

Antimicrobial activity of *Allium cepa* and *Cinnamomum zeylanicum* against common bacteria causing urinary tract infections: in vitro study

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ABSTRACT

Background: This study was undertaken to investigate and compare the antimicrobial effect of Onion bulbs (*Allium cepa*) and cinnamon bark (*Cinnamomum zeylanicum*) against some common bacteria causing Urinary tract infection.

Methods: Antimicrobial activity of the plant extract in different concentrations was observed. Commonly isolated bacteria from the urine samples of suspected and untreated patients which were found to be *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Results: The comparison between zones of inhibition showed a statistically significant data of both *Allium cepa* and *Cinnamomum zeylanicum*. The antimicrobial activity of cinnamon extract showed the maximum effect against *Klebsiella pneumoniae*, zone of inhibition 25.50 mm±3.72 with 2±1 of extract and *E. coli* with zone of inhibition 11.72mm±1.86. The onion (*Allium cepa*) extract exhibited some antimicrobial effect, it was most effective against *E. coli*, though the effect was minimal.

Conclusions: From the current study we conclude that the extract of onion and cinnamon bark are promising but were not as effective as the conventional antibiotics such as ciprofloxacin and nitrofurantoin but can be a good alternative in selected group of patient.

Keywords: *Allium cepa*, antimicrobial activity, *Cinnamomum zeylanicum*, plant extract, urinary tract infection

INTRODUCTION

Urinary tract infection (UTI) is a common and painful bacterial disease occurring in all age groups. It affects about 40 to 50% of women population of reproductive age groups. The increased risk of urinary tract infection in specific subpopulation majorly includes infants, pregnant women, the elderly patients with diabetes, catheterized patients with serious injuries, acquired immunodeficiency syndrome patients and patients with underlying urological abnormalities. In pregnant women the treatment becomes difficult due to restricted use of antimicrobial therapy.

urinary tract infection is also common in rural areas of India, this may be attributed to lack of personal hygiene. In such areas the major problem faced by clinicians and the patients is burden of cost of medicine leading to lack of compliance by patients, which further results in drug resistance.

Most common causative organisms observed is *Escherichia coli*, a commensal found in rectum is responsible for maximum incidences of urinary tract infection. Many studies suggested that *E. coli* was the most common isolated microorganism 37-44% in cases of

urinary tract infection, Infrequent causative agents of urinary tract infections include bacteria such as *Staphylococcus aureus*, *Gardnerella vaginalis*, *Corynebacterium* and *Lactobacilli*, yeasts such as *Candida*. High level of resistance was shown by these isolates to commonly prescribed antibiotics specially Fluoroquinolones.¹

There is a considerable variation among general practitioners in the management of patients presenting with symptoms of urinary tract infection.² Mild cases of urinary tract infection are managed conservatively with plenty of water and urine alkalinizers, but sever cases require prompt antimicrobial therapy.

Fluoroquinolones e.g. Norfloxacin, Ciprofloxacin are most commonly prescribed antibiotics in treatment of urinary tract infection cases, though less commonly used antimicrobials are Amoxicillin, Gentamicin, Amikacin, Tetracycline, Nitrofurantoin, Cotrimoxazole (sulfamethoxazole + trimethoprim) and penicillin. But especially in pregnancy drug safety is an important concern and a good percentage of pregnant women is not willing to use conventional antibiotics but has inclination towards the use of plant derived products.

The natural products found commonly in kitchen along with other spices is Cinnamon and Onion is the most common vegetable used around the world. The antimicrobial and anti-inflammatory properties of these common household items was well known to mankind since ancient times.³

Cinnamon (*Cinnamomum zeylanicum*) is one such spice that can potentially help to combat the infectious diseases. It also has antidiabetic, antioxidant and antiulcer activity. The bioactive compound was identified as cinnamaldehyde. Which is responsible for the antimicrobial activity of this spice.⁴

In folk medicine as alternative medication, onion has been used for asthma, bronchitis, whooping cough and similar ailments. Other uses include the treatment of stingray wounds, warts, acne, appetite loss, urinary tract disorders and indigestion.

Onion (*Allium cepa*) is one of the most commonly found vegetable in the world, it has wound healing properties, used for hair regrowth and antimicrobial effect as well, the active compounds: a flavinol compound Quercetin along with its glycosides and allicin an organosulfer compound, these chemicals of onion are responsible for their antifungal, antibacterial and antiviral properties

The present study is undertaken to determine the antimicrobial activity of *Cinnamomum Zeylanicum* and *Allium Cepa* against some common bacteria causing urinary tract infection. These kitchen herbs are with known and presumed antimicrobial activity already in use. And hence this project was undertaken to explore and evaluate

their antimicrobial activity to add on the list of easily accessible medicine with almost no side effects as compared to the allopathic counterpart.

METHODS

Locus

The present study was undertaken in the Department of Pharmacology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha, Maharashtra.

The Phytochemical extraction and identification procedures were done in Central Research Laboratory, Datta Meghe Institute of medical Sciences, Sawangi (Meghe), Wardha, Maharashtra.

The microbiological procedures were executed in the Department of Microbiology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha, Maharashtra, India,

Duration of study was from January 2017 to September 2018.

Study population

A total of 301 patient were included in the study who were clinically assessed and suspected cases but untreated patients of urinary tract infection and were admitted in ward or ICU of Acharya Vinoba Bhave Rural Hospital, Sawangi (MEGHE), Wardha, Maharashtra, India, 442001. 301 urine samples were taken in this study.

Inclusion criteria

- Urine samples of suspected untreated cases of Urinary Tract Infection
- Patients who were not prescribed any antimicrobial therapy.
- Patients admitted in In-patient department

Exclusion criteria

- Suspected cases of Urinary tract infection who were prescribed antimicrobial therapy.
- Patients receiving treatment from outpatient department.

Statistical Analysis

Statistical analysis was done by using descriptive and inferential statistics using z-test for difference between two means and software used in the analysis was SPSS22.0 version and $p < 0.05$ are considered as level of significance.

Plant material and extraction procedure

The Bark of *Cinnamomum zeylanicum* was purchased from Ayurvedic College, Salod, Wardha, India. The barks

were washed with water, air dried at ambient temperature, then ground into coarse powder in a grinder. For the preparation of ethanolic extracts, 50g of coarsely powdered bark of cinnamon was extracted with 250 ml ethanol in soxhlet apparatus optimized at 70°C for 8h and ~200 ml of product was recovered then air dried in an open glass plate at ambient room temperature.

Onion bulbs were purchased from local vegetable market in Wardha city. The onion bulbs were cleaned of any dirt from outside, the roots were cut using a knife and the tunic layer was removed by hands, 100 gm of onion was grinded to a smooth paste, the paste was then strained. A pinkish white liquid was obtained, 5 ml of extract was mixed with 5ml of acetone and filtered using whatman filter paper no. 1. It was transferred in a borosilicate glass bottle and stored in a refrigerator at 4°C until further use.

Preparation of agar media

Himedia Hichrome Muller Hinton agar and disposable sterile petri dishes were purchased from local vendor in Wardha, India. A 40% of agar solution in distilled water was made. The Agar was autoclaved, allowed to cool and poured in sterile petri dishes, cooled further until solidified.

Preparation of culture for antimicrobial susceptibility test

The research protocol was approved from Institutional Ethic Committee at Datta Meghe Institute of Medical Sciences. (Deemed to be university), Sawangi (M), Wardha.

The morning urine samples of the suspected & untreated (without antimicrobial therapy) 301 cases of urinary tract infection patients after thorough checking of history were collected after consent from the patients. The samples were centrifuged and the bottom residue of sample were inoculated on the culture media plate by streak culture method, next day the colonies of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* were identified with the help of their color on the Hi chrome agar and isolated (Figure 1).

The isolated colonies were incubated in peptone water for 3 h then inoculated again by lawn culture method on the culture media for antimicrobial activity of Ciprofloxacin, nitrofurantoin, cinnamon extract, cinnamaldehyde standard, onion extract, quercetin standard was observed. Results were analyzed and compared.



Figure 1: Isolated colonies of *E. coli*. Isolated colonies of *Pseudomonas aeruginosa*. Isolated colonies of *Klebsiella pneumoniae*.

Statistical analysis

was done by using descriptive and inferential statistics using z-test for difference between two means and software used in the analysis was SPSS 22.0 version and $p < 0.05$ is considered as level of significance.

RESULTS

The mean zone of inhibition (Table 1) of Ciprofloxacin against *E. coli* was 32.55 mm±2.48, for Nitrofurantoin 21.02 mm±2.05 and Cinnamon extract 11.72±1.86, for Cinnamaldehyde 24.39 mm±4.05, for onion extract 6.29 mm±1.23 and for quercetin 14.58±1.86 By using Z-test for the difference between 2 means, statistically significant

difference was found in mean zone of inhibition between ciprofloxacin and nitrofurantoin in comparison with cinnamon extract, cinnamaldehyde, onion extract and quercetin (Figure 2).

The mean zone of inhibition of ciprofloxacin against *Klebsiella pneumoniae* was 32.03 mm±2.38, for nitrofurantoin 27.01 mm±2.34, for cinnamon extract 25.50 mm±3.72, for cinnamaldehyde 18.45±3.42, for onion extract 0.30±0.73 and for quercetin 10.22±1.63 by using Z-test for difference between 2 means, statistically significant difference was found in mean zone of inhibition (Table 2) between ciprofloxacin and nitrofurantoin in comparison with cinnamon extract, cinnamaldehyde, onion extract and quercetin (Figure 3).

Table 1: Zone of Inhibition in mm of ciprofloxacin, nitrofurantoin, cinnamon extract, cinnamaldehyde standard 98%, onion extract and quercetin standard 98% against *E. coli*.

	N	Mean (mm)	Std. deviation	Std. Error Mean	z-value	p-value
Ciprofloxacin	301	32.55	2.48	0.14	116.32	0.0001, S
Nitrofurantoin	301	21.02	2.05	0.11	58.24	0.0001, S
Cinnamon extract	301	11.72	1.86	0.10	-	-
Cinnamaldehyde	301	24.39	4.05	0.23	-	-
Onion Extract	301	6.29	1.23	0.07	-	-
Quercetin	301	14.58	1.86	0.10	-	-

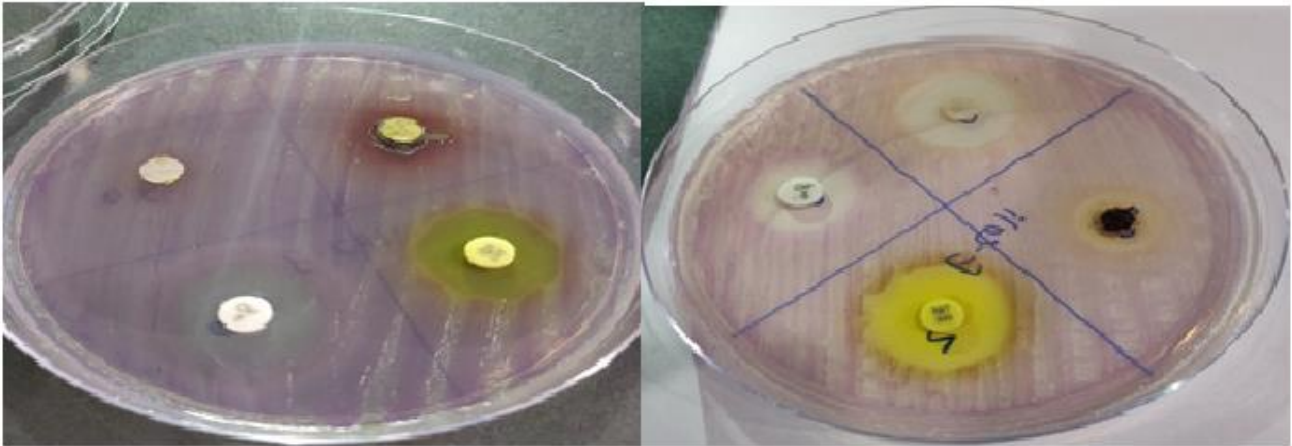


Figure 2: Zones of inhibition of ciprofloxacin, nitrofurantoin, onion extract, quercetin against *E. coli*. Zones of inhibition of ciprofloxacin, nitrofurantoin, cinnamon extract, cinnamaldehyde against *E. coli*.

Table 2: Zone of inhibition in mm of ciprofloxacin, nitrofurantoin, cinnamon extract, cinnamaldehyde standard 98%, onion extract and quercetin standard 98% against *Klebsiella pneumoniae*.

	N	Mean	Std. Deviation	Std. Error Mean	z-value	p-value
Ciprofloxacin	301	32.03	2.38	0.13	25.65	0.0001,S
Nitrofurantoin	301	27.01	2.34	0.13	5.98	0.0001,S
Cinnamon Extract	301	25.50	3.72	0.21	-	-
Cinnamaldehyde	301	18.45	3.42	0.19	-	-
Onion Extract	301	0.30	0.73	0.04	-	-
Quercetin	301	10.22	1.63	0.09	-	-

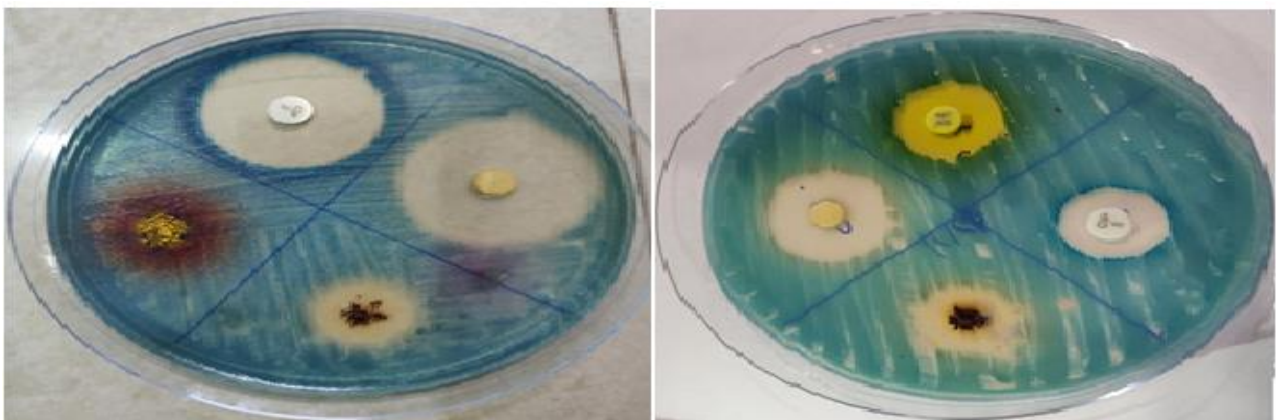
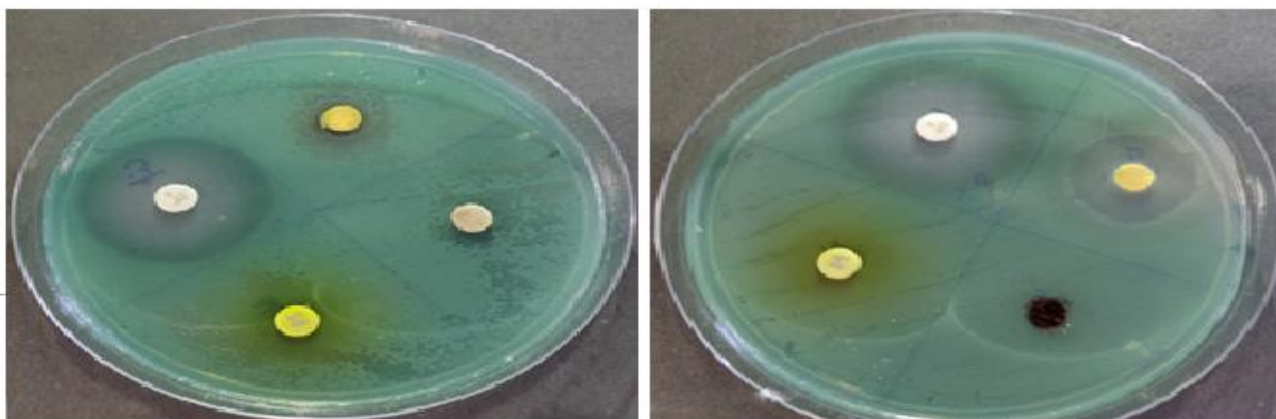


Figure 3: Zone of inhibitions of ciprofloxacin, quercetin, cinnamaldehyde and cinnamon extract against *Klebsiella pneumoniae*. Zone of inhibitions of ciprofloxacin, nitrofurantoin, cinnamon extract and quercetin against *Klebsiella pneumoniae*.

Table 3: Zone of Inhibition in mm of ciprofloxacin, nitrofurantoin, cinnamon extract, cinnamaldehyde standard 98%, onion extract and quercetin standard 98% against *Pseudomonas aeruginosa*.

	N	Mean	Std. Deviation	Std. error mean	z-value	P-value
Ciprofloxacin	301	38.50	2.16	0.12	84.06	0.0001, S
Nitrofurantoin	301	23.25	4.33	0.24	10.04	0.0001, S
Cinnamon Extract	300	20.16	3.10	0.17	-	-
Cinnamaldehyde	301	14.82	3.01	0.17	-	-
Onion Extract	301	5.06	0.24	0.01	-	-
Quercetin	301	14.30	1.96	0.11	-	-

**Figure 4: Zones of inhibition of ciprofloxacin, nitrofurantoin, onion extract, quercetin against *Pseudomonas aeruginosa*. Zones of inhibition of ciprofloxacin, nitrofurantoin, cinnamon extract, cinnamaldehyde against *Pseudomonas aeruginosa*.**

The mean zone of inhibition of ciprofloxacin against *Pseudomonas aeruginosa* (Table 3) was 38.50 mm \pm 2.16, for nitrofurantoin it is 23.25 mm \pm 4.33 and for cinnamon extract 20.16 mm \pm 3.10, for cinnamaldehyde is 14.82 mm \pm 3.01, for onion extract is 5.06 mm \pm 0.24, for quercetin is 14.30 \pm 1.96 by using Z-test for difference between 2 means, statistically significant difference was found in mean zone of inhibition between ciprofloxacin and nitrofurantoin in comparison with cinnamon extract, cinnamaldehyde, onion extract and quercetin.(Figure 4).

DISCUSSION

The present study was undertaken to investigate and compare the antimicrobial effect of *Allium cepa* and *Cinnamomum zeylanicum* against some common bacteria causing urinary tract infection. The antimicrobial activity of the cinnamon extract was due to the presence high concentration of cinnamaldehyde which was about 85.06% w/w. The susceptibility test disc containing the standard of cinnamaldehyde 98% was placed over the inoculated culture media, thus confirming that the antimicrobial activity of the cinnamon extract was due to the presence of cinnamaldehyde and the presence of quercetin was thought be the reason for antimicrobial property.

In the current study the mean zone of inhibition of cinnamon extract against *E. coli* was 11.72 mm \pm 1.86. On the contrary to above findings another study conducted by B. Kaskatepe et al, observed that mean zone of inhibition of cinnamon extract against *E. coli* was 38 mm.5

In this study the mean zone of inhibition of cinnamaldehyde against *E.coli* was 24.39 mm \pm 4.05, while another study conducted by Gupta et al. in 2008 the mean zone of inhibition of cinnamaldehyde against *E.coli* was found to be 16 mm.⁶ This variation in the results may be due to different manufacturing processes of the standard.

In this study the mean zone of inhibition of onion extract against *E. coli* against was observed to be 6.29 mm \pm 1.23 however completely contrary to the above finding another study conducted be J. Santas et al, observed no antimicrobial activity of onion extract against *E. coli*.⁷

From It was observed in this study that, the mean zone of inhibition of Quercetin against *E.coli* against was 14.58 mm \pm 1.86, in the similar study conducted by be J. Santas et al. in 2010 observed the mean zone of inhibition obtained by quercetin was 9.8 mm \pm 0.6.⁷ This difference in the observations may be because of the different solvents used by the researchers.

Current study has observed that the mean zone of inhibition of cinnamon extract against *Klebsiella pneumoniae* was 25.50 mm±3.72 similarly, the study conducted by Chang et al. in 2001 also found that cinnamon extract shows inhibitory activity against *Klebsiella Pneumoniae* through different parameters.⁸

In this study the mean zone of inhibition of cinnamaldehyde against *Klebsiella pneumoniae* was 18.45 mm±3.42, in another study conducted by Dhara and Tripathi et al, observed that most study samples of bacterial isolates inhibited between the range of 21-30 mm.⁹ Thus both studies have observed the antibacterial activity of cinnamaldehyde.

In the current study the mean inhibition zone of onion extract against *Klebsiella pneumoniae* was found to be 0.30 mm±0.73, similarly on study carried out by Lekshmi N. C. J et al. in 2015 observed similar results and the water extract of onion displayed no antimicrobial activity against *Klebsiella pneumoniae*.¹⁰ In the above study the mean zone of inhibition of quercetin was found to be 10.22 mm±1.63, the study conducted by Elzbieta Woenicka et al, also found that quercetin shows inhibitory activity against *Klebsiella Pneumoniae* through different parameters.¹¹

In this study the mean zone of inhibition of cinnamon extract against *Pseudomonas aeruginosa* was found to be 20.16 mm±3.10, similarly in the study carried out by Grullon et al, observed that the inhibition zone of Cinnamon extract against *Pseudomonas aeruginosa* was 25. mm.¹² In the present study the zone of inhibition of cinnamaldehyde against *Pseudomonas aeruginosa* was found 14.82 mm±3.01, on the contrary study done by Kaskatepe et al. in the year 2016 observed that the zone of inhibition of cinnamaldehyde against *Pseudomonas aeruginosa* was more than 22 mm. This variation in the results may be due to different manufacturing processes of the standard.^{5,13}

In this study it was observed that the mean zone of inhibition of onion extract against *Pseudomonas aeruginosa* was 5.06 mm±0.24, similarly in a study conducted by N Azu et al, found that the zone of inhibition of onion extract against *Pseudomonas aeruginosa* was 17mm.¹⁴ In the current study the mean zone of inhibition of quercetin against *Pseudomonas aeruginosa* was found to be 14.30 mm±1.96, Jaisinghani et al, conducted similar study and found the antimicrobial effect of quercetin against *Pseudomonas aeruginosa* through different parameters.

CONCLUSION

We conclude with our study that the phytochemicals have different activity against some common bacteria which cause urinary tract infection. The comparison between zones of inhibition showed a statistically significant data of both *Allium cpea* and *Cinnamomum zeylanicum*. The antimicrobial activity of cinnamon extract showed the

maximum effect against the bacteria causing urinary tract infection and maximum activity was seen against *Klebsiella pneumoniae* and *E. coli*.

The onion (*Allium cepa*) extract exhibited some antimicrobial effect, it was most effective against *E. coli*, though the effect was minimal. Cinnamon bark (*Cinnamomum zeylanicum*) extract showed promising antimicrobial activity against *Klebsiella pneumoniae* but it was also effective against *E. coli* and *Pseudomonas aeruginosa*. From the current study we also conclude that the extract of onion and cinnamon bark were not as effective as the conventional antibiotics such as ciprofloxacin and nitrofurantoin but can be a good alternative in selected group of patient. Further extensive preclinical and clinical studies are required to evaluate the safety, efficacy and dose range in human subjects.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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