



## Studies on antibacterial activity of *Tamarindus indica* against bacteria causing urinary tract infection.

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### Abstract

*Tamarindus indica* is well-known all over the world for its high nutritional and health-promoting properties. This is a plant that is utilized in customary medication for the treatment of cold, fever, stomach turmoil, looseness of the bowels and jaundice, urinary lot disease (UTI) and as skin more clean. The purpose of this study is to assess the scientific justification for using plants. UTI-causing pathogens were tested for the antimicrobial properties of leaf extracts. Biochemical and morphological methods were used to characterise the bacteria that were isolated from the urinary tracts of women who had been infected. The zones of inhibition were measured and the antibacterial activity of ethanolic leaf extracts against bacteria isolated from UTI-infected patients was evaluated. Urine samples from UTI patients containing three different bacteria *Escherichia coli*, *Klebsiella*, and *Staphylococcus aureus* were used to isolate them. Concentrates on antibacterial defencelessness test ampicillin showed the most significant level of MIC against *Staphylococcus aureus* and most minimal level of *Klebsiella* and *E. coli*. The leaves of *Tamarindus indica* were found to be most effective against *Staphylococcus aureus*, *Klebsiella*, *E. coli* when extracted with ethanol.

**Keywords:** *Tamarindus indica*, Antibacterial activity, Urinary Tract Infection, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC)

## Introduction

The most typical bacterial infection that affects both sexes in urinary tract infection. The primary cause of urinary tract infection, *E.coli*, is responsible for more than 85% of recurrent cystitis and at least 35 % of recurrent pyelonephritis. The urinary tract infection bacteria isolates were characterised species level by using different laboratory procedures. Traditional healers have reported using 31 different species medicinal plants for UTI. The term *Tamarind* in this context refers to *Tamarindus indica* Linn. *Tamarindus indica* is a member of the caesalpinaceae subfamily of the leguminosae family. It is a tropical green tree that can grow up to 24 metres tall and 7 metres wide. Its flowers are a light yellow colour. *Tamarindus indica* has a lot of sugar and vitamin C. Today, 70% of the world's population relies on medicinal plants, which are the primary source of therapeutic agents used to treat human diseases. In particular, the last 10 years have seen more rigorous research for natural medicines, making medicinal plants an useful source of natural materials for sustaining human health.

This plant has anti- hyperlipidemic, antidiabetic, antibacterial, anti-venomic, hepato-protective, anti- asthmatic and laxative properties. It includes a variety of bioactive compounds in the leaves. The antiemetic and liver - protective properties of leaves. Protein, lipid, fibre, fatty acids, flavonoids and vitamins like thiamine, riboflavin, niacin, ascorbic acid and beta - carotene are all abundant in these leaves. **Escalona et al** looked into the pharmacological effects and toxicity of *Tamarind* leaf extract of erythrocytes. No negative effects were discovered .when tested using the agar well diffusion method with enteric bacterial isolates like *Klebsiella* and *E.coli*. The understanding of the epidemiology and clinical significance of medication resistance among uropathogens that cause simple urinary tract infections or cystitis has made significant strides in recent years. The highest inhibition zones against isolates of both gram positive and gram negative bacteria were found in the ethanolic and aqueous forms of

*Tamarindus indica*. The goal of the current study is to determine the antibacterial activity of herbs extract against isolates of bacteria from urine samples of patients with UTI.

## Materials and Methods

### Collection of urine sample

The samples were collected from urinary tract infected patients in Government hospital from Thiruvannamalai. The urine samples were inoculated into nutrient broth and incubated at 37°C for 24 - 48 hours. Finally, observe the microbial growth.

### Isolation and identification of bacteria from UTI infected patients

The bacteria present in urine samples of UTI infected women patients were cultured in the laboratory media. The isolated bacterial culture was identified by gram staining technique and biochemical tests.

### Collection of plant materials

Leaves of *Tamarindus indica* were collected from Thiruvannamalai. Leaves of the plant were washed with running water and removed dust and rinsed with distilled water, air dried for 2 weeks at room temperature, grinded to powder and stored in a tight bag after drying at low temperature.

### Preparation of plant extracts

Air dried leaves of *Tamarindus indica* is grinded to powder and dissolved in a known amount of organic solvents (ethanol) and kept for 24 hours at room temperature with continuous shaking.

The content was filtered to obtain clear decoction. Each preparation was filtered through a Whatman's No:1 filtered paper extracts were stored in a sterile air tight container and stored at room temperature (**Saeed and Tariq, 2008**).

### Disc diffusion method

The disc diffusion method is widely used to evaluate the antimicrobial activity of plant leaves extracts. The Mueller hinton agar medium was prepared and poured into sterile Petriplate and solidified. The agar disc diffusion method, the filter paper disc is dipped into a different extract solution, air dried and then placed on the agar surface. It was then inoculated at 37 °C for 16-18 hours. Clear zones of inhibition were measured after 24 hours of incubation. The diameter of the clear zone was measured to the nearest millimetre using a transparent ruler. Results were tabulated.

### Minimum inhibitory and bactericidal concentration

The turbidity method or tube dilution method was used for determination of minimum inhibitory concentration. The extracts were taken at different concentrations of 5,10,15,20, and 25 mg/ml in test tubes containing 1 ml sterile muller hinton broth. Then, 0.1 ml of bacterial suspension were inoculated in test tubes. The control tube

was maintained. All the tubes were then incubated at 37°C for 24 hours and then examined for growth by observing turbidity.

A loopful of broth was collected from each tube of MIC, that is used for determination of MBC. The broth was inoculated into a sterile nutrient agar by streaking. The inoculated plates were incubated at 37 °C for 24 hours. After incubation the concentration at which there was no visible growth, was noted as the minimum bacterial concentration.

### Results and Discussion

The present study reveals that the urine samples were collected from UTI patients, at Government Hospital Tiruvannamalai. The sample inoculated into nutrient broth. After incubation observe the microbial growth. Based on their morphological shape, visualised by gram staining technique. The Gram negative rod and Gram positive cocci were observed. The nutrient broth culture inoculated into different selective media and observed the colony morphology. The results shown in Table:1

**Table 1:** Observation of colonies from selective media

S.no	Selective media	Colony morphology
1.	Eosin methylene blue	Green metallic sheen colonies
2.	Mannitol salt agar	Golden yellow colonies
3.	MacConkey agar	Pink colonies

*Escherichia coli*, is a gram negative, facultatively anaerobic, rod shaped, coliform bacteria (*Bhadoriya et al., 2018*), can cause serious complications like *pneumonia, urinary tract infections*. In this study the isolated culture is

further characterised by biochemical tests. Finally, *E. coli*, *Klebsiella*, and *Staphylococcus aureus* were identified from culture plates. The result were shown in Table: 2

**Table 2:** Biochemical test results

S.no	Name of the test	<i>E.coli</i>	<i>Klebsiella</i>	<i>S.aureus</i>
1.	Gram stain	–	–	+
2.	Indole test	+	–	–
3.	Methyl red test	+	–	+
4.	Voges Proskauer test	–	+	+
5.	Citrate utilisation test	–	+	+
6.	Triple sugar iron test	+	–	+
7.	Urease test	–	+	+
8.	Catalase test	+	+	+
9.	Oxidase test	–	–	–
10.	Coagulase test	–	–	+

The ability of microorganisms like *E.coli*, *Klebsiella*, and *Staphylococcus aureus* ferment glucose, sucrose, lactose, and could not ferment maltose (*Klebsiella*), fructose (*Staphylococcus aureus*). The results were shown in Table: 3. An organoleptic character in terms of morphology is

the preliminary experiment for identification and detection for the quality of the raw herbs. Looking at that morphological identification was carried out and results were reported. The morphological features of *Tamarindus indica* shown in Table :4

**Table 3:** Sugar fermentation test results

S.no	Sugars	<i>E.coli</i>	<i>Klebsiella</i>	<i>S.aureus</i>
1.	Glucose	+	+	+
2.	Fructose	+	+	–
3.	Maltose	+	–	+
4.	Sucrose	+	+	+
5.	Lactose	+	+	+

**Table 4:** Organoleptic characters of *Tamarindus indica*

S.no	Characters	<i>Tamarindus indica</i>
1.	Colour	Green
2.	Odour	Honey
3.	Taste	Sour, sweet
4.	Size	12m to 30m

The antibacterial activity of the *Tamarindus indica* leaf extract was evaluated using disc diffusion method. The solvent extracts of ethanol plants were used. The results were shown in Table :5

**Table 5:** Zone of inhibition of the bacterial culture against ethanol extract.

S.no	Organisms	Zone of inhibition in (mm)
		Ethanol
1.	<i>Escherichia coli</i>	20mm
2.	<i>Klebsiella</i>	16mm
3.	<i>Staphylococcus aureus</i>	12mm

Leaves of *Tamarindus indica* extracted for evaluation of antibacterial activity against UTI pathogens. Results showed that the crude ethanol extract there was a strong activity against *Escherichia coli* (22.5mm) followed by *Klebsiella* (19mm) and *Staphylococcus aureus* (15mm). The

ethanol extract had activity against *E.coli* (20mm) *Klebsiella* (16mm). Based on the result *Escherichia coli* and *Klebsiella* and *Staphylococcus aureus* are more sensitive to the extract.

**Table 6:** Results of Minimum inhibitory and Minimum bactericidal concentration.

S.No	Organisms	MIC ( mg/ml)	MBC ( mg/ml)
1.	<i>Escherichia coli</i>	15	10
2.	<i>Klebsiella</i>	10	5
3.	<i>Staphylococcus aureus</i>	20	15

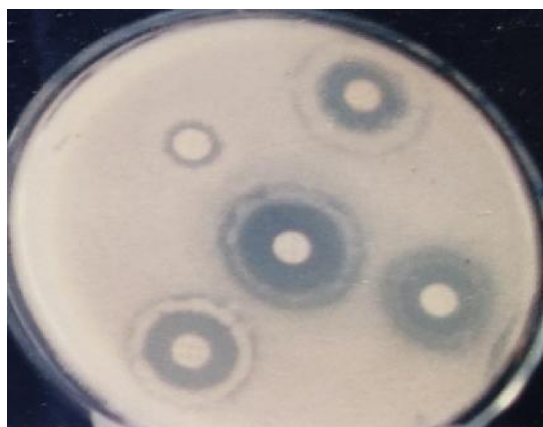


Fig: 1 This figure shows the antibacterial activity of the *Tamarindus indica* against *Escherichia coli*

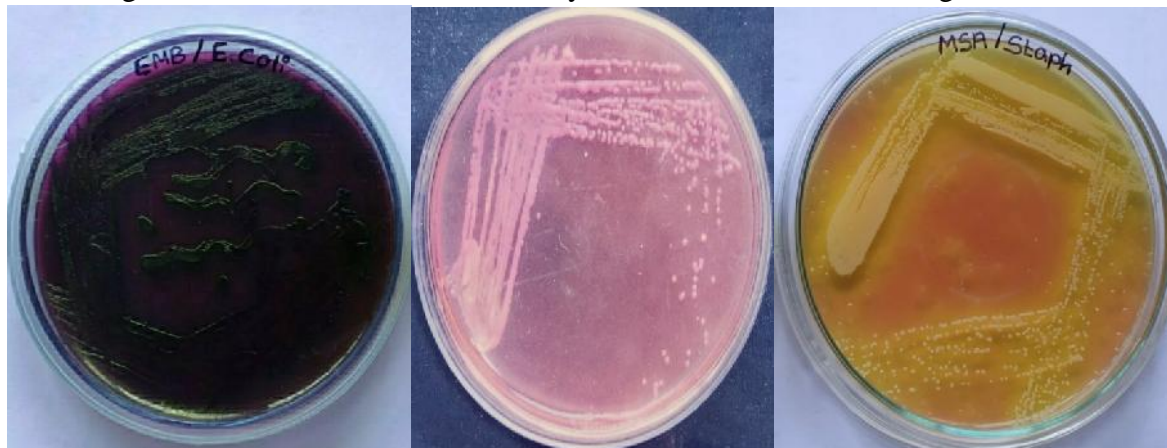


Fig: 2 This figure shows the bacterial colonies from different selective media (*E.coli*, *Klebsiella* and *S.aureus*)



The presence of phytochemicals like alkaloids, flavonoids, tannins and saponins is responsible for the antibacterial activity in the leaf extract of *Tamarindus indica*. These compounds have been reported to inhibit bacteria growth and are capable of protecting certain plants against, Bacterial infection (Clark, 1981; Mather and Gonzalez, 1982).

Results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are shown in table:6. The result showed various concentrations of ethanol leaves extracts can inhibit the growth of the isolates. The results showed that *S. aureus* had the highest MIC (20mg/ml) and MBC (15mg/ml). The *Escherichia coli* also the highest MIC (15mg/ml), MBC(10mg/ml). While the *Klebsiella* had the lowest MIC (10mg/ml), MBC (5mg /ml).

The highest Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) values of *Staphylococcus aureus* and *E. coli* is an indication that either the plant extracts are less effective on some bacteria or that the organisms has the potential of developing antibiotic resistance, while the low MIC and MBC value for *Klebsiella* bacteria is an indication of the efficacy of the plant extracts. The obtained results are in agreement with those reported by Doughari (2006) and Julio et al (2010).

## Conclusion

The current study has revealed that *Tamarindus indica* ethanolic extract inhibits *Escherichia coli*, *Klebsiella*, and *Staphylococcus aureus* for common UTI pathogens. The minimum inhibitory concentration of ethanol extract of the plant showed various concentrations can inhibit growth of the isolates. Findings from this work support the use of *Tamarindus indica* leaves extract for medicinal purposes. This work has opened up the possibility of using this plant leaf extract in the treatment of UTI infections in near future.

## Summary

The current study was Antibacterial activity of *Tamarindus indica* against bacteria causing Urinary tract infection (UTI). It was identified by collection of urine sample from UTI patients. The sample inoculated into nutrient broth. After incubation observe the microbial growth. Further analysis by gram staining, Biochemical tests and carbohydrate fermentation test. Further carried out by Disc diffusion Method, Minimum Inhibitory concentration (MIC), and Minimum bactericidal concentration (MBC). *Tamarindus indica* leaf extract inhibit the growth of *Escherichia coli*, *Klebsiella*, *Staphylococcus aureus*. So, the leaf extract is used to medicinal purpose. *Tamarind* polyphenol, flavonoids, and antioxidants may benefit heart health by reducing the risk of oxidative damage brought on by LDL (low density lipo protein) cholesterol. It has capacity to enhance liver function. In particular, it aids in the battle against renal cell carcinoma.

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