributions:

Dr. S. Sandhya made substantial contributions to the conception and design of the study, acquisition of data, and analysis and interpretation of the results. She played a key role in the literature review, homoeopathic case studies, and the collection of clinical data related to *Tribulus terrestris* and its potential in addressing urinary tract infections and male infertility. Dr. S. Sandhya also actively participated in drafting the manuscript, critically revising it for intellectual content, and ensuring its accuracy regarding the homoeopathic aspects of the study.

Dr. G. Senthil Kumaran played a vital role in the conception and design of the study, providing valuable guidance and expertise in the field of homoeopathy. His extensive knowledge of homoeopathic principles and practice was instrumental in shaping the research methodology and ensuring its alignment with homoeopathic principles.

Dr. Kirtida Desai served as the primary guide and mentor for this research project. Her extensive experience in the field of homoeopathy and research methodology was instrumental in shaping the research objectives and methodology. Dr. Desai provided continuous guidance throughout the research process, from the initial proposal to the final manuscript. She was actively involved in the critical analysis of the study's design, data collection methods, and interpretation of results from a homoeopathic standpoint. Dr. Desai's contributions extended to the supervision of Dr. S. Sandhya's work as a Ph.D. Scholar and Dr. G. Senthil Kumaran's contributions as a co-author.