



COMPARATIVE STUDY ON THE ANTIBACTERIAL ACTIVITY OF TWO MEDICINAL PLANTS AGAINST URINARY TRACT INFECTION PATHOGENS

^{1*}Jeevitha. M, ²Gnanamuthu. G and ³Kutty Jusker Jebaraja. P

¹Assistant Professor, Department of Botany, Pope's College (Autonomous), Sawyerpuram, Thoothukudi, TamilNadu, India.

²Assistant Professor, Department of Zoology, Pope's College (Autonomous), Sawyerpuram, Thoothukudi, TamilNadu, India.

³Associate Professor, Department of Zoology, Pope's College (Autonomous), Sawyerpuram, Thoothukudi, TamilNadu, India.

Abstract : Urinary Tract Infections (UTIs) are an age old problem in the human development. UTIs are generally caused by several pathogens. A number of medicinal plants may have potential effect on this UTIs disease. The present study was carried out to compare the antibacterial activity of two different species of Medicinal plant like fruit of *Tribulus terrestris* and stem of *Saccharum spontaneum* against five urinary tract infection bacterial strain like *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The phytochemical analysis carried out revealed the presence of flavonoids, carbohydrate, amino acid, phylobactanins, volatile oil, glycosides, terpenoids, saponin, phenols, tannins, and sterols. The antibacterial activity of the plant extracts were carried out by disc diffusion method. The results showed that *Tribulus terrestris* are potential antibacterial agent for urinary tract infections (*Staphylococcus aureus* and *Enterococcus faecalis*) when compared with *Saccharum spontaneum*.

IndexTerms: Urinary Tract Infections, *Tribulus terrestris*, *Saccharum spontaneum*, Phytochemical, Antibacterial activity

1. INTRODUCTION

Antibacterial as well as antiviral activity of a molecule is completely associated with the compounds that provincially kill bacteria and virus or slow down their rate of growth, without being extensively toxic to nearby tissues. Most recently discovered antimicrobial agents are modified natural compounds and this modification is done through chemical mode. For example, b-lactams (penicillin's), Carbapenems or Cephalosporin. Antibacterial agents are the most important in fighting infectious diseases. But with their wide use as well as abuse, the appearance of bacterial resistance toward antibacterial agents has become a major problem for today's pharmaceutical industry. Resistance is most commonly based on developmental processes taking place, for example, antibiotic therapy, that leads to inheritable resistance. This increasing resistance of the microorganisms toward antibacterial agents has been responsible in recent years for serious health issues. Most infections bacteria

are resistant to a minimum of one of the antibiotics that are generally used to eliminate the infection.

Urinary tract infections (UTIs), accounting for 25% of all infections (Ahmed, 2016), are caused by a range of pathogens, most common are *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus saprophyticus* (Sharm, 2009 and Divya, 2015). UTIs are commonly caused by the bacteria which enter through the urethra (Nbavi, 2017). UTIs commonly develop in the bladder and spread to renal tissues (Nabbavi, 2017). This can occur in any part of the urinary tract such as bladder, Kidney, ureters and urethra (Sureda, 2017). UTIs affect nearly 150 million people each year (Stamm, 2001). UTIs can occur in any populations and in any age groups but more prevalent among the females of reproductive age group (Ahmed, 2016 and Karki, 2004). Some recent study reported that 40-50% suffer at least one clinical episode during their lifetime (Singh, 2014). The risk factors of UTIs are personal hygiene, lower socio-economic status, increased parity, increased age, sickle cell trait and anemia, lack of prenatal care, diabetes mellitus and the functional urinary tract abnormalities (Nowiciki, 2002 and Manjula, 2013).

Uncomplicated urinary tract infection is usually considered to be cystitis or pyelonephritis that occurs in premenopausal adult women with no structural or functional abnormality of the urinary tract and who are not pregnant and have no significant comorbidity that could lead to more serious outcomes. Also, some experts consider urinary tract infections (to be uncomplicated even if they affect postmenopausal women or patients with well-controlled diabetes. In men, most urinary tract infections (occur in children or elderly patients, are due to anatomic abnormalities or instrumentation, and are considered complicated (Hooton *et al.*, 2013).

It is reported that about 35% of healthy women suffer symptoms of Urinary tract infection and about 5% of women each year suffer with the problem of painful urination (dysuria) and frequency (Hootan, 2003). The incidence of UTI is greater in women as compared to men. Several potent antibiotics are available for the treatment of UTI, but increasing drug resistance among bacteria has made therapy of UTI difficult. Bacteria have the genetic ability to transmit and acquire resistance to drugs (Soulsby, 2005). Essential oils and extracts of certain plants have been shown to have antimicrobial effects, as well as imparting flavor to foods (Burt, 2004). The synergistic effect of the mixture of phytochemicals plays important role to use plant extracts as antimicrobial agents (Pauli, 2013). It has been suggested that volatile oils, either inhaled or applied to the skin, act by means of their lipophilic fraction reacting with the lipid parts of the cell membranes, and as a result, modify the activity of the calcium ion channels (Buchbauer and Jirovetz, 1994). The antimicrobial and other biological activities of the essential oils varied depending upon the origins and cultivars (Hussain *et al.*, 2008).

In this regard, the traditional medicines are the best alternatives, as these medicines are used from the time of immemorial with same efficacy (Das, 2013; Sinka, 2015 and Das, 2015). The antimicrobial efficacy of some plants for the treatment of UTIs has been beyond belief. The plants bio-constituents have been a good source of antimicrobial agents but still many of the plant species remained unexplored. A recent study reported that there are nearly 500,000 plant species in the world, of which only 1% has been phytochemically investigated (Kim, 2005). Keeping this in view, the present study was initiated with an aim to comparative study the antibacterial effect of *Tribulus terrestris* and *Saccharum spontaneum* extracts on some urinary tract infection causing bacteria.

II. MATERIALS AND METHODS

2.1. STUDY BACTERIA

Tested bacteria included *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains from the microbiology department laboratory of the Kamaraj College, Thoothukudi. Assay plates were prepared by inoculating 100 μ L–1 of each sample (1×10^5 colony-forming units [cfu]) onto Mueller-Hinton agar (OXOID CM 337).

2.2. PLANT SAMPLE

2.2.1. Source and collection of plant samples

The fruit of *Tribulus terrestris* and stem of *Saccharum spontaneum* in this study were collected from Sawyerpurem, Thoothukudi, Tamil Nadu state, India (Fig 1).

2.2.1.1. *Tribulus terrestris* Linn (Zygophyllaceae)

Tribulus is a plant that produces fruit covered with spines. Rumor has it that *Tribulus* is also known as puncture vine because the spines are so sharp, they can flatten bicycle tires. People use the fruit, leaf, and root as medicine. People use *Tribulus* for conditions such as chest pain, eczema, enlarged prostate, sexual disorders, infertility, and many others, but there is no good scientific evidence to support these uses.

2.2.1.2. *Saccharum spontaneum* Linn (Poaceae)

Saccharum spontaneum is an herb mentioned in Ayurveda for the treatment in conditions of purning urination, renal calculi, maeorrhagia, bleeding piles and to improve the quality of breast milk in lactating women. It is a perennial grass, growing up to three meters in height, with spreading rhizomatous roots. The stem is little hard and the leaves are thin, blunt edged. The flowers are white in color and appear in clusters, seen in winter season. The plant is found all over the plains of India. (Wild sugarcane) (Munci)

2.2.2. Authentication of plant samples

The plants were authenticated at the Department of Plant Science and Biotechnology, Kamaraj College, Thoothukudi, Tamil Nadu state, India.



Tribulus terrestris



Saccharum spontaneum



Fruit



Stem

Fig 1: The plant used for this comparative study

2.3. PREPARATION OF PLANT SAMPLES

The seed of *Tribulus terrestris* and stem of *Saccharum spontaneum* collection were first washed thoroughly with sterile distilled water and appropriately air dried at room temperature for two weeks to ensure the samples lose most of their moisture content. The specimen after being air dried, was powdered and milled at the department of Zoology, Pope's College, Sawyerpuram, Thoothukudi district, Tamil Nadu state, India.

2.4. EXTRACTION OF PLANTS

For the extraction of plant part, 500g of powdered plant sample was weighed into corked containers containing 1500ml each of water, acetone, ethanol, methanol, chloroform and petroleum ether, the mixtures were initially shaken rigorously and left for 7 days. The mixtures were filtered using sterile whatman filter papers, and the filtrates were collected directly into sterile crucibles. The filtrate was extracted using rotary evaporator, and the residues obtained were kept at room temperature (Osuntokun, 2015).

2.5. PHYTOCHEMICAL SCREENING:

Phytochemical examinations were carried out for all the extracts as per the standard methods (Roopashree, *et al.*, 2008; Obasi, *et al.*, 2010; Audu, *et al.*, 2007).

1. **Detection of carbohydrates:** Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.
Felling test: Filtrates were treated with Felling solution a and b and heated gently. Orange red precipitate indicates the presence of reducing sugars.
2. **Detection of saponins:**
Foam Test: 2 ml of extracts was shaken with 2ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.
3. **Detection of phenols:**
Ferric chloride test: Extracts were treated with 2 ml of ferric chloride solution. Formation of dark blue colour indicates the presence of phenols.
4. **Detection of tannins:**
2 ml of extracts and add for few drops of 10% ferric chloride solution were added. Formation of Blue or green colour precipitate indicates the presence of tannins.
5. **Detection of flavonoids:**

NaOH Tests: To 2 ml of extracts, few drops of sodium hydroxide solution were added in a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicated the presence of flavonoids.

6. **Detection of Glycosides:**

Glycosides test: To small amount of extract, add 1 ml of water and shake well. Then NaOH was added. Yellow colour appeared that indicates the presence of glycosides.

7. **Detection of sterols:**

Salkowski's test: To 2 ml of extract, add 2 ml Chloroform and 2 ml concentrated H₂SO₄ and shaken well. Chloroform layer appeared green and acid layer showed greenish yellow fluorescence indicated the presence of sterols.

8. **Detection of terpenoids:**

To 2 ml of extracts, add 2ml chloroform and 2 ml concentrated H₂SO₄ and was shaken well. Chloroform layer appeared brown and acid layer showed brownish yellow fluorescence indicated the presence of terpenoids.

9. **Detections of phylobactannis:**

To 2ml of extracts add 1ml of Dilute HCL. Red colour appeared that indicates the presence of Phylobactannis.

10. **Detection of volatile oil:**

To 2ml of extracts, add 0.1ml of dilute NaOH. Then small amount of dilute HCL added. White precipitate indicates presence of violet oils and it's disappearing immediately.

11. **Detection of amino acid:**

Ninhydrin test: To 2ml of extracts, 1 ml of Ninhydrin solution were added. Then heated for few minutes. Violet colour indicates presence of protein.

2.6. PREPARATION OF INOCULUM

Slants of the various organisms are reconstituted using an aseptic condition. Using a sterile wire loop, approximately one isolated colony of each pure culture was transferred into 5ml of sterile nutrient broth and incubated for 24 hours. After incubation, transfer 0.1ml of the isolated colony using a sterile needle and syringe into 9.9ml of sterile distilled water contained in each test tube and then mixed properly. The liquid now serve as a source of inoculum containing approximately 10⁶ cfu/ml of bacterial suspension.

2.7. PREPARATION OF NUTRIENT AGAR PLATES

About 8.5 g of nutrient agar was dissolved in 300 mL of distilled water. The resulting mixture was properly shaken and sterilized by autoclaving at 121 °C at 15 lbs inch⁻² for 15 min. The Petri-dishes were similarly sterilized. The mixture was allowed to cool and poured into sterilized Petri dishes and then allowed to solidify. The sterile discs (6 mm in diameter) were impregnated with 20 and 2.5 µl of above extract solution to achieve desired concentration of 500 and 250 µg/disc and placed in inoculated agar. Gentamicin (G) (10 µg/disc) and Ciprofloxacin (CF) (25 µg/disc) were used as standards. The controls were prepared using the same solvents employed to dissolve the extracts. The inoculated plates with the test and standard discs on them were incubated at 37⁰ for 24 h.

III. RESULT AND DISCUSSION:

The phytochemical screening of the different plants show the presence of some chemical constituents of the *Tribulus terrestris* seed and *Saccharum spontaneum* stem: Tannins, Phenol, Flavonoids, Carbohydrate, Amino acid, Phylobactanins, Volatile oil, Terpenoids, Steroids, Saponin and Glycoside (Table 1 and 2). The tests were only qualitative as described earlier.

Table 3 showed that both, the acetone and ethanol extracts of *Tribulus terrestris* had strong (IZD = 6 mm) inhibitory action against *S. aureus* but failed to show any promising (IZD = 1 mm or more) inhibitory effect against the Gram-negative bacteria *P. aeruginosa* and *E. coli*. Ethanol extract of *Tribulus terrestris* showed intermediate (IZD = 9 mm -11mm) inhibitory action against all the test strains. But their aqueous extracts failed

to show any promising inhibitory effect (IZD = 8 mm or more) against all the strains evaluated except *K. pneumoniae*. The aqueous extracts of *Tribulus terrestris* showed intermediate (IZD = 3 mm). In Table 4 showed that maximum inhibitory zone is obtained for the chloroform extract of *Saccharum spontaneum* against *Enterococcus faecalis*. There is no inhibitory zone is reported in *E.coli*.

S. aureus owes its clinical significance due to the fact that it causes a variety of supportive (pusforming) infections and toxins in the humans. It causes superficial skin lesions such as boils, sties and furunculosis; more serious infections such as pneumonia, mastitis, phlebitis, meningitis and urinary tract infections. *S. aureus* is a major cause of hospital acquired (nosocomial) infection of the surgical wounds and infections associated with indwelling medical devices. *S. aureus* causes food poisoning and toxic shock syndrome by the release of superantigens into the blood stream (Michael *et al.* 1999).

P. aeruginosa owes its clinical significance to the fact that it is an etiology of a good number of infections such as septic burns and wounds, conjunctivitis, endocarditis, meningitis, and urinary tract infections. Notably, it serves as a reference species in susceptibility testing on account of its notorious resistance to most antimicrobial compounds (Ho *et al.* 1998).

S.No	Chemical compound	Chemical extracts					
		Water	Acetone	Ethanol	Methanol	Chloroform	Petroleum ether
1	Saponing	-	+	+	+	+	-
2	Flavonoids	-	+	-	-	-	-
3	Carbohydrate	-	+	+	-	-	-
4	Amino acid	-	+	+	+	-	-
5	Phylobactanins	+	-	+	+	+	-
6	Volatile oil	+	+	-	-	+	-
7	Glycosides	+	+	-	-	+	+
8	Terpenoids	+	-	+	+	+	+
9	Steroids	+	-	+	+	+	+
10	Tannin	-	+	+	+	-	-
11	Phenol	-	+	-	+	-	-

- = Absent; + = Present

Table 1: Phytochemical constituent of the *Tribulus terrestris* using different extracts

S.No	Chemical compound	Chemical extracts					
		Water	Acetone	Ethanol	Methanol	Chloroform	Petroleum ether
1	Saponing	-	-	+	-	+	-
2	Flavonoids	-	-	+	+	-	-
3	Carbohydrate	-	+	+	-	-	-
4	Amino acid	+	-	-	+	-	-
5	Phylobactannis	-	-	-	-	-	-
6	Volatile oil	+	+	-	-	-	-
7	Glycosides	-	-	-	+	+	-
8	Terpenoids	-	+	-	+	+	-
9	Steroids	-	+	-	+	+	+
10	Tannin	-	-	+	+	-	-
11	Phenol	-	-	-	+	-	-

- = Absent; + = Present

Table 2: Phytochemical constituent of the *Saccharum spontanium* using different extracts

Similarly, *E. coli*, though normally a gut commensal, has attracted the clinical significance owing to the recognition of several strains of diarrhoeagenic *E. coli* with distinct virulent factors and also an important organism in urinary tract infections (UTIs) (Johnson 1991).

The present study revealed that all the plant materials possessed strain specific antibacterial activity and ethanol was the suitable solvent for the extraction of bioactive plant materials. According to Baur et al. (1966) classification, the bactericidal activity is classified into (i) resistant: if the zone of inhibition is less than 8 mm, (ii) intermediate: if it is 8 to 11 mm, and (iii) sensitive: if the zone of inhibition is 12 mm or more. On the basis of this classification, the present findings revealed that all the test strains were sensitive to the ethanol extract of *T. chebula* and *A. marmelos*, Intermediate action was observed for *M. indica* and *O. sanctum* and both aqueous and ethanol extracts of *A. indica* were sensitive only against Gram-positive *S. aureus*.

Chemical analysis revealed that *Tribulus terrestris* contained 24-32% tannin (Chung et al. 1988). The chief constituent of this tannin was chebulagic acid, chebulinic acid, corilagin and gallic acid. It had 18 amino acids. Resin and purgative principle of anthroquinone and sennoside nature was also present. Since both, the ethanolic and aqueous extract of *T. chebula* possessed antibacterial activity. It was presumed that the antibacterial activity of *T. chebula* could be due to the presence of high content of tannins in it.

Thus, the present study has shown that *Tribulus terrestris* has the wide spectrum antibacterial activity and could be a more potent antibacterial agent among the plant materials used. The high content of tannin present in it might be responsible for its antibacterial activity. This reinforces the importance of ethnomedical approach as a potential source of bioactive substances for the treatment of infectious diseases caused by these pathogenic microorganisms.

IV. CONCLUSION

In conclusion, the results showed that *Tribulus terrestris* are potential antibacterial agent for **urinary tract infections** (*Staphylococcus aureus* and *Enterococcus faecalis*) when compared with *Saccharum spontaneum*. We know that organisms are gaining resistance day by day towards the antibiotics, so that some

natural product should be try to overcome these antibiotic resistant organisms. More over plants can be grown easily and the production of extract is sophisticated than antibiotic Expense on these material is bearable than antibiotic. From these properties of extract we can say that natural medicine can take place of antibiotics in future.

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S.No	Name of the Urinary Tract Infection Bacteria	Zone of Inhibition in mm in diameter							
		Control	Antibiotic	Water	Acetone	Ethanol	Methanol	Chloroform	Petroleum ether
1	<i>Enterococcus faecalis</i>	0	12 ±1	-	3 ±0.1	6 ±0.3	4 ±0.2	-	-
2	<i>Escherichia coli</i>	0	15 ±1.5	3 ±0.1	1 ±0.03	2 ±0.1	2 ±0.1	-	2 ±0.1
3	<i>Klebsiella pneumoniae</i>	0	12 ±1.2	-	-	-	-	-	-
4	<i>Pseudomonas aeruginosa</i>	0	10 ±1.4	-	1 ±0.02	2 ±0.1	2 ±0.1	1 ±0.1	-
5	<i>Staphylococcus aureus</i>	0	13 ±1.1	-	6 ±0.2	6 ±0.2	4 ±0.2	2 ±0.1	1 ±0.1

Table 3: Shows the antibacterial activity of *Tribulus terrestris* against urinary tract infection pathogens

.No	Name of the Urinary Tract Infection Bacteria	Zone of Inhibition in mm in diameter							
		Control	Antibiotic	Water	Acetone	Ethanol	Methanol	Chloroform	Petroleum ether
1	<i>Enterococcus faecalis</i>	0	12 ±1.2	-	-	-	-	4 ±0.1	2 ±0.1
2	<i>Escherichia coli</i>	0	15 ±1.9	-	-	-	-	-	-
3	<i>Klebsiella pneumoniae</i>	0	12 ±1.6	-	-	3 ±0.1	-	-	-
4	<i>Pseudomonas aeruginosa</i>	0	10 ±1	1 ±0.02	-	2 ±0.04	-	2 ±0.05	-
5	<i>Staphylococcus aureus</i>	0	13 ±1.2	-	2 ±0.01	3 ±0.1	2 ±0.1	3 ±0.1	-

Table 4: Shows the antibacterial activity of *Saccharum spontaneum* against urinary tract infection pathogens