# Phytochemical scrutiny, antioxidant and bactericidal potential of selected medicinal plants against urinary tract pathogens

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## Abstract

According to recent researches on the incidence of urinary tract pathogenicity, nearly 1/3 of the world's population is infected. The current study used agar well and disc diffusion methods to compare the antibacterial activity of hydro-ethanoic extracts of *Rheum emodi (RE), Tribulus terrestris (TT), and Piper cubeba (PC)* against Escherichia coli, Staphylococcusaureus by agar well and disc methods with standard amoxicillin. Antioxidant activity were measured using DPPH radical scavenging methods, and phytochemical analysis was used to identify phyto constituents. All of the plants have strong antibacterial properties. The disc diffusion test produced a zone of inhibition of 25±.34mm, 18±0.64mm, 22±.50mm, 32±0.02 mm, while the agar well diffusion test produced a zone of inhibition of 22±0.67mm, 20±0.46mm, 22±0.46mm, 33±0.00 mm against Rheum emodi, Tribulus terrestris and Piper cubeba. Similarly, for antioxidant potential, % inhibition was 77.74, 58.75, 81.88 and 92.35, respectively. Antibacterial and antioxidant properties were found in abundance in the plants studied.

## Keywords

Bacteriacidal, Urinarytract infection, Herbs, Plants

## 1. INRODUCTION

The urinary tract stands as the body's most susceptible region to infectious diseases due to its direct connection with the external world through a conduit for liquid waste disposal. Urinary tract infections (UTIs) are prevalent ailments that encompass pyelonephritis affecting the renal pelvis, urethritis affecting the urethra, cystitis affecting the urinary bladder, and prostatitis affecting the prostate gland in the upper and lower urinary tracts, respectively. Symptoms of lower urinary tract infections typically include discomfort during urination, frequent urination, and urine leakage, while upper urinary tract infections are characterized by symptoms such

as fever, lower back pain, and occasionally blood in the urine (Bao *et al*., 2017).

One study revealed a UTI incidence rate of 0.7 percent, with prominent risk factors including age, previous UTI history, sexual activity, obesity, and structural abnormalities. Annually, approximately 151 million individuals experience urinary tract infections (Ullah *et al*., 2018). The primary culprits responsible for UTIs are *Escherichia coli (E. coli)* and *Staphylococcus aureus* (Hashemzadeh *et al*., 2021). Urinary tract infections often lead to blood-borne illnesses and are typically caused by pathogens such as E. coli, E. faecalis, S. aureus, and P. mirabilis (Pannu *et al*., 2020). Due to the proximity of the female urethra to the anus, UTIs are more commonly observed in women (Paude *et al*., 2018).There is an increase in antibiotic resistance, which leads to recurrent infection and its associated hazard effects (Wagenlehner FM., 2016). As a result, attention is drawn to alternative natural therapies that have been shown to be effective and safe at various degrees. Medicinal florae have also played a part in the treatment of UTIs since they have less side effects, are inexpensive, and have little bacterial resistance (Yatoo *et al*., 2018). In recent years, there has been a surge in the popularity of medicinal plants, as indicated by a report from the World Health Organization, which states that 81% of the global population rest on herbal remedies (Aziz *et al*., 2017). Phytoconstituents, particularly secondary metabolites, are found in medicinal plants and have therapeutic benefits in a variety of disorders.

Many therapeutic herbs, such as *Tribulus terrestris, Vaccinium macrocarpon*, and *Cucumis sativus* seeds, were traditionally used to do UTI. Cranberry juice plays an important function in preventing UTIs (Shaheen *et al*., 2019).

Holds sialic acid, which has the capacity to decrease inflammation and relieve pain (Luczak *et al*., 2018). Carbohydrate, Phenols, lignans, anthrones flavonoids, stilbenesoxanth-rone ethers / esters and oxalic acid are phyto- constituents found in *Rheum emodi* (Singh *et al*. 2018).

A variety of chemicals are found in Piper species, including amides, lignans, benzoic acids, chromepes, phenylpropanoids, phenolicsand a number of alkaloids (Andriana *et al*.. 2019). Chlorgenin, gitogenin and diosgenin, Kaempferol nitinoide, and tribuloside are among the active ingredients of *Tribulus terrestris* (Semerdeva IB and Zheljarkov XD, 2019).

In the current study, antibacterial and antioxidant properties were studied using *Rheum emodi, Tribulus terrestris*and *Piper cubeba.*

## 2. MATERIALS AND METHODS

### 2.1. Plant material collection and identification

#### 2.1.1. Preparing the extract

The plants were purchased from the Ansari Pansar shop, local market in Multan. In a shaded area, the acquired plant materials were carefully prepared, washed, and subsequently dehydrated. The unprocessed herb material was finely ground into a powder, and it was then immersed in 70 percent aqueous-ethanol for a period of 72 hours, with occasional stirring and mixing. To obtain a final semi-solid substance, the mixture underwent sequential filtration through muslin fabric and Whatman filter-paper. This process yielded a final product with a ratio yield of 30.4 percent (w/w) (Balogun *et al*., 2016).

#### 2.1.2. Analysis of phytochemicals

Using established techniques, the existence

of saponins, glycosides, carbohydrates, flavonoids, proteins, alkaloids, phenols, tannins and sterols in the selected plants was assessed qualitatively (Madhavan and Tharakan, 2017).

**2.1.3. In vitro Analyses**

Plants bactericidal activity was tested at concentrations of 50, 100, 150, 250 (µg/ml), and 500 (µg/ml). The disc diffusion method and agar well methods were used to test *E. coli, S. aureus*. To investigate the antibacterial properties, both approaches were used to determine the zone of inhibition.

**2.1.4. Culture preparation**

8 g of Merck's nutritional broth were mixed with 1 litter of distil water. Broth was autoclaved for 20 min., at 15 Psi at 121°C and then added to Erlenmeyer-flasks with 50µL of bacteriological culture. Flasks were shaken for 24 hours at normal temperature at 200 rpm on a flat shaker. After 24 hours, the culture's optical-density was measured b/w 0.12-0.19 according to the 0.5McFarland standard (Harathi *et al*., 2017).

**2.1.5. Broth dilution method**

Antibacterial activity was measured using sterile 96-well micro plates. For pre read absorbance at 540 nm, 200µL was poured to wells containing 180µL of microbial culture suspension and 20µL of tested extract. Petri plates were incubated at 37°C for 24 hours. The difference between pre and after read was used as a record of bacterial migration, as was the difference between pre and after read. The results were calculated the average of three replicates (n=3, SEM). Amoxicillin and ethanol were utilized as positive and negative controls, respectively. All of the evaluations were done three times (Srinivasan *et a*l., 2001). PI= 100 * (X-Y)/X was used to compute percentage inhibition.

Where X denotes the negative control absorbance and Y denotes the test sample absorbance as determined by bacterial culture.

**2.1.6. Evaluation of the MIC**

Aimed at MIC, serial dilutions of the tested sample process were performed as previously described. EZ-Fit5 Perrella Scientific Inc. Amherst USA was used to calculate the results.

**2.1.7. Agar wells diffusion**

In distilled water 28 gm Muller Hinton agar was dissolved. Sterilization was achieved by autoclaving at 121°C for 15 min. at 15 Psi. Tapping Sterile Muller Hinton (20ml) agar in Petri dishes completes the solidification process. The bacterial culture was completely streaked on agar and dried. In each Petri plate, four 6mm holes were punched in the agar. With the use of a micro pipette, 20µL of amoxicillin was placed in 1st hole and 20µL of prepared solution was placed in the remaining three holes. For a total of 24 hours, Petri dishes were kept at 37°C. The zone of inhibition was estimated at the end of the incubation time to check antibacterial activity.

**2.1.8. Antioxidant properties**

Assay for scavenging DPPH radicals the extracts' antioxidant activity was tested using the DRSA(DPPH radicals scavenging-assay) (Jiao *et al*., 2015). 0.1mM DPPH solution in ethanol was produced, and 2.4 ml from the solution was combined with 1.6 ml of hydro-ethanolic tested extracts at various concentrations (50, 100, 150, 250, and 500 µg/ml each ). The reaction mixture was briskly shook and maintained at room temper-

ature 30 minutes in the dark. At 517 nm, a spectrophotometer (Hunter Associates Laboratory, Inc.) was used to test the mixture's absorance. Ascorbic acid was employed as a control.

The DRSA percentage was computed as follows:

DRSA percent =(Ao-A1)/Ao x 100

The absorbance of the control and extractives / standard, respectively, is $A_O$ and A1. The % inhibition was then designed against concentration, and the $IC_{50}$ was derived from the graph.

### 2.1.9. STATISTICAL ANALYSIS

All data was analysed using SPSS version 20 and expressed as the mean S.D. of three replicates. Post-hoc Tukey For the purpose of determining differences between means, a one-way analysis of variance (ANOVA) was used. Values of $P<0.05$ were observed as significant.

## 3. RESULTS AND DISCUSSION

### 3.1. Antibacterial properties

### 3.1.1. Disk diffusion technique antibacterial activity

The antibacterial activity of individual plant and chemical formulations was tested using the disc diffusion method against E. coli and S. aureus at concentrations of 50, 100, 150, 250, and 500 (µg/ml). As a positive control, amoxicillin was used. The zone of inhibition was calculated by reproducing the results three times and averaging the results with ± SEM. Table 1 summarises the findings. By using the well method, antibacterial activity can be measured. Five dilutions (25, 50, 100, 250, and 500 g/ml) of various plant extracts and a standard medication were used to test antibacterial activity against the bacteria indicated. The zone of inhibition was calculated by reproducing the results three times and averaging the results with ± SEM. Table 2 summarises the findings.

**Table 1. Bactericidal effect by Disk Diffusion Method**

| Inhibition Zone in mm | | |
|---|---|---|
| **Plants (500 µg/ml)** | ***E. coli*** | ***S. Aureus*** |
| TT | 25±0.34 | 23±0.66 |
| RE | 18±.64 | 19±0.41 |
| PC | 22±.50 | 21±0.26 |
| Amoxicillin | 32±0.00 | 31±0.43 |

**Table 2. Bactericidal effect by Agar Well Method**

| Inhibition Zone in mm | | |
|---|---|---|
| **Herb (500 µg/ml)** | ***E. coli*** | ***S. Aureus*** |
| TT | 22±0.67 | 16±0.33 |
| RE | 20±0.77 | 21±0.31 |
| PC | 22±0.00 | 17±0.47 |
| Amoxicillin | 33±0.00 | 31±0.47 |

### 3.1.2. DPPH Radical Scavenging activity has Antioxidant action.

The dose-response curve of DPPH radical scavenging activity of the aqueous ethanolic extracts of all tested plants shows the dose-response curve of DPPH radical scavenging activity of the aqueous ethanolic extracts. The Piper cubeba (PC) extract had better activity than the other extracts, but it was still less than comparable to a normal medicine. Table 3 summarises the findings.

**Table 3. Antioxidant activity**

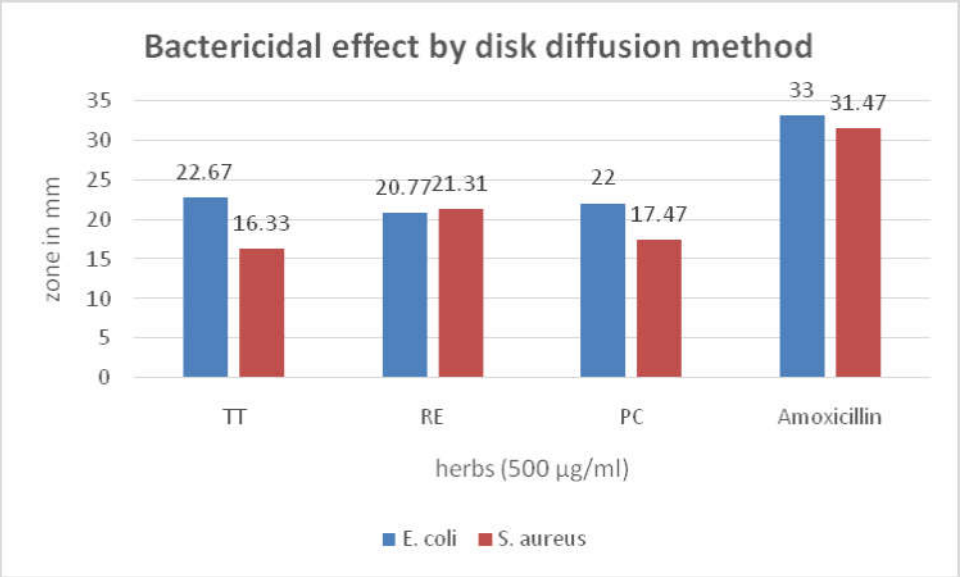
| Antioxidant screening | | |
|---|---|---|
| Herb (500 µg/ml) | DPPH (%) | IC50 |
| TT | 81.78 | 45.05 |
| RE | 79.75 | 40.05 |
| PC | 61.76 | 31.04 |
| A. A | 91.45 | 45.00 |

### 3.1.3. Analysis of phytochemicals

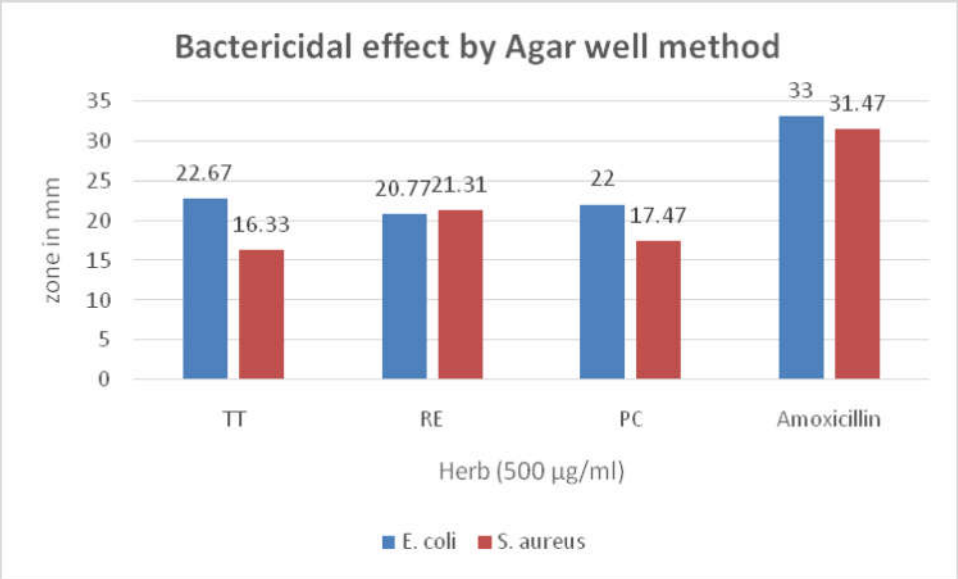
Alkaloids, glycosides, proteins, flavonoids, saponins, sterols, phenols,Carbohydrates, and tannins were all found in phytochemical examination according to (Madhavan and Tharakan, 2017).. The presence and estimated quantities of phytochemicals in experimental plants were variable, but they were suspected of having antibacterial and antioxidant activities (Table 4).

**Table 4. Phytochemicals screening**

| Test for phytochemicals screening | | TT | RE | PC |
|---|---|---|---|---|
| Alkaloids | Mayer's Test | P | P | P |
| Glycosides | Born Trager's test | P | P | A |
| Tannins | Bromine water | A | P | P |
| Sterols | Salkowski reaction | P | P | P |
| Phenols | Ferric chloride test | P | P | P |
| Protein | Biuret Test | P | A | A |
| Flavonoids | Alkaline reagent test | P | P | P |
| Carbohydrates | Molisch Test | A | P | A |




**Graph 1: Bactericidal effect by Disk Diffusion Method**




**Graph 2: Bactericidal effect by Agar Well Method**

Herbs have been shown to be extremely safe and operative, and they are currently crucial in the growth of novel antimicrobial medicines to treat infectious illnesses and a variety of other disorders. As a result, anti-infective ingredients in herbal medications have been documented from Pakistan, India, Turkey, Taiwan, Japan and other parts of the map (Dar *et al*., 2017). Appropriate screening and systematic studies on these natural medicine's mechanisms of act and therapeutic qualities are urgently needed.

The utmost sensitive bacterium was E. coli, inhibited by *TT*, *RE* and *PC* with 25±0.35, 22±.50mm 18±0.64mm, correspondingly, followed by *S. aureus* by *PC* and amoxicillin with 21±0.51, and 33±0.37 mm respectively, as determined by disc diffusion techniques. The utmost sensitive bacterium was *E. coli*, inhibited by extract of *T.T, R.E, P.C* and amoxicillin with 22±0.67 mm, 20±0.46mm, 22±0.46mm and 33±0.00 respectively, followed by *S. aureus* by *R.E* with 22±0.31 mm and Amoxicillin with 33± 0.05 mm.

The antibacterial efficacy of the medicinal herbs we selected is on par with findings from various other studies. In a previous investigation, the antibacterial properties were examined counter to both pathogenic gram-positive and gram-negative bacteria (Mostafa *et al.*, 2018). The combination of an alcoholic extract and cumin oil successfully impeded the growth of *K. pneumoniae* and its variants, leading to enhancements in capsule appearance, cell morphology, and reduced urease activity.

The antimicrobial potential of an ethanolic extract from *T. terrestris* fruit was assessed against six different bacteria strains, namely Streptococcus mutans, Actinomyces viscosus Staphylococcus aureus, Streptococcus sanguis, Enterococcus fecalis and Escherichia coli (Soleimanpour *et al*., 2015).

Another recent study investigated the anti-

bacterial properties of 13 communal flavonoids (including flavanones flavones and flavonols,) and 6 organic acids (both aliphatic and aromatic acids). The micro-dilution method was employed to assess the Minimum Inhibitory Concentrations (MICs) of selected plant compounds using clinical strains of E. coli, E.faecalis, P. aeruginosa, and S. aureus (Adamczak *et al*., 2020).

In a different study, varying concentrations of ethanol extracts from Aloe vera roots and leaves were tested against fungal and bacterial strains (Mostafa AA *et al.*, 2018 ; Semerdjieva IB *et al*., 2019 ; Sintara M *et al.*, 2018 ; Yatoo M *et al.*, 2018). The study included *Bacillus megaterium B. cereus, B. subtilis, Streptococcus pyogenes, Escherichia coli, Staphylococcus aureus, P. aeruginosa* and other bacterial strains. The research concluded that using traditional medicinal plants to treat diseases triggered by these pathogenic strains was advantageous (Danish *et al*., 2020).

Furthermore, at a concentration of 500µg/mL, the scavenging activity of the extracts was assessed in decreasing order as TT-81.75 percent, RE=79.75, PC-61.76, and percent, while the ascorbic acid was 91.45percent. TT 45.05, RE 40.05, and PC 31.04 µg/ml were the $IC_{50}$ of aqueous ethanolic extracts, while the $IC_{50}$ of A.A (standard) were 45.01µg/mL, respectively. Plant polyphenols have been demonstrated in numerous studies to be effective antioxidants in the treatment of oxidative stress-related illnesses. In higher plants, including edible ones, thousands of polyphenolic compounds have been discovered (Pawlowska *et al*., 2019).

## 4. CONCLUSION

Plants have substantial bactericidal and antioxidant properties, according to the results.

Piper cubeba (PC), Tribulus terrestris (TT), Rheum emodi (RE), can thus be utilized for bacterial contaminations, particularly UTI, based on these findings.

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