

# ANTIBACTERIAL ACTIVITY OF SELECTED SPICES AGAINST MULTI- DRUG RESISTANT URINARY TRACT MICROFLORA

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## Abstract

Now a days, Urinary tract infections (UTIs) are one of the important bacterial infections seen in hospitals. In this study, the pathogens were isolated from urine samples of urinary tract infected patients. The antibacterial property of ethanol extract of selected spices was tested against the urinary tract pathogens. *Allium sativum* ethanol extract greatly inhibited the growth of all urinary tract pathogens. Antibacterial assay of selected commercial antibiotics showed that isolated organisms were found to be resistant against the antibiotics. It can be concluded that *Allium sativum* extracts revealed effective antimicrobial compounds against resistant UTI pathogens

Key words: *Allium sativum*, antibacterial, UTI pathogens etc.

## Introduction

Urinary tract infection (UTI) is a collective term that describes any infection involving parts of the urinary tract, namely the kidney, ureter, bladder and urethra. Urinary tract infections (UTIs) are responsible for nearly 10 million doctor visits each year. One in five women will have at least one UTI in her lifetime. It is a familiar contamination among men and women but the frequency is quite elevated in women due to their physiology. It is a common source of infection in children and infants and is the most common bacterial infection in children < 2 years of age, both in the community and hospital setting (Hanna-Wakim *et al.*, 2015). In the urinary tract infection, bacteria get into the urinary tract (the bladder), multiply and adherence to the uroepithelium. The result is redness, swelling and pain in the urinary tract (<https://www.kidney.org/sites/default/files/uti.pdf>). Frequent use of several antibiotics has been made bacteria to develop resistance in their population which have become a burning predicament. However, with the increased resistance among uropathogens and changes in the prevalence of UTI-causing organisms, new guidelines have emerged (Tan and Chlebicki, 2016). As a result there is an urgent need to find the alternative of chemotherapeutic drugs in diseases treatment particularly those of plants origin which are easily available and have considerably less side effects (Khulbe and Sati, 2009). Spices are important natural products, which have been used since ancient times and until now. Spices have been used for not only flavor and aroma of the foods but also to provide antimicrobial properties (Nanasombat *et al.*, 2002). Some of the natural compounds found in various spices possess antimicrobial (Indu *et al.*, 2006). Grohs and Kunz (2000) observed that spices mixtures were able to inhibit the growth of various meats spoiling microorganism. The

present study deals with the study of antibacterial activity of selected spices against urinary tract infected pathogens.

## Materials and methods

### 1. Collection of samples

Urine samples were collected from urinary tract infected patients in a sterile container from V.O.C Port Trust hospital, Thoothukudi, Tamil Nadu, India. The urine samples were streaked on Nutrient agar plates. The plates were incubated at 37°C for 24 hours. After incubation, the individual colonies having different colony morphologies were streaked on different selective agar plates such as Eosin Methylene Blue agar, Mannitol Salt agar, MacConkey agar, Blood agar etc. The isolated organisms were stored in agar slants and subjected to biochemical identification.

### 2. Activity of selected commercial antibiotics against the isolated pathogens

Antibiotic assay was performed by Kirby-Bauer disc diffusion method. The Mueller-Hinton agar plates were prepared and the organism was swabbed over it using a sterile cotton swab. Commercially available antibiotics such as Streptomycin, Gentamycin, Chloramphenicol, Oxacillin, Vancomycin, Ciprofloxacin and Methicillin were tested against the urinary tract pathogens. The antibiotic discs were placed on the surface of the agar plates and then, the plates were incubated at 37°C for 24 hrs. After incubation, the zone of inhibition (mm) was measured.

### 3. Collection of some selected spices

Fresh spices such as *Allium cepa* (Onion), *Allium sativum* (Garlic), *Myristica fragrans* (Nutmeg) *Zingiber officinale* (Ginger) and *Piper nigrum* (pepper) were obtained from the local market, Thoothukudi, Tamil Nadu, India.

#### 3.1 Solvent Extraction

Plant material was washed with distilled water and air dried in the laboratory for two weeks and grounded. 50g of each ground material was soaked in 500ml of ethanol (polar) and chloroform (nonpolar) solvents separately for 72 hours with frequent shakings. The samples were then filtered through Whatman No:1 filter paper. The filtrate was evaporated to dryness under reduced pressure at 40°C. Dimethyl sulfoxide (DMSO) was made for each extract preparation and was kept at 4°C, until used (Samie *et al.*, 2005).

### 3.2 Preparation of spices extracts

The spices were cleaned, descaled when necessary and washed in sterile distilled water. In order to obtain the spices extracts, about 100g of each washed spice were crushed with mortar and pestle. The extracts were sieved through a fine mesh cloth and sterilized using a membrane filter.

Garlic extract was made in a different ways due to the difficulty to filter the crushed material. One hundred grams of the descaled and cleaned garlic were taken and surface sterilized using ethanol. The ethanol was allowed to evaporate in a sterile laminar flow chamber and the garlic was homogenized aseptically using a sterile mortar and pestle. Twenty grams of fine grounded pastes of *Allium sativum* was dispensed into a beaker containing 80ml of 95% ethanol and chloroform. They were soaked for 72hours while the resulting supernatant was decanted into a conical flask of 100ml and kept in the refrigerator for further study (Olayemi and Opaleye, 1999).

### 4. Antimicrobial property of selected spices against the UTI pathogens

Antibacterial activity of selected spices was carried out against the isolated UTI pathogens using agar well diffusion method. Urinary tract infected sample were inoculated into 10ml of sterile nutrient broth and incubated at 37°C for 24 hours. Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile Mueller- Hinton agar plates. Agar wells were prepared with the help of sterile cork borer with 10mm diameter. Using a micropipette, different concentrations of spices extracts were added to different wells in the plate. The plates were incubated in an upright position at 37°C for 24 hours. The diameter of inhibition zones was measured in mm and the result were recorded.

#### Determination of minimal bactericidal concentration (MBC)

MBC was determined for those extract which showed the antibacterial activities by two fold dilution method. Determination of MICs of the spice extracts was done by broth dilution techniques and the concentrations of the extracts used were 3.12, 6.25, 12.5, 25, 50 mg/ml. The lowest concentration that did not permit any visible growth on nutrient agar medium when compared with the control was considered as the minimum inhibitory concentration.

## RESULTS

The urine samples from urinary tract infected patients were found to be *Citrobacter freundii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus saprophyticus*, *Enterobacter aerogenes*. These isolated pathogens were found to be highly resistant against all the selected commercial antibiotics such as

Streptomycin(30mcg), Gentamycin(30mcg), Chloramphenicol(30mcg), Oxacillin(30mcg), Vancomycin(30mcg), Ciprofloxacin(30mcg) and Methicillin(30mcg) (Table-1).

**Table-1 Selected commercial drugs for Urinary tract Microorganisms**

Organisms	Antibiotics(30mcg)						
	Strepto- mycin	Gentomycin	Chloram- phenicol	Oxacillin	Vancomycin	Ciprofloicacin	Methicillin
<i>Escherichia coli</i>	-	-	-	-	-	-	-
<i>Enterobacter aerogenes</i>	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-
<i>Citrobacter freundii</i>	-	-	-	-	-	-	-
<i>Proteus vulgaris</i>	-	-	-	-	-	-	-
<i>Klebsisella pneumonia</i>	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-

- = Resistant.

The chloroform extracts (100mg/ml) of *Allium sativum*, *Myristica fragrans*, *Zingiber officinale*, *Piper nigrum* and *Allium cepa* did not show any inhibitory action against UTI microflora.

Among the five spices tested, garlic, zinger and nutmeg ethanol extract showed effective antibacterial activity against UTI pathogens is given in Table 2. The ethanol extract of *Zingiber officinale* inhibited *Staphylococcus saprophyticus* and *Proteus mirabilis* only. The ethanol extracts of *Myristica fragrans* showed inhibitory against *Enterobacter aerogenes* and *Escherichia coli*. But the ethanol extracts of *Piper nigrum* and *Allium cepa* did not show any inhibitory action against isolates of urinary tract microflora. The ethanol extract of *Allium sativum* was found to be effective inhibition against all isolates of urinary tract infections.

The *Myristica fragrans* ethanol extract showed maximum zone of inhibition of 25mm at 200mg /ml concentration against *Enterobacter aerogenes*, 18mm at 200mg /ml concentration against *Pseudomonas aeruginosa* and 17mm/ml against *Escherichia coli*. The ethanol extracts of *Myristica fragrans* showed effective antibacterial activity against UTI pathogens is given in Table- 3.

The Garlic ethanol extract showed excellent antibacterial activity at all concentrations (50, 100,150 and 200mg/ml) against all UTI pathogens. In the present investigation, the activity increased in a linear manner of concentration. The extract showed better antibacterial

activity against UTI pathogens at a concentration of 200mg/ml. At concentration of 200mg/ml, *Citrobacter freundii* was least sensitive (20 mm) and *E. coli* more sensitive (27 mm) (Fig.1).

**Table-2 Antimicrobial Activity of ethanol extract of selected spices against Urinary tract Microflora**

Spices(ethanol extract - 100mg)	Organisms						
	<i>Enterobacter aerogenes</i>	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>	<i>Proteus mirabilis</i>	<i>Staphylococcus saprolyticus</i>	<i>Citrobacter freundii</i>	<i>Klebsiella pneumoniae</i>
<i>Allium sativum</i>	+++	+++	+++	+++	+++	+++	+++
<i>Myristica Fragrans</i>	+++	+++	+++	-	-	-	-
<i>Zingiber officinale</i>	-	-	-	++	++	-	-
<i>Piper nigrum</i>	-	-	-	-	-	-	-
<i>Allium cepa</i>	-	-	-	-	-	-	-

- Resistant,+ Intermediate,++ Sensitive,+++ Highly Sensitive

**Table – 3 Antimicrobial activity of ethanol extract of *Myristica fragrans* against UTI microflora**

Organism	Ethanol extract of <i>Myristica fragrans</i> (Concentration)			
	50 mg	100mg	150mg	200mg
<i>Enterobacter aerogenes</i>	7	15	19	25
<i>Pseudomonas aeruginosa</i>	5	9	14	18
<i>Staphylococcus saprophyticus</i>	-	-	-	-
<i>Escherichia coli</i>	5	10	13	17
<i>Proteus mirabilis</i>	-	-	-	-
<i>Citrobacter freundii</i>	-	-	-	-
<i>Klebsiella pneumoniae</i>	-	-	-	-

Figure.1 Antibacterial activity of ethanol extract of *A. sativum* against UTI pathogens

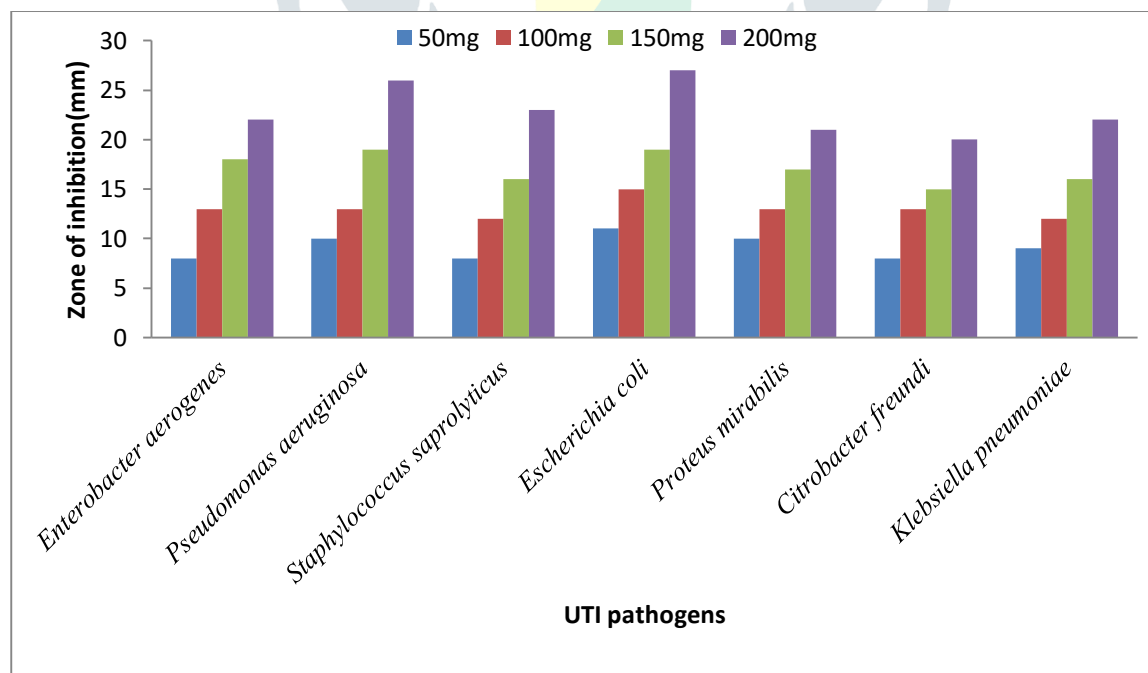


Table-4 The MBC of ethanol extract of garlic against UTI pathogens

UTI pathogens	<i>Enterobacter aerogenes</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus saprophyticus</i>	<i>E. coli</i>	<i>Proteus mirabilis</i>	<i>Citrobacter freundii</i>	<i>Klebsiella pneumoniae</i>
MBC (mg/ml)	25	12.5	25	6.25	12.5	25	12.5

Table 4 showed the MBC of different effective concentration of ethanol extract of garlic. The MBC ranged from 6.25mg/ml to 25mg/ml in case of ethanol extract of garlic. The MBC value was given by ethanol extract of garlic against *E. coli* was 6.25mg/ml. The MBC value of 12.5mg/ml was *Pseudomonas mirabilis* and *Klebsiella pneumoniae*.

## Discussion

In the present work, isolated UTI pathogens were *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus saprophyticus*, *Proteus mirabilis*, *Citrobacter freundii* and *Klebsiella pneumoniae*. Similarly, Sobel (2014) also reported that the most common pathogen for uncomplicated UTIs is *E. coli* (75%–95%), followed by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B streptococci and *Proteus mirabilis*.

Antibiotic toxicity and multi drug resistant pathogens are the two greatest challenges being faced by today's medical world. In the present study, the antimicrobial activity of spices has been investigated as a choice to antibiotics in order to undertake these dangers. In recent years, Sobel (2014) stated that worldwide spread of extended-spectrum  $\beta$ -lactamase – producing *E. coli* has evolved as an important cause of community-associated UTIs. In the present study, isolated UTI pathogens were mostly resistant to commercially available antibiotics. To avoid this problem, alternative chemotherapeutic compounds were needed. Antimicrobial activity of spices has been used for medicinal purposes for several centuries (Bagamboula *et al.*, 2003). In this work, *Allium cepa* (Onion), *Allium sativum* (Garlic), *Myristica fragrans* (Nutmeg) *Zingiber officinale* (Ginger) and *Piper nigrum* (pepper) were tested for antimicrobial activity against Urinary Tract microflora.

This study compares the sensitivity of selected UTI pathogens against chloroform and ethanol spice extracts (100mg/ml) by agar well diffusion method. Ethanol extracts of *A. sativum* showed highest antibacterial activity against UTI pathogens. Antibacterial activity of chloroform extract of nutmeg seeds against both gram-positive and gram-negative bacteria was reported by Narasimhan and Dhake (2006). Contrary to the above, in my present work, chloroform extract (100 $\mu$ g) of nutmeg seeds did not show any antibacterial activity against UTI pathogens. And also, chloroform extracts of onion and pepper did not show any antibacterial activity against the UTI pathogens.

Garlic is one of the important bulb spices and its ethanol extract has potent antimicrobial activity against UTI pathogens. Ethanol should be a good solvent for most active compounds. Caccioni *et al.* (2000) reported that antibacterial activity of spices disrupts membrane of pathogens; followed by destruction of electrons transport systems (Tassou *et al.*, 2000) and cell wall perturbation (Odhav *et al.*, 2002).

*Allium sativum*, commonly known as garlic is a species in the onion family Alliaceae and belongs to the plant order liliales (Rocio, 1982). Of all the *Allium* species, garlic is the most important (Alli *et al.*, 2011) and it has the typical odour (Block, 1985). Research by Moore and Atkins (1977) had revealed that garlic stimulates the activity of the defensive cells of the body such as the lymphocytes and macrophages. When the Garlic cell is crushed/injured/sliced, the enzyme allinase comes in contact with alliin and causes its breakdown into the sulphur containing product of allicin. Allicin is the most important antibacterial substance of garlic (Levetin and McMahon, 2006) and it plays an important role in killing bacteria to prevent infection (Kyolic, 2003). Increasingly, there is a big problem with harmful and infectious organisms adapting and mutating to become drug-resistant, dangerous strains. Based on the above result, it can be concluded that the ethanol extract of *A. sativum* possess excellent antibacterial properties against UTI pathogens. Lowest MBC values were given by ethanol extract of *A. sativum* against pathogens. So spices of ethanol extract of *A. sativum* could be remedies for certain ailments and active compounds of garlic may be an alternative medicine to resistant UTI pathogens. This paper established ethanol extract of garlic for its potential uses in preventing resistant UTI pathogens. Today, with the ever-growing resistant organisms, taking of garlic ethanol extract remains powerful antimicrobial active compounds should be an excellent alternative medicine.

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