

# In Vitro Antimicrobial Activity Evaluation of Ginger (*Zingiber officinale*) Absolute Ethanol Extract against Uropathogenic *Escherichia coli* (UPEC)

Angeline Felicia<sup>1</sup> , Kartuti Debora<sup>2\*</sup> , Ramadhani Ramadhani<sup>3</sup>

<sup>1</sup>Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

<sup>2</sup>Department of Medical Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

<sup>3</sup>Department of Anatomy, Histology, and Pharmacology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

## ABSTRACT

**Introduction:** Uropathogenic *Escherichia coli* (UPEC) is commonly found in the urine culture of women with urinary tract infections (UTIs) and is often resistant to several antimicrobials. Herbals, such as ginger (*Zingiber officinale*), are known to have antimicrobial activity against various microorganisms. This study was conducted to determine the antimicrobial activity of ginger against UPEC.

**Methods:** This was a true experimental study to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using agar dilution method. The McFarland 0.5 suspension of UPEC was inoculated on agar with 6 different ginger concentrations, i.e. 2,000 mg/ml, 1,000 mg/ml, 500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml. The MIC and MBC were read as the lowest concentration without visible growth.

**Results:** No visible growth of bacteria on agar at a concentration of 2,000 mg/ml and 1,000 mg/ml. Thus, the value of MIC and MBC for UPEC was 1,000 mg/ml.

**Conclusion:** Ethanol extract of ginger has antimicrobial activity against UPEC. In this study, the MIC and MBC for UPEC was 1,000 mg/ml.

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\* Correspondence: kartutidebora@gmail.com

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## Introduction

Urinary tract infections (UTIs) in Indonesia are quite prevalent. It occurs due to the colonization of bacteria/other pathogens in the urinary tract. Due to anatomical predisposition (shorter urethra), UTIs are more common in women than men. Uropathogenic *Escherichia coli* (UPEC) (75-90%) is the most common causative organism of UTIs.<sup>1</sup> The ability of UPEC to cause the symptoms of UTIs is associated with their virulence factors which allow them to colonize the urinary tract and survive the host's immune system.<sup>2</sup> Prolonged use of antibiotics to kill this bacteria often causes antimicrobial resistance and leads to recurrence of UTIs.<sup>3</sup> Therefore, it is necessary to develop research on herbals for the treatment of recurrent UTIs.

Ginger (*Zingiber officinale*) is a herbal medicine available in Indonesia. There are several kinds of ginger, but this study focused on the small ginger (*jahé emprit*), considering this type of ginger is the most commonly used by people and because it has higher levels of gingerol and shogaol compared to the other kinds of ginger.<sup>4</sup>

In the book Herbal Medicine by Ann M. Bode and Zigang Dong: Biomolecular and Clinical Aspects, ginger plays important role in inhibiting several diseases. In addition, ginger has been proven to have antimicrobial activity due to the components of mono- and oxygenated sesquiterpenes, phenolic compounds (shogaol, gingerol), which are fat-soluble phenols isolated from ginger rhizome.<sup>5</sup>

Gull, *et al.* studied the effect of ginger extract (in a concentration of 100 mg/ml to 0.01 mg/ml) against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *E. coli*, *Shigella sp.* The minimum inhibitory concentration (MIC) varied between each type of bacteria.<sup>6</sup>

Ginger has also been reported effective to treat various diseases, including infectious diseases. Antimicrobial susceptibility testing of ginger against various bacteria has been performed to determine its MIC and minimum bactericidal concentration (MBC). In an *in vitro* study, it was reported that a specific concentration of ginger can be either bacteriostatic or bactericidal to enteric *Escherichia coli*.

Most strains of *E. coli* can produce UTIs, but the disease is more common with certain specific serogroups, such as UPEC compared to enteric *E. coli*, because they have higher virulence factors. Those virulence factors are encoded in the plasmid or the DNA of a bacteriophage.<sup>7,8</sup> Genes encoded in plasmids also play a role in their resistance to penicillins, cephalosporins, aminoglycosides, tetracyclines, and so on.<sup>9</sup> Concerning the growing problem of antibiotic-resistant UTIs and a previous *in vivo* research had reported that ginger could decrease UPEC colony counted in menopausal women with asymptomatic urinary tract infection,<sup>10</sup> this study wanted to evaluate ginger *in vitro* antibacterial activity against UPEC as the main cause of UTIs.

## Methods

### Instruments and Materials for Extraction

Scales, spatula, beaker glass, 1-liter measuring cylinder, jar, stirring rod, fan, vacuum pump, Buchner funnel, Erlenmeyer flask, thermostatic water bath, *Zingiber officinale* (*jahé emprit*), and ethanol 96%.

### Plant Extraction Methods

The extraction of ginger in this study was performed by maceration methods. The ginger rhizome was washed, minced, washed once again, and dried. The dried rhizome was then processed into a powder. After that, the ginger powder was weighed at 250 grams and then soaked with 1 liter of ethanol for 24 hours. Then it was filtered using a Buchner funnel, Erlenmeyer flask, and vacuum pump. Finally, the liquid extract was evaporated using a thermostatic water bath at 50°C to remove the ethanol. The extract was kept in the refrigerator at 4°C until used.

### Instruments and Materials for Antimicrobial Susceptibility Testing

Calibrated loops, spiritus, matches, pipette, test tubes, incubator (at 37°C), petri dish, Muller Hinton agar (MHA), aqua dest, autoclave, and UPEC stock from Laboratory of Medical Microbiology Universitas Airlangga.

### Identification of UPEC

Before performing the antimicrobial susceptibility testing using agar dilution method, UPEC from the stock was initially streaked on Mac Conkey agar for purification to obtain separated colonies. Then UPEC was inoculated on Eosin Methylene Blue (EMB) agar for confirmation. The result showed colonies of Green Metallic Sheen.

### Preparation of Inoculum

3-4 colonies of UPEC was diluted in 4 ml NaCl. The turbidity was adjusted to a standard suspension of 0.5 Mc Farland ( $1.5 \times 10^8$  CFU/mL).

### Preliminary Study

The preliminary study was performed to determine the estimated concentration that might inhibit the growth of UPEC. It was performed using the broth macrodilution method. The initial concentration tested at the beginning was 1.6 mg/ml. The broth's turbidity caused by the ginger extract and the turbidity as a result of bacteria growth could not be distinguished visually. Thus, the determination of MIC was not possible. Each sample was inoculated on MHA to determine the MBC. The result showed that the concentration was too low. It could be increased to the maximum of 500 mg/ml because a higher concentration could not be diluted completely. The 500 mg/ml ginger extract dilution was still unable to kill the bacteria. Considering that a higher concentration of ginger might have antibacterial activity against UPEC, this study decided to use agar dilution method for this study.

**Antimicrobial Susceptibility Testing**

The MIC and MBC was determined using agar dilution method. The experiment was performed in 3 days.

Day 1:

1. Preparation of instruments and materials.
2. Determination of concentration.
3. Medium preparation (MHA).
4. Sterilization of MHA medium using autoclave 121°C 15 minutes.
5. Allowing the molten MHA to reach ± 50°C before diluting the ginger extract. The extract was dilluted serially in 6 test tubes to obtain a concentration of 2,000 mg/ml, 1,000 mg/ml, 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml.
6. Preparation of control test tubes (MHA medium without ginger extract).
7. Pouring the series of dilution into 6 plates. 2 control plates were also prepared.
8. Allowing the agar to solidify before incubating overnight to ensure their sterility.

Day 2:

1. Preparation of 0.5 McFarland UPEC suspension.
2. Inoculation of UPEC to 6 plates and 1 positive control plate. The negative control plate remained sterile.
3. Incubation of the plates at 37°C for 20 hours.

Day 3:

Visual observation of bacterial growth on each plate. The lowest ginger extract concentration without visible bacterial growth was determined as MIC and MBC.

**Results**

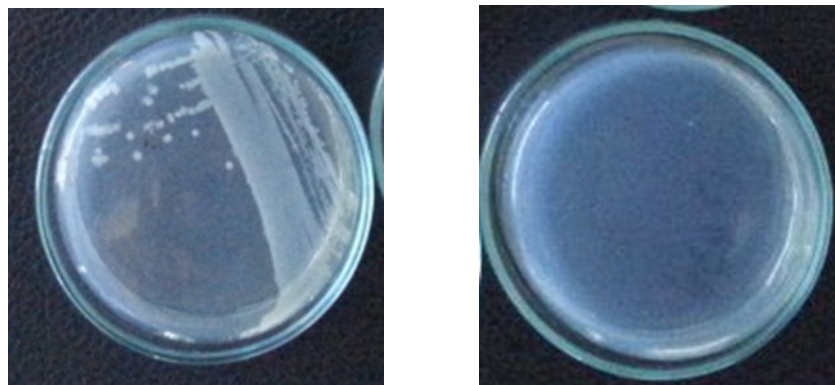
The 4 times of replication showed the same result – visible growth of UPEC at 500 mg/ml, 250 mg/ml, 125 mg/ml, dan 62.5 mg/ml, and no bacterial growth at 2,000 mg/ml dan 1,000 mg/ml (Table 1, Table 2). Thus, the MIC and MBC obtained from the serial twofold dilutions (2,000 mg/ml, 1,000 mg/ml, 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml) was 1,000 mg/ml. The growth of UPEC was observed in the positive control plate, whereas the negative control plate was sterile (Picture 1).

Table 1. Antimicrobial activity of ginger extract against UPEC

Replication	Final concentration of ginger ( <i>Zingiber officinale</i> )						Control	
	P1 (2,000 mg/ml)	P2 (1,000 mg/ml)	P3 (500 mg/ml)	P4 (250 mg/ml)	P5 (125 mg/ml)	P6 (62.5 mg/ml)	Positive (Kp)	Negative (Kn)
1	O	O	X	X	X	X	X	O
2	O	O	X	X	X	X	X	O
3	O	O	X	X	X	X	X	O
4	O	O	X	X	X	X	X	O







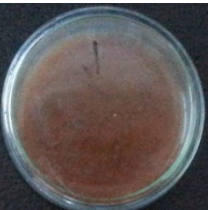

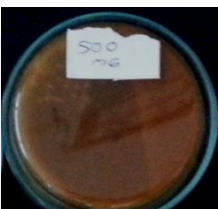

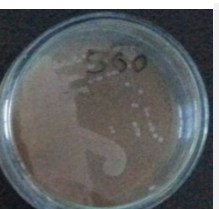





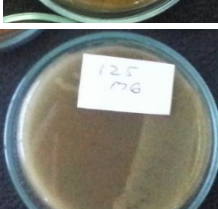

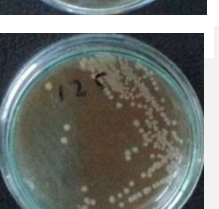
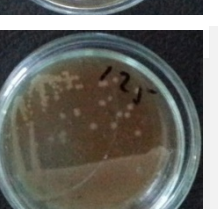
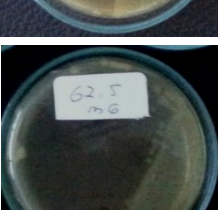


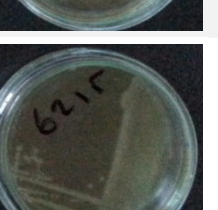
\*X = bacterial growth (+)

\*O = no bacterial growth



Picture 1. Positive (left) and negative control plates

Table 2. UPEC growth on 6 agar plates

	Replication 1	Replication 2	Replication 3	Replication 4
2,000 mg/ml				
1,000 mg/ml				
500 mg/ml				
250 mg/ml				
125 mg/ml				
62.5 mg/ml				

## Discussion

Ginger is commonly used in Indonesia for cooking and as a traditional medicine. Bioactive compounds in ginger include gingerol, shogaol, zingerone and its derivatives.<sup>4,11</sup> Containing the active ingredients, ginger extract was effective to prevent or treat various diseases, including cancer, asthma, dementia, diabetes, ulcerative colitis, cardiovascular disease, and so forth.<sup>5</sup> The potential of ginger to kill a wide variety of pathogenic microorganisms has been studied, thus it can be used for the treatment of infectious diseases.

A number of research on antimicrobial potential of ginger extract have been performed in Indonesia and worldwide. One of them was Gull, *et al.*<sup>6</sup> who studied the antimicrobial activity of *Zingiber officinale* against *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Shigella sonnei*, *Staphylococcus epidermidis*, and *Salmonella typhi*. Most of the previous experiments were performed using agar diffusion method or a modified method that calculated the diameter of zones of inhibition. The weakness of this method was there had not been an approved standard of the diameter of the zone of inhibition which might indicate that ginger extract was considered effective to inhibit the growth of UPEC. Therefore, the preliminary study was performed by broth dilution method, as this method had been considered practical. Moreover, it had been commonly used for antimicrobial activity assessment against *Enterobacteriaceae* family.<sup>12</sup> However, as it was not possible to dissolve the extract completely in the broth, and as the turbidity due to the growth of bacteria and the turbidity due to extract could not be distinguished visually, the broth dilution method was considered unsuitable to use in this study. This study decided to use agar dilution method. Actually, this method was not used routinely in most laboratories because it was labor intensive. Nevertheless, it had an advantage to test fastidious microorganisms, such as *N. Gonorrhoeae*, that could not grow sufficiently in the broth.<sup>13</sup> Considering the advantage of this method compared to broth dilution method, agar dilution was chosen to be the method in this study.

Based on a number of references, agar dilution method could only determine the MIC, but it could be concluded that MBC = MIC because there was no bacteria growth observed visually. Thus, this study proved that *Zingiber officinale* has the ability to both inhibit the growth and kill UPEC. UPEC was streaked on each MHA containing a specific concentration of ginger extract. In the fourth times of replication, the result was constant. There was no bacterial growth at the concentration of 1,000 mg/ml. Hence, the MIC obtained was 1,000 mg/ml.

In this study, ginger (*jahe emprit*) extract was potent to inhibit UPEC growth but with relatively high concentrations. A study conducted by Gull, *et al.*<sup>6</sup> reported that ginger extract could inhibit the growth of *E. coli* at a much lower concentration, the MIC obtained was 0.08 mg/ml. The MIC determination was performed by modified agar diffusion method by measuring the diameter of zone of inhibition, which was different from this study.

This study used agar dilution method to obtain a concentration that could kill the bacteria completely. There was no growth of UPEC at 1,000 mg/ml of extract. Another significant difference was the study by Gull, *et al.* evaluated antimicrobial activity of ginger on enteric *E. coli*.<sup>6</sup> UPEC has special characteristics compared to the enteric *E. coli*. A previous study revealed that UPEC has urovirulent factors that was not expressed by most enteric *E. coli*. Those urovirulent factors included hemolysin, Mannose Resistant Haemagglutination (MRHA), and Cell Surface hydrophobicity (CSH). The percentage of UPEC that had those virulence factors were much higher than faecal *E. coli*.<sup>8</sup> Virulence factors were encoded in the plasmid or bacteriophage DNA.<sup>7</sup> The plasmids encoded the genes responsible for antimicrobial resistance. It also regulated formation of enzymes that could damage the antimicrobial agent.<sup>9</sup> However, a specific mechanism of the resistance of UPEC against ginger has yet to be understood.

Further studies are needed to determine different patterns of antibiotic resistance against ginger. Likewise, the exact maximum dose of ginger that is safe for human is still being studied. A previous concluded that the lethal dose (LD50) of ethanol extract of ginger injected into rats intraperitoneally was  $1,551 \pm 75$  mg/kg.<sup>14</sup> Another study by reported that ginger up to 1-1.5 grams per day was safe for pregnant women and did not cause fetal malformations.<sup>15-17</sup> More than 6 grams could irritate the stomach and ginger powder inhalation might stimulate allergies mediated by IgE.<sup>18,19</sup>

This study discovered the ability of ginger extract's antimicrobial activity against UPEC to inhibit growth and kill the bacteria at a concentration of 1,000 mg/ml. A previous in vivo study discovered the potential of ginger to treat UTIs by decreasing bacterial colonies counted in the urine culture of menopausal women until zero colonies (no bacteria) although it studied a different type of ginger (*jahe merah*).<sup>20</sup>

The type of ginger observed in this study, *jahe emprit*, might be a cause of a different result of the experiment compared to other studies that used other types of gingers.

## Conclusion

Ethanol extract of ginger has inhibitory and bactericidal effects on UPEC bacteria. In this in vitro study using the agar dilution method, both the MIC and MBC of ginger against UPEC were 1,000 mg/ml.

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## Conflict of Interest

The authors declared there is no conflict of interest.

## References

1. Hooton T. Uncomplicated Urinary Tract Infection. *N Engl J Med* 2012; 366: 1028–1037. [[WebPage](#)]
2. Bien J, Sokolova O, Bozko P. Role of Uropathogenic Escherichia coli Virulence Factors in Development of Urinary Tract Infection and Kidney Damage. *Int J Nephrol* 2012; 2012: 1–15. [[PubMed](#)] [[CrossRef](#)]
3. Rané A, Dasgupta R. *Urinary Tract Infection*. London: Springer London. Epub ahead of print 2013.
4. Wijaya CH, Fathona D. *Kandungan Gingerol dan Shogaol, Intensitas Kepedasan dan Penerimaan Panelis terhadap Oleoresin Jahe Gajah*. Institut Pertanian Bogor. 2011. [[WebPage](#)]
5. Bode AM, Dong Z. *The Amazing and Mighty Ginger*. 2011. [[PubMed](#)] [[CrossRef](#)]
6. Gull I, Saeed M, Shaikat H, *et al*. Inhibitory Effect of Allium Sativum and Zingiber Officinale Extracts on Clinically Important Drug Resistant Pathogenic Bacteria. *Ann Clin Microbiol Antimicrob* 2012; 11: 8. [[PubMed](#)] [[CrossRef](#)]
7. Murray PR, Rosenthal KS, Pfaller MA. *Medical Microbiology E-Book, 8th Edition*. Elsevier, 2016.
8. Bharathi D., Kumari R., Lakshmi G., *et al*. Identification of Urovirulent Markers in Uropathogenic Escherichia Coli. *Int J Curr Res Rev* 2015; 7: 51–54. [[WebPage](#)]
9. Brooks G. *Jawetz, Melnick, & Adelberg's Medical Microbiology, Twenty-Fifth Edition (LANGE Basic Science)*. Atlanta (US): McGraw-Hill Professional Publishing, 2010.
10. Rosmana D, Hardianto G, Debora MS K. Jahe Mengurangi Jumlah Koloni Uropathogenic Escherichia Coli pada Wanita Menopause dengan Infeksi Saluran Kemih Asimtomatis. *Maj Obstet Ginekol* 2018; 24: 1. [[CrossRef](#)]
11. Semwal RB, Semwal DK, Combrinck S, *et al*. Gingerols and Shogaols: Important Nutraceutical Principles from Ginger. *Phytochemistry* 2015; 117: 554–568. [[PubMed](#)] [[CrossRef](#)]
12. Li Y, Tran VH, Duke CC, *et al*. Preventive and Protective Properties of Zingiber Officinale (Ginger) in Diabetes Mellitus, Diabetic Complications, and Associated Lipid and Other Metabolic Disorders: A Brief Review. *Evidence-Based Complement Altern Med* 2012; 2012: 1–10. [[PubMed](#)] [[CrossRef](#)]
13. Tille P. *Bailey & Scott's Diagnostic Microbiology*. 13th ed. Mosby, 2013.
14. Hasan HA, Raauf A, Razik BMA. Chemical Composition and Antimicrobial Activity of the Crude Extracts Isolated from Zingiber Officinale by Different Solvents. *Pharm Anal Acta*; 3. Epub ahead of print 2012. DOI: 10.4172/2153-2435.1000184. [[CrossRef](#)]
15. Wang S, Zhang C, Yang G, *et al*. Biological Properties of 6-Gingerol: a Brief Review. *Nat Prod Commun* 2014; 9: 1027–30. [[PubMed](#)] [[CrossRef](#)]
16. Mesomo MC, Corazza ML, Ndiaye PM, *et al*. Supercritical CO<sub>2</sub> Extracts and Essential Oil of Ginger (Zingiber officinale R.): Chemical Composition and Antibacterial Activity. *J Supercrit Fluids* 2013; 80: 44–49. [[ScienceDirect](#)] [[CrossRef](#)]
17. Butt MS, Sultan MT. Ginger and its Health Claims: Molecular Aspects. *Crit Rev Food Sci Nutr* 2011; 51: 383–393. [[PubMed](#)] [[CrossRef](#)]
18. Viljoen E, Visser J, Koen N, *et al*. A Systematic Review and Meta-Analysis of the Effect and Safety of Ginger in the Treatment of Pregnancy-Associated Nausea and Vomiting. *Nutr J* 2014; 13: 20. [[PubMed](#)] [[CrossRef](#)]
19. Kubra IR, Rao LJM. An Impression on Current Developments in the Technology, Chemistry, and Biological Activities of Ginger (Zingiber officinale Roscoe). *Crit Rev Food Sci Nutr* 2012; 52: 651–688. [[PubMed](#)] [[CrossRef](#)]
20. Chen H, Soroka DN, Hu Y, *et al*. Characterization of Thiol-Conjugated Metabolites of Ginger Components Shogaols in Mouse and Human Urine and Modulation of the Glutathione Levels in Cancer Cells by [6]-Shogaol. *Mol Nutr Food Res* 2013; 57: 447–458. [[PubMed](#)] [[CrossRef](#)]