

**Anti-virulence effects of aqueous  
pomegranate peel extract on *E. coli*  
urinary tract infection**

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# Introduction

- Urinary Tract Infection (UTI) is defined as the microbial invasion of any tissues in different parts of the urinary tract.
- It is the second most common infectious presentation in community medical practice.
- It has been reported in several studies that the Gram negative bacteria of *E. coli* cause 70-95% of upper and lower UTIs.

# Introduction



# Introduction

Treatment strategy



A fluoroquinolone for 7-10 days



Or a third-generation oral  
cephalosporin could be an alternative





## Problematic

- It has been found that the numbers of fluoroquinolone-resistant *E. coli* have increased in some parts of the world.
- Thus restricting the use of fluoroquinolones.

## Solution

- Medicinal plants have always been a good source to find new remedies for human health problems.
- Recently, a wide range of these plants have been screened for antimicrobial property.





# Why?

- Cranberry products in a variety of formulations have undergone extensive evaluation over several decades in the management of UTIs.
- At present, there is no evidence that cranberry can be used to treat UTIs.
- Hence, the focus has been on its use as a preventative strategy.



# Why?

- Cranberry has been effective in vitro and in vivo in animals for the prevention of UTI.
- Cranberry appears to work by inhibiting the adhesion of type of E. coli to the uroepithelium, thus impairing colonization and subsequent infection.
- Reasonable evidence suggests that the anthocyanidin/proanthocyanidin moieties are potent antiadhesion compounds.

# Introduction

- Pomegranate peels (*Punica granatum*) are considered wastes or byproduct obtained through juice processing.
- It is characterized by significant presence of ellagitannins and polyphenols, gallic acid and ellagic acid as well as flavonoids—associated with biological properties such antimicrobial agents.





# Previous studies on the antimicrobial efficacy of pomegranate peels

80% methanolic extract of peels was a potent inhibitor for *Klebsiella pneumonia*, *Proteus vulgaris*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*.

In contrast Nuamsetti et al. found that the hot water extract of the peels was most potent against *E. coli* compared to 95% ethanol and acetone extracts.

Ethanol extract of pomegranate peels has lowest MIC against *E. coli*, *P. aeruginosa* and *S. aureus* compared to MICs of methanolic and hot water extracts.

The inhibitory zones of all the three extracts were greater than that of the standard antibiotic Tetracycline.



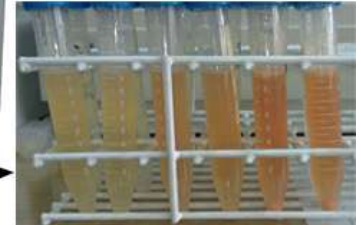


# Objective

- The objective of this study was to explore the efficacy of aqueous pomegranate peel extract to reduce pathogenicity of *E. coli* responsible for UTI (antibacterial and antivirulence activity).

# Materials and methods

- Pomegranate peel extract





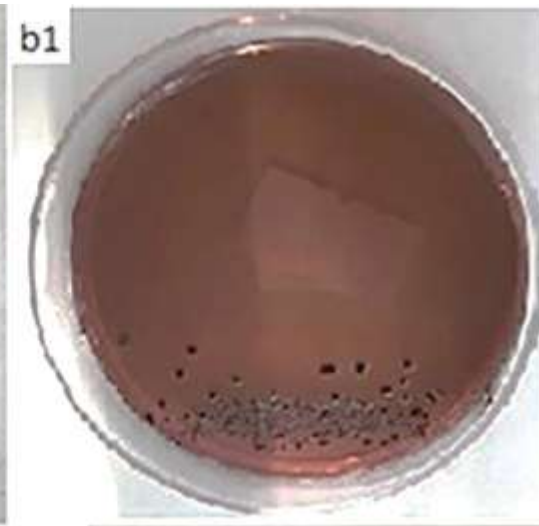
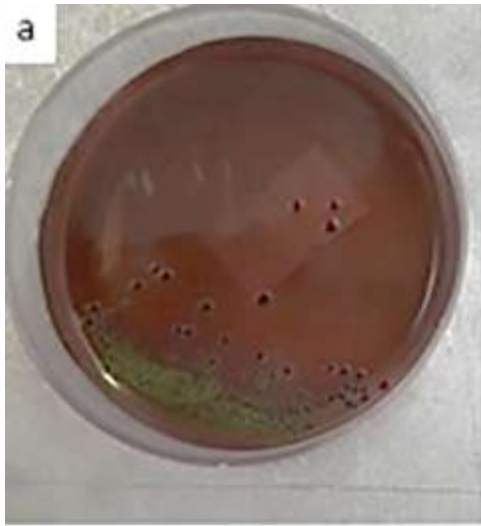
# Materials and methods

- **Microbial cultures**
- Cultures of *E. coli* were provided from urinary culture collections.
- Bacteria were incubated at  $37 \pm 0.1^\circ\text{C}$  for 24 h in Nutrient Broth.
- A standardized suspension:  $1.5 \times 10^8$  CFU/ml.



# Antibacterial efficacy

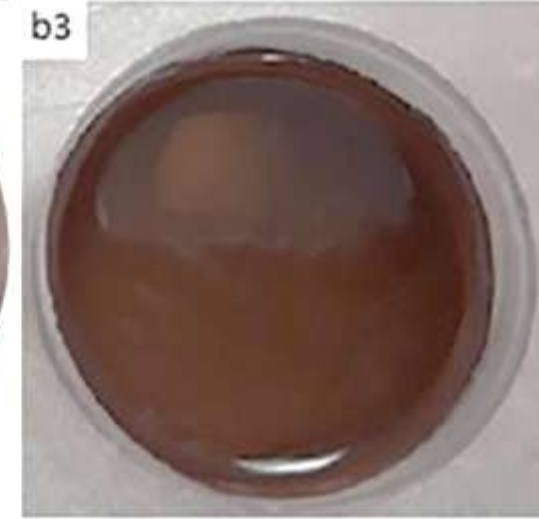
- **Determination of MIC:** using microdilution broth method (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mg/ml)
- **Determination of MBC:** on Eosin Methylene Blue agar (EMB) plates.
- After 24 h of incubation at 37°C, the concentration of antibacterial agent in the tube that showed no bacterial growth was recorded as MBC.



E. coli growth on EMB

(a): before.

(b1, b2 and b3): after.

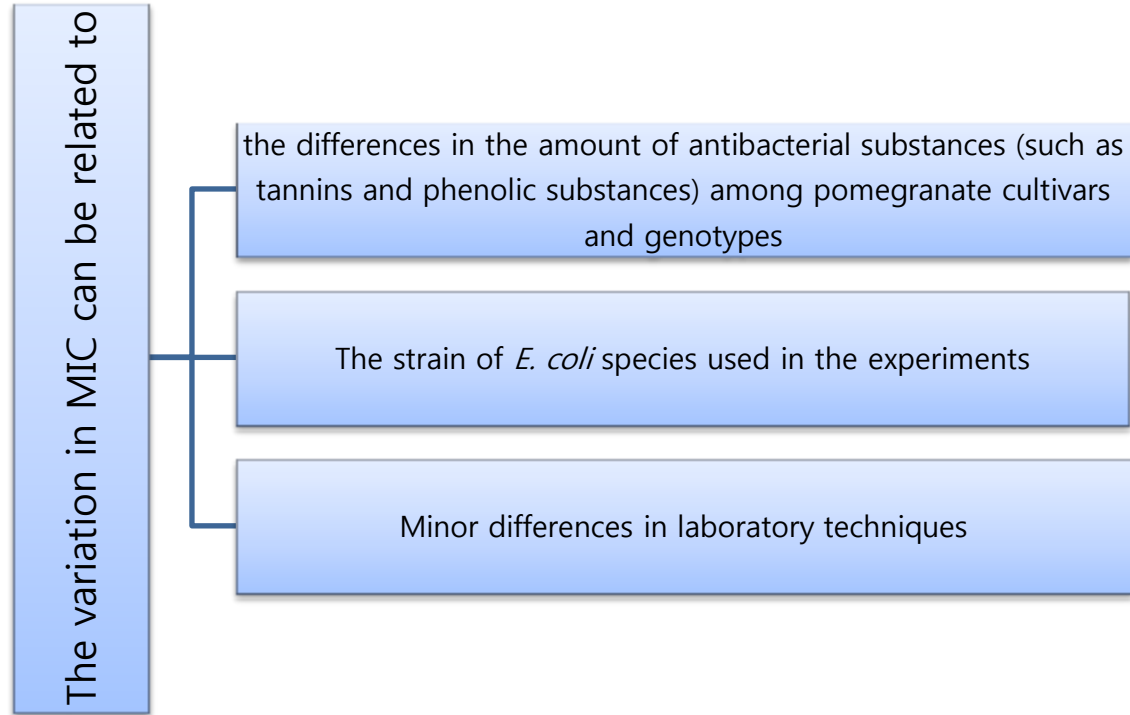


MIC = 0.6 mg/ml  
MBC = 1.2 mg/ml.



# MIC & MBC

- In different recent studies, MIC varied from 0.19–25 mg/ml against several strains of *E. coli*.



# Antivirulence efficacy

## Adhesion assay

### Collection of uroepithelial cells

Uroepithelial cells were obtained from fresh urine from normal healthy women with no history of urinary or vaginal infections and who are not taking contraceptive or antimicrobial agents.

The urine was immediately centrifuged at 4000 rpm for 15 minutes, the supernatant was discarded and the uroepithelial cells were harvested

The number of cells was calculated by direct light microscopy

# Antivirulence efficacy

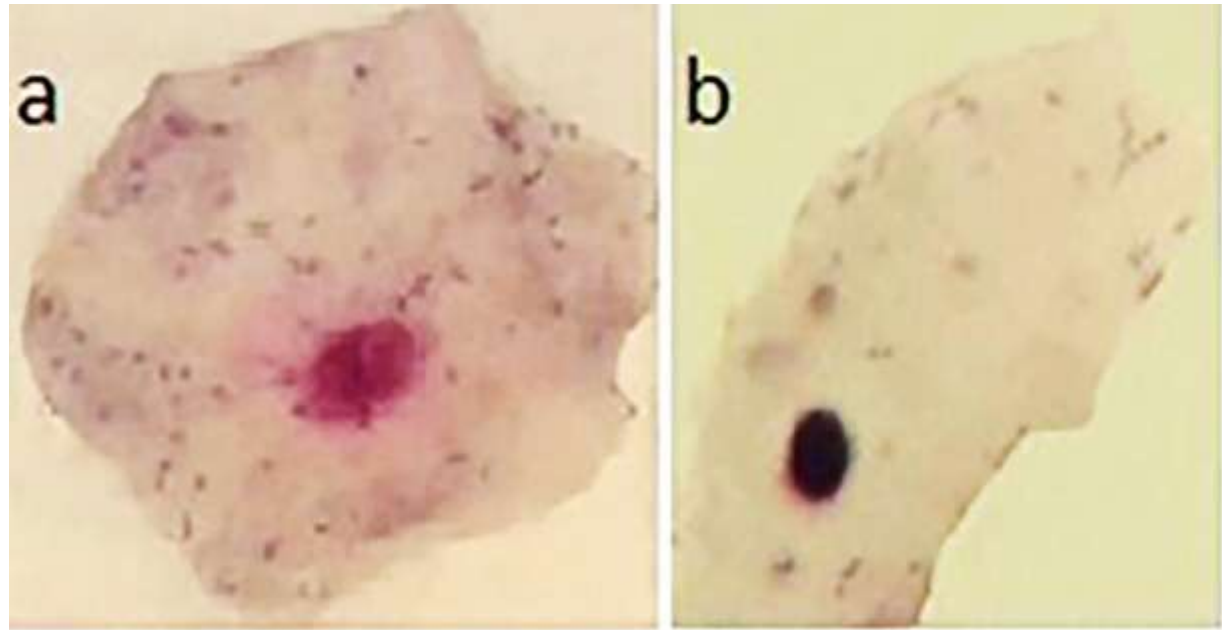
## Adhesion assay

### In-vitro assay

One ml of bacterial suspension was mixed with one ml of epithelial cell suspension. The mixture was incubated in shaking water bath at 37°C for 3 hours.

A portion of the final cell suspension was placed on a slide, air dried, methanol fixed and stained with Giemsa stain (10%) for 30 minutes and examined under light microscopy (X100).

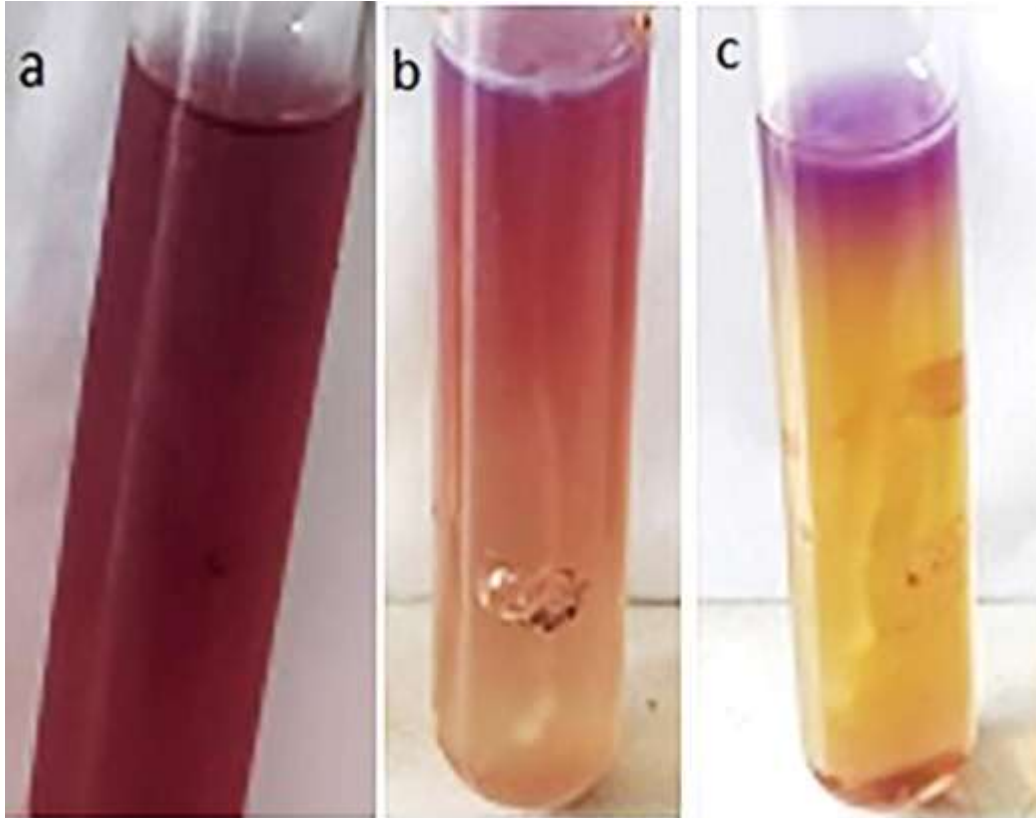
The average number of adhering bacteria per cell was obtained from an examination of 50 cells. Each test was performed in triplicate.



- a: *E. coli* adherent to a cell on a control slide.
- b: *E. coli* adherent to cells after treatment with aqueous pomegranate peel extract (1.0 mg/ml).  
With a reduction of up to 80% of the adhesion index.

# Antivirulence efficacy

## Motility Assay



MIO test

a: the MIO medium

b: MIO medium + *E. coli*  
untreated

c: MIO medium + *E. coli*  
treated at MIC

# Antivirulence efficacy

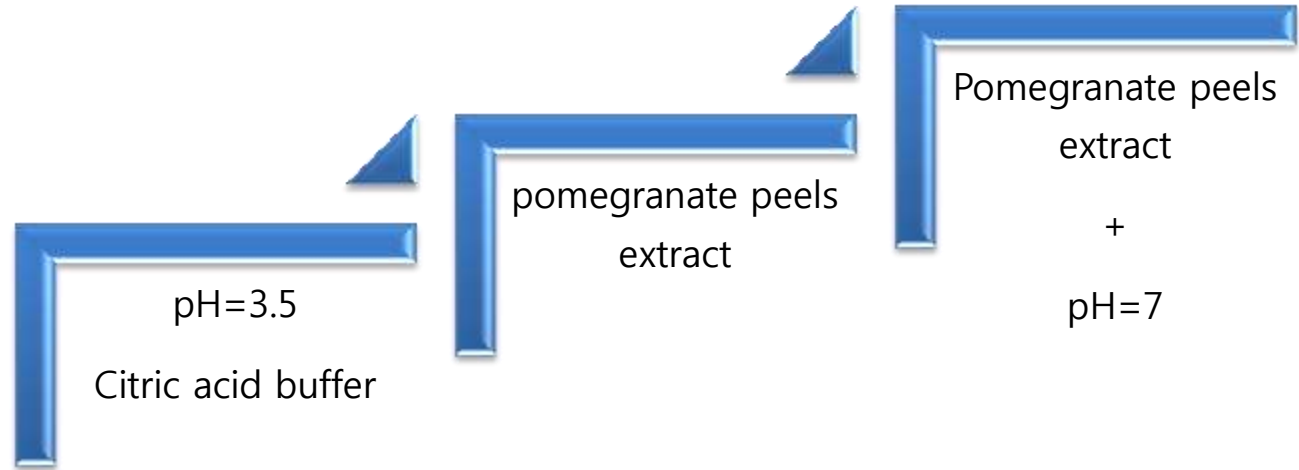
- A positive motility test is indicated by a diffuse cloud of growth away from the line of inoculation.
- Whereas ornithine decarboxylation is indicated by a purple color in the medium.
- A negative ornithine reaction produces a yellow color at the bottom of the tube.
- Activity of ornithine decarboxylase results in production of polyamines such as putrescine and spermidine which play an important role in biofilm formation and so in cellular adherence of *E. coli*





# Antivirulence efficacy

pH effect



# Antivirulence efficacy

## pH effect

The use of citric acid buffer could inhibit the *E. coli* growth with no bactericidal properties.

The aqueous pomegranate peel extract adjusted to pH=7 showed no bacteriostatic effect on *E. coli*.

PPO effect





# Bioavailability

- Ellagitannins are hydrolyzed to ellagic acids.
- Ellagic acid was found in urine and lungs of mice fed pomegranate ellagitannins.
- Plasma bioavailability was reached after  $0.94 \pm 0.06$  hours of taking pomegranate polyphenol liquid extract, judged based on ellagic acid levels.
- Urolithin-A glucuronide, a urinary metabolite of ellagic acid, reaches levels of approximately 1,000 ng/mL.



# Conclusion

- The aqueous pomegranate peel extract exhibited bacteriostatic, bactericidal and anti-virulence activities against urinary tract infectious *E. coli*.
- The use of the extract caused a reduction in the adhesion index accompanied with a reduction in motility and ornithine decarboxylation of the *E. coli* strains.
- The presence of phytochemicals including phenols, tannins and flavonoids may be responsible for these activities.



# Conclusion

- Further studies are required to identify and isolate the active compounds present in the pomegranate's peel which exhibits the antimicrobial effect and also to confirm these effects in vivo.
- The synergy between the extract active compounds and drug should be attentively studied which will probably solve the problem of multiple drug resistance, toxicity and overdose since when they combine a little concentration of two agents is required.

