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ORIGINAL ARTICLE

# Antimicrobial activity of five traditionally used medicinal plants on bacterial infection of urinary tract

Pradip Samanta[1], Nirmalya Kumar Sinha[2]

1. Dept. of Microbiology, Raja N.L. Khan Women's College, Midnapore, West Bengal, India.
2. Dept. of Nutrition, Raja N.L. Khan Women's College, Midnapore, West Bengal, India.

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ABSTRACT

Urinary tract infection (UTI) has become a serious public health problem worldwide. Antimicrobial resistance of the pathogenic bacteria makes the situation more serious. Medicinal plants used as crude form by the communities have potential effect on this disease. The present study was carried out to investigate the antibacterial activities of five medicinal plants viz. *Allium sativum*, *Cinnamomum verum*, *Syzygium aromaticum*, *Terminalia arjuna*, *Zingiber officinale* against five Urinary Tract Infection (UTI) causing bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Staphylococcus aureus*. The antimicrobial activity of the plant extracts by agar well diffusion assay and Minimum Inhibitory Concentration (MIC) method. The diameter of the zone of inhibition (DZI) were measured. Antioxidant action of five plants was observed by thin layer chromatography (TLC) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay. Phytochemical screening was done by following the standard procedure. Among the two extracts used, highest antibacterial activity was recorded with ethanolic extract of *Terminalia arjuna* on *E.coli* and least against extracts of *Zingiber officinale* on *P. aeruginosa* with DIZ of 3.2 mm and 1.1 mm respectively. MIC values of 100 µg/ml indicated that *Z. officinale* is more effective on *E. coli*, *K. pneumonia* and *P. vulgaris* than the other plant extracts, but less effective on *S. aureus* and *P. aeruginosa*. The presence of high anti-oxidative biomolecules were noted in *Z. officinale* extracts. Preliminary phytochemical analysis of the plant parts revealed the presence of active compounds such as phenolics, steriods, alkaloids, glycosides, and flavonoids. The results obtained in this analysis clearly demonstrated that the antibacterial activity of selected plant extracts on all UTI causing bacteria is satisfactory and this is due to the presence of anti-oxidative biomolecules or active compounds such as phenolics, steriods, alkaloids, glycosides, and flavonoids. Further research is needed.

## INTRODUCTION

Urinary tract infection (UTI), a bacterial infection caused by *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae, Staphylococcus aureus*, has become a serious public health problem worldwide [1]. The quality of life is poorly affected by the UTI induced lower urinary tract symptoms like urinary urgency, frequency, painful urination, hesitancy, and the sense of incomplete bladder emptying [2]. Globally, nearly 150 million people are encountered with UTI each year [3]. It has become the most common hospital-acquired infection. In the community, the prevalence of UTI has been reported in all age groups and in both sexes. Though, it is reported that the incidence of UTI is greater among the women than the men. It may be due to anatomical predisposition or urothelial mucosal adherence to the mucopolysaccharide lining or other host factors [4].

***Correspondence to:**

**Pradip Samanta**
In-Charge, Dept. of Microbiology,
Raja N.L. Khan Women's College,
Midnapore, West Bengal, India.
Email: pradip2012samanta@gmail.com

It is reported that about thirty five percent of the healthy women encounter with UTI infection at some stage in their life.

Nowadays, drug resistance is a huge growing problem for the treatment of infectious diseases including UTI. The improper and uncontrolled use of the antibiotics is the main cause of antimicrobial resistance [5]. The antimicrobial resistance has become a serious problem worldwide. So, there is some urgent need of restriction in the unnecessary use of antibiotics and also to search out some new drugs. The traditional medicines are the best alternatives to it, as these medicines are used from the time of immemorial with same efficacy. The antimicrobial efficacy of some plants in the treatment of diseases 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. It is reported that the local communities have used only 10% of all flowering plants on earth for this purpose. But, only 1% of these plants were recognition by modern scientists [6]. The plants are the rich source of secondary metabolites like tannins, alkaloids and flavonoids. These secondary metabolites have proven their antimicrobial properties in many in vitro studies [7]. Keeping in view, the growing problem with UTIs and drug resistance, the present study

was undertaken with an objective to identify the antimicrobial activity and phytochemicals present in five medicinal plants that are used by the tribal people of Paschim Medinipur district for the treatment of UTI.

## MATERIALS AND METHODS

### Plant materials

A total of five plants and their parts such as *Allium sativum* L. (rhizome), *Cinnamomum verum* J. Presl (bark), *Syzygium aromaticum* (L.) Merrill & Perry (flower), *Terminalia arjuna* (Roxb.) Wight & Arn. (bark), *Zingiber officinale* Roscoe (rhizome) were used in this study. The taxonomic identities of these plants were confirmed in the Dept. of Botany, Raja N.L. Khan Women's College, Midnapore.

### Preparation of plant extracts

The fresh plant parts mentioned earlier were washed with running tap water immediately after collection followed by washed with distilled water and kept at an incubator for 24h. The plant parts were powdered by grinder by maintaining the standard protocol [8]. Aqueous and ethanol extracts of the plant parts were prepared from 60 g of dried powder of each plant parts mixed with the said solvent in 1:5 ratio. The extraction was carried out in shaker at 37˚C for 24 h. The extracts were concentrated to dryness by a rotary evaporator at 50˚C and those are kept at 4˚C for drying.

### Bacterial strain

Five standard bacterial strains which caused UTI among the people were used as tested microorganisms. The microbial cultures such as *Escherichia coli* (MTCC No. 40), *Klebsiella pneumoniae* (MTCC No. 4030), *Staphylococcus aureus* (MTCC No. 3160), *Proteus vulgaris* (MTCC No. 426), *Pseudomonas aeruginosa* (MTCC No. 424) were collected from IMTECH, Chandigarh. All the microorganisms were incubated into Mueller-Hinton broth for 24 h at 37°C. The turbidity of the medium indicates the growth of organisms. All the cultures were kept at 4°C until further use.

### In vitro antibacterial assay

**Agar well diffusion method:** This method was used to determine the antimicrobial activity of said plants. Bacterial suspensions were spread on the Mueller Hinton agar (MHA) plates. The wells were cut from the agar plates using a sterile cork borer. The extracts were poured into the well. One standard antibiotic (Norfloxacin) was also used for study the susceptibility of five UTI causing bacteria[9]. The plates were incubated at 37˚C for 24 h. After incubation the diameter of the zone of inhibition were measured in mm and recorded [8].

**Minimal Inhibitory Concentration (MIC):** MIC of these plant extracts was carried out using broth dilution method. For bacteria, nutrient broth (4ml) in each test tubes containing different concentrations (50, 100, 150, 200 and 250 µg/ml) of ethanol extracts as well as tested antibiotic were prepared separately and inoculated with 0.1 ml inoculums. The inoculated cultures were incubated at 37˚C for 24 h. After keeping the test tubes for 24 h incubation the OD values are recorded at 520 nm.

### Phytochemical analysis

The presence of several phytochemicals (like phenolics, alkaloids, flavonoids, tannins, saponins, steroids and glycosides) in the plant extracts were determined by following the methods described by Harborne [10] and Kolkate et al. [11].

**Thin layer chromatography (TLC) analysis for Antioxidant activity:** About 2µg of the five plant extracts was loaded on TLC plates (Merck, 20 cm×20 cm). The plates were developed in methanol: chloroform: hexane (7:2:1 v/v/v) to separate various constituents of the extracts. The developed plates were air dried. Then antioxidant constituents were analyzed by DPPH technique. The DPPH radical scavenging activity percentage was calculated by using the following formula:

DPPH radical scavenging activity (%) = $\{[A_{control} - A_{extract}]/A_{control}\} \times 100$ Where $A_{control}$ is the absorbance of a DPPH solution without extract; $A_{extract}$ is the absorbance of the tested extract.

**TLC analysis for flavonoid constituents :** About 2 µg of plant extracts was loaded on TLC plates (Merck, 20 cm X 20 cm). The plates were developed in toluene: chloroform: methanol (4:4:1, v/v/v) to separate flavonoid compounds of the extracts. The developed plate was air dried. Then anisaldehyde sulfuric acid was sprayed on the surface of the plate and incubated for 20 min at 100°C. The present flavonoid compound of this extracts was detected as blue spot on developed TLC plate.

## RESULTS

Table 1 shows the antibacterial activity of aqueous and ethanol extract of five medicinal plants against five UTI bacteria. The study indicated that the diameter of inhibition zones (DIZ) ranging from 0.1 to 3.2 mm with the highest zone observed against *E. coli* of *Terminalia arjuna* (Table - 1). It was also noted that this plant is highly effective in the case of *K. pneumonia* and *P. vulgaris,* but no such effectiveness was observed in the cases of *S. aureus* and *P. aeruginosa.*

Quantitative evaluation of antibacterial activity (MIC) was carried out for ethanolic extracts. The MIC values of selected plant extracts on five UTI causing bacteria is shown in Table 2. A wide range of MIC values were recorded depending on the bacterial strain.

Phytochemical studies revealed that flavonoids, alkaloids, phenolics, steroids, glycosides are present in these selected plants. Results of other phytochemical constituents are shown in Table-3.

The antioxidant activity of selected plant parts have been represented in Table 4. From the experimental findings it has been found that *Zingiber officinale* showed greater antioxidant activity than other plant materials.

## DISCUSSION

Medicinal plants are used to cure the diseases throughout the globe from the ancient times [12]. Even at the 21st

century the herbal medicines achieved the reliability in mind set of global people as because it have no such side effects, relatively less expensive and better patient tolerance [13]. It is reported that nearly 80% of people from developing countries depending on traditional medicines for primary health care and in modern medicine also approximately 25% are based on plant-derived drugs [14-16].

**Table 1 Diameter of zone of inhibition shown by five UTI causing bacteria against standard antibiotic and plant extracts.**

| Organisms | Diameter of inhibition zone (mm) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Zingiber officinale* | | *Allium Sativum* | | *Syzygium aromaticum* | | *Cinnamomum verum* | | *Terminalia arjuna* | | Norfloxacin |
| | Aq | Et | Aq | Et | Aq | Et | Aq | Et | Aq | Et | |
| *E. coli* | 1.8 | 3.0 | 1.3 | 2.9 | 1.2 | 2.9 | 1.5 | 2.6 | 2.5 | 3.2 | 3.0 |
| *K. pneumoniae* | 1.6 | 2.5 | 0.8 | 1.7 | 0.9 | 1.7 | 1.0 | 1.2 | 1.7 | 2.8 | 4.0 |
| *P. vulgaris* | 0.5 | 1.8 | 1.1 | 2.3 | 1.1 | 2.3 | 0.4 | 1.5 | 2.3 | 3.0 | 1.3 |
| *S. aureus* | 0.2 | 1.5 | 0.8 | 2.0 | 0.8 | 2.0 | 1.4 | 2.4 | 1.1 | 1.8 | 3.2 |
| *P. aeruginosa* | 0.1 | 1.1 | 0.1 | 1.2 | 0.1 | 1.1 | 0.8 | 1.8 | 0.5 | 1.5 | 1.6 |

Aq=Aqueous extract; Et=Ethanol extract

**Table 2 Comparison of MIC values of *different plant extract* and Norfloxacin**

| | ***E. coli*** | ***K. pneumonia*** | ***P. vulgaris*** | ***S. aureus*** | ***P. aeruginosa*** |
|---|---|---|---|---|---|
| *Allium sativum* | | | | | |
| 250 µg/ml | 1.10 | 0.04 | 0.90 | 0.27 | 0.30 |
| 200 µg/ml | 1.12 | 0.04 | 1.02 | 0.48 | 0.49 |
| 150 µg/ml | 1.21 | 0.05 | 1.03 | 0.49 | 0.52 |
| 100 µg/ml | 1.21 | 0.05 | 1.36 | 0.54 | 0.58 |
| 50 µg/ml | 1.54 | 0.06 | 1.45 | 0.74 | 0.75 |
| $Control_E$ | 1.64 | 0.20 | 1.81 | 1.13 | 1.14 |
| *Cinnamomum verum* | | | | | |
| 250 µg/ml | 1.15 | 0.08 | 0.67 | 0.35 | 0.67 |
| 200 µg/ml | 1.20 | 0.08 | 0.70 | 0.74 | 0.75 |
| 150 µg/ml | 1.21 | 0.09 | 0.73 | 0.87 | 0.89 |
| 100 µg/ml | 1.25 | 0.10 | 0.88 | 0.90 | 0.94 |
| 50 µg/ml | 1.28 | 0.13 | 1.05 | 1.11 | 1.09 |
| $Control_E$ | 1.64 | 0.20 | 1.81 | 1.13 | 1.14 |
| *Syzygium aromaticum* | | | | | |
| 250 µg/ml | 0.46 | 0.01 | 0.62 | 0.57 | 0.64 |
| 200 µg/ml | 0.56 | 0.05 | 0.64 | 0.60 | 0.71 |
| 150 µg/ml | 0.56 | 0.07 | 0.71 | 0.68 | 0.75 |
| 100 µg/ml | 0.67 | 0.09 | 0.78 | 0.76 | 0.83 |
| 50 µg/ml | 0.78 | 0.11 | 0.90 | 0.89 | 0.93 |
| $Control_E$ | 1.64 | 0.20 | 1.81 | 1.13 | 1.14 |
| Terminalia arjuna | | | | | |
| 250 µg/ml | 1.11 | 0.06 | 0.51 | 0.43 | 0.63 |
| 200 µg/ml | 1.25 | 0.06 | 0.58 | 0.45 | 0.77 |
| 150 µg/ml | 1.24 | 0.08 | 0.69 | 0.56 | 0.92 |
| 100 µg/ml | 1.25 | 0.09 | 0.76 | 0.63 | 1.03 |
| 50 µg/ml | 1.22 | 0.12 | 0.87 | 0.98 | 1.12 |
| $Control_E$ | 1.64 | 0.20 | 1.81 | 1.13 | 1.14 |
| *Zingiber officinale* | | | | | |
| 250 µg/ml | 0.15 | 0.04 | 0.12 | 0.50 | 0.55 |
| 200 µg/ml | 0.23 | 0.05 | 0.17 | 0.57 | 0.60 |
| 150 µg/ml | 0.25 | 0.05 | 0.20 | 0.61 | 0.69 |
| 100 µg/ml | 0.47 | 0.05 | 0.24 | 0.67 | 0.72 |
| 50 µg/ml | 0.87 | 0.06 | 0.27 | 0.78 | 0.84 |
| $Control_E$ | 1.64 | 0.20 | 1.81 | 1.13 | 1.14 |
| Norfloxacin | | | | | |
| 250 µg/ml | 0.51 | 0.05 | 0.57 | 0.39 | 0.38 |
| 200 µg/ml | 0.75 | 0.06 | 0.79 | 0.78 | 0.74 |
| 150 µg/ml | 0.83 | 0.09 | 0.89 | 0.93 | 0.84 |
| 100 µg/ml | 1.00 | 0.16 | 0.95 | 0.97 | 0.94 |
| 50 µg/ml | 1.01 | 0.19 | 1.13 | 1.18 | 1.00 |
| $Control_A$ | 1.64 | 0.20 | 1.81 | 1.13 | 1.14 |

**Table 3. Phytochemical analysis of five medicinal plants in ethanolic extracts**

| Phytochemicals | *Zingiber officinale* | *Allium Sativum* | *Syzygium aromaticum* | *Cinnamomum verum* | *Terminalia arjuna* |
|---|---|---|---|---|---|
| Phenolics | - | - | + | - | - |
| Alkaloids | + | + | - | - | + |
| Flavonoids | - | - | - | - | + |
| Tannins | - | - | - | - | - |
| Saponins | - | - | - | - | - |
| Steriods | + | + | - | - | + |
| Glycosides | + | + | - | - | - |

**Table 4. DPPH radical scavenging activity of ethanol extract of five medicinal plants (Test) and Ascorbic acid (Standard)**

| Concentration (µg/ml) | Percentage inhibition | | | | | |
|---|---|---|---|---|---|---|
| | *Zingiber officinale* | *Allium sativum* | *Syzygium aromaticum* | *Cinnamomum verum* | *Terminalia arjuna* | Ascorbic acid |
| 10 | 11.66 | 8.41 | 5.40 | 4.76 | 7.16 | 4.45 |
| 20 | 23.32 | 16.82 | 10.80 | 9.52 | 14.32 | 8.90 |
| 30 | 34.98 | 25.23 | 16.20 | 14.28 | 21.48 | 13.35 |
| 40 | 46.64 | 33.64 | 21.60 | 19.04 | 28.64 | 17.80 |
| 50 | 58.30 | 42.05 | 27.00 | 23.80 | 35.80 | 22.25 |

In the present study aqueous and ethanolic plant extracts was exhibited antibacterial activity towards all UTI pathogens, with more activity observed with ethanolic extracts. Ethanol extracts may have the potential role of extracting plant biomolecules that are actually responsible for higher antimicrobial activity than the aqueous one. Among all plant extracts highest DIZ values were recorded for *Terminalia arjuna* against UTI bacteria, followed by *Zingiber officinale, Allium Sativum, Syzygium aromaticum, Cinnamomum verum.* Presence of alkaloids, flavonoids and steroids in the *Terminalia arjuna* may be the cause of such activity.

Phytochemicals exert this antimicrobial activity through different mechanisms, like act by iron deprivation, hydrogen bounding or non-specific interactions with vital proteins such as enzymes [17]. Sawer et al. [18] demonstrated that the main indoloquinoline alkaloid, cryptolepine, causes cell lysis and morphologicalchanges of S. aureus, but the antimicrobial effects of the alkaloid may be through another mechanism, since the compound is known to be a DNA intercalator and an inhibitor of DNA synthesis through topoisomerase inhibition [19-26].

In the case of MIC values of100 µg/ml, it was found that *Zingiber officinale* is more effective on *E. coli, K. pneumonia* and *P. vulgaris* than the other plant extracts, but less effective on *S. aureus* and *P. aeruginosa*. It may be due to the presence of high anti-oxidative biomolecules of *Zingiber officinale.*

## CONCLUSION

All selected plants used in this study expressed broad spectrum antibacterial activity on UTI causing bacteria with highest activity recorded for ethanolic extracts. By comparing the antibacterial activity of selected plant extracts with standard antibiotic used in this study, it is found that ethanolic extracts exerted better antibacterial activity. Importantly the results suggest that these plants contain active ingredients which qualify them for medicinal use. The presence of phytocompounds in the extracts including phenols, tannins and flavonoids as major constituents may be responsible for the antibacterial activity. Further research is needed to isolate, purify, and characterize the active constituents responsible for the bioactivity study.

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