Research Article

Potential of Single-Bulb Garlic and Single-Bulb Black Garlic Extracts to Eradicate Uropathogenic *Escherichia coli*

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Abstract

Uropathogenic Escherichia coli (UPEC) is the main causative organism of urinary tract infections (UTIs). The increasing resistance of UPEC to conventional antibiotics necessitates the exploration of alternative treatments, including the use of herbal remedies such as single-bulb garlic. This study aimed to evaluate and compare the antibacterial potential of single-bulb garlic extract and single-bulb black garlic extract against clinical isolates of UPEC. The study was conducted at the Microscopic Laboratory, Faculty of Medicine, Universitas Tanjungpura, in June 2023. Ethanol (96%) was used for the extraction process and the antibacterial activity was assessed using the disc diffusion method. The study included 10 treatment groups, comprising single-bulb garlic and single-bulb black garlic extracts at concentrations of 25%, 50%, 75%, and 100%, with nitrofurantoin (300 µg/disc) as the positive control and 1% DMSO as the negative control. The results indicated that the single-bulb garlic extract exhibited no antibacterial activity against UPEC at any concentration. In contrast, the single-bulb black garlic extract demonstrated antibacterial activity at 75% and 100% concentrations, with inhibition zones of 7.28 mm and 7.24 mm, respectively. These findings suggest that single-bulb black garlic extract is more effective than single-bulb garlic extract in inhibiting the growth of UPEC (p = 0.001).

Keywords: *antibacterial, single bulb garlic, single bulb black garlic, uropathogenic Escherichia coli.*

Potensi Bawang Putih Siung Tunggal dan Bawang Hitam Siung Tunggal terhadap *Uropathogenic Escherichia coli*

Abstrak

Uropathogenic Escherichia coli (UPEC) adalah organisme penyebab utama infeksi saluran kemih (ISK). Meningkatnya resistensi UPEC terhadap antibiotik konvensional mengharuskan eksplorasi pengobatan alternatif, termasuk penggunaan obat herbal seperti bawang putih umbi tunggal. Penelitian ini bertujuan untuk mengevaluasi dan membandingkan potensi antibakteri ekstrak bawang putih siung tunggal dan ekstrak bawang hitam siung tunggal terhadap isolat klinis UPEC. Penelitian dilakukan di Laboratorium Mikroskopis Fakultas Kedokteran Universitas Tanjungpura pada bulan Juni 2023. Etanol (96%) digunakan untuk proses ekstraksi dan aktivitas antibakteri dinilai menggunakan metode difusi cakram. Terdapat 10 kelompok perlakuan, yang terdiri atas ekstrak bawang putih siung tunggal dan bawang hitam siung tunggal konsentrasi 25%, 50%, 75%, dan 100%, dengan nitrofurantoin (300 µg/cakram) sebagai kontrol positif dan DMSO 1% sebagai kontrol negatif. Hasil penelitian menunjukkan bahwa ekstrak bawang putih siung tunggal tidak menunjukkan aktivitas antibakteri terhadap UPEC. Namun, ekstrak bawang hitam siung tunggal menunjukkan aktivitas antibakteri pada konsentrasi 75% dan 100%, dengan zona hambat masing-masing 7,28 mm dan 7,24 mm. Hasil tersebut menunjukkan bahwa ekstrak bawang hitam siung tunggal lebih efektif dari ekstrak bawang putih siung tunggal dalam menghambat pertumbuhan UPEC (p=0,001).

Kata kunci: *antibakteri, bawang putih siung tunggal, bawang hitam siung tunggal, uropathogenic Escherichia coli.*

Introduction

Urinary tract infections (UTIs) is a prevalent pathological condition within the fields of urology and nephrology, affecting millions of individuals worldwide, with an estimated annual incidence of 150 million cases globally.[1] In Indonesia, the incidence of UTIs is reported to be between 90 and 100 cases per 100,000 people annually, equating to approximately 180,000 new cases each year.[2] Most UTIs are caused by uropathogenic *Escherichia coli* (UPEC).[1] Over time, the resistance of UPEC increased due to various antibiotics. Resistance rates have been reported in multiple countries, with the highest resistance observed for the antibiotic trimethoprim-sulfamethoxazole (TMP-SMZ), 14.6%-82%.[3] In West Kalimantan province, a study on antibiotic sensitivity patterns in *E. coli* among UTI patients reported a resistance rate of 100% for amikacin and cefepime.[4] Study conducted at dr. Cipto Mangunkusumo Hospital found that *E. coli* was most sensitive to meropenem (90%).[5] These alarming trends in antibiotic resistance underscore the urgent need for alternative interventions, including the exploration of herbal antibiotics such as garlic.[5]

Garlic can inhibit the growth of both Gram-positive and Gram-negative bacteria. Compounds in garlic, such as allicin, ajoene, and S-allyl cysteine, are known for their antibacterial properties.[6] Garlic comes in various varieties, including single-bulb garlic. Single-bulb garlic is a variety formed accidentally due to suboptimal growth conditions. The garlic contains active compounds equivalent to 5-6 regular garlic cloves.[7] Single-bulb garlic can be further processed through fermentation by heating at 70-90°C for 12-40 days, resulting in single-bulb black garlic. Fermentation produced more active compounds in single-bulb black garlic than in single-bulb garlic, particularly S-allyl cysteine (SAC), which increases by as up to six times, which enhances effectiveness in inhibiting bacteria.[8]

Previous study indicated that *Pseudomonas aeruginosa* is susceptible to both garlic and black garlic extract.[9] Additional research has shown that *Staphylococcus aureus* and *E. coli* are susceptible to single-bulb garlic extract.[10,11] In the context of UPEC, garlic has been found to inhibit biofilm formation, which is critical for the pathogenicity of UPEC.[12] However, no study has yet compared the antibacterial activity of single-bulb garlic and single-bulb black garlic against UPEC. Therefore, this study aims to evaluate the antibacterial activity of these extracts against clinical UPEC isolates.

Methods

This study was conducted in June 2023 at the Microscopic Laboratory, Faculty of Medicine, Universitas Tanjungpura. Ethical approval for the study was obtained from the Faculty of Medicine Ethics Committee, Universitas Tanjungpura, under registration number 2552/UN.29/PG/2023.The single-bulb garlic samples were purchased from Surabaya City, East Java, and imported from China. The cloves were separated, peeled and washed. A portion of single-bulb garlic bulbs are processed into single-bulb black garlic by heating them in an oven (Memmert, Germany) at 70°C for 21 days.

The extraction process utilized 96% ethanol as a solvent in the maceration method. The bulbs are crushed using a blender. The resulting simplicia are placed in containers, and then 96% ethanol was added at a 1:4 ratio for 24 hours, with periodic stirring. The maceration product was then filtered using Whatman No. 1 filter paper.[9,13] This process was repeated three times. Then, the filtrate was evaporated at 40°C to reduce the solvent volume and concentrate the extract.[14] The final extract was stored in a tightly sealed dark glass bottles and kept at 4°C. For the antibacterial activity assay, the extract was diluted to concentrations of 25%, 50%, 75%, and 100% using 1% dimethyl sulfoxide (DMSO) (PhytoTech, Kansas).

Bacterial Isolate Preparation

The bacterial isolates used in this research were clinical isolates of UPEC obtained from the Microbiology Department collection at the Faculty of Medicine, Universitas Tanjungpura. The isolates were identified as *E. coli* using API 20E (bioMérieux). Bacterial isolates were cultured on MacConkey (Oxoid, United Kingdom) and eosin methylene blue (Merck, German) media and incubated at 37°C for 18 hours. The bacterial suspensions were prepared from these 18-hour cultures. One loop of bacterial colonies was suspended in 5 mL of 0.9% NaCl solution. The turbidity of the suspension was measured by comparing the bacterial suspension tube to the 0.5 McFarland standard.[15]

Antibacterial Activity Test

The antibacterial activity of the extracts was assessed using the *Kirby-Bauer* disc diffusion method. A sterile cotton swab was dipped into the bacterial suspension and inoculated onto Mueller-Hinton Agar (Oxoid, United Kingdom) by gently streaking the plate from top to bottom with three repetitions while rotating the plate 60 degrees each time to ensure uniform coverage.[16]

This study comprised ten treatment groups, each with three replicates for each group. The paper discs were soaked in the extract solution for 15 minutes before being placed on a single media plate with a minimum distance of 24 mm. The media plates were then incubated at 37°C for 24 hours.[17] The antibacterial activity of the extracts was classified based on the diameter of the inhibition zones: strong (>10-20 mm), moderate (>5-10 mm), and weak (<5 mm).[18]

Nitrofurantoin (300 µg/disc) served as the positive control, with inhibition zone diameters interpreted according to the 2023 Clinical and Laboratory Standards Institute (CLSI) guidelines.[19] DMSO 1% was used as the negative control.

Statistical Analysis

The data from this study were were subjected to a normality test using the Shapiro-Wilk test. Depending on the normality results, statistical analysis was conducted using either one-way analysis of variance (ANOVA) or the Kruskal-Wallis test. Post hoc comparisons between groups were performed using the Mann-Whitney test to identify significant differences.

Table 1. The Antibacterial Activity of Single-Bulb Garlic and Single-Bulb Black Garlic Extracts Against Clinical UPEC Isolates

Treatment Group		Inhibition Zone Diameter Average (mm)	Interpretation
Single bulb garlic extract	25%	0[a,b,c]	No activity
	50%	0[a,b,c]	No activity
	75%	0[a,b,c]	No activity
	100%	0[a,b,c]	No activity
Single bulb black garlic extract	25%	0[a,b,c]	No activity
	50%	0[a,b,c]	No activity
	75%	7,28[c,d,e]	Moderate
	100%	7,24[c,d]	Moderate
DMSO 1% (negative control)		0	No activity
Nitrofurantoin 300µg (positive control)		21,84[d]	Susceptible

[a]Significantly different from black garlic 75%; [b]Significantly different from black garlic 100%;
[c]Significantly different from positive control; [d]Significantly different from negative control;
[e]Not significantly different from black garlic 100%.

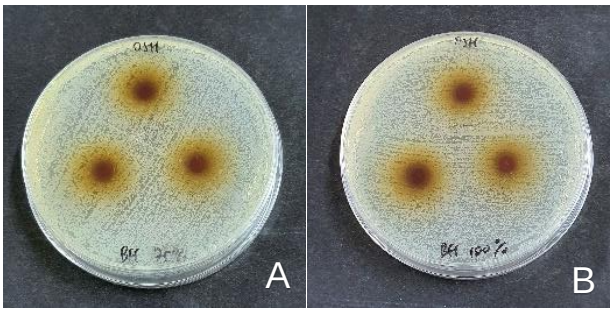

Figure 1. The Inhibition Zone of Single-Bulb Black Garlic. A. 75%; B. 100%

Result

The antibacterial activity in this test was assessed by observing and measuring the inhibition zones formed on the test media (Table 1). The results of the antibacterial activity assay against UPEC revealed that single-bulb garlic extract did not exhibit any antibacterial activity at any of the tested concentrations. In contrast, the single-bulb black garlic extract demonstrated antibacterial activity at concentrations of 75% and 100%, with inhibition zones of 7.28 mm and 7.24 mm, respectively (Figure 1). The positive control, Nitrofurantoin (300 µg/disk), produced an average inhibition zone of 21.84 mm, indicating susceptibility of the UPEC isolates. The negative control (1% DMSO) showed no antibacterial activity.

The Kruskal-Wallis test yielded a significance value of 0.0001, indicating that the diameters of the inhibition zones formed by each treatment group were significantly different. To further explore these differences, the analysis was continued with the Post Hoc Mann-Whitney test. The Post Hoc Mann-Whitney test results demonstrated that the inhibition zone diameter for the 75% single-bulb black garlic extract differed significantly from those of the 25% and 50% concentrations (p=0.037), and from the positive control (p=0.050). Similarly, the 100% concentration showed significant differences compared to the 25% and 50% single-bulb black garlic extracts, as well as the positive control (p=0.037). However, there was no significant difference between the 75% and 100% concentrations (p=0.827). These results suggest that the 75% concentration of single-bulb black garlic extract is as effective as the 100% concentration in inhibiting the growth of UPEC. Therefore, the effective single-bulb black garlic extract concentration is 75%.

Discussion

The results of this study reveal varied outcomes regarding the antibacterial activity of single-bulb garlic extract. Notably, the single-bulb garlic extract did not show any inhibition zones at any concentrations, indicating a lack of antibacterial activity against clinical UPEC isolates. This finding contrasts with the study by Aini et al[9] which reported inhibition zones for *E. coli* when using garlic extract.[9] The discrepancy is likely due to several factors, such as variations in garlic varieties and growth locations, the duration of extract storage, and the specific isolates used.[20] The inhibition zones produced by the garlic extract used in this study may be influenced by factors related to the plant's growth location, such as pH, light intensity, soil type, and temperature. These factors can affect the plant's composition or concentration of antibacterial compounds.[21]

The clinical UPEC isolates used in this study were resistant to beta-lactam antibiotics like ampicillin, cephazolin, ceftriaxone, and cefepime, which inhibit bacterial cell wall synthesis, causing cell lysis.[22] In addition to beta-lactam resistance, the bacterial isolates also showed resistance to sulfamethoxazole-trimethoprim, which interferes with the production of dihydrofolate acid and inhibits essential nucleic acid and protein biosynthesis in bacteria.[23]

Single-bulb garlic contains organosulfur compounds, including allicin and tannins, that can interfere with the synthesis of bacterial cell walls. However, the resistance mechanisms associated with these compounds differ from those associated with beta-lactam antibiotics. Allicin affects bacterial cell wall synthesis by inhibiting phospholipid synthesis,[24] while tannins inhibit synthesis in the inner membrane of bacteria.[25] Allicin and tannins also have other antibacterial mechanisms, such as inhibiting protein and nucleic acid synthesis and interfering with bacterial metabolism.[24,26]

In addition to allicin and tannins, alkaloids in single-bulb garlic can inhibit cell wall synthesis by interfering with the function of peptidoglycan in bacterial cells.[27] Although alkaloids have some resistance mechanisms found in bacterial isolates, they also have other antibacterial mechanisms, such as inhibiting nucleic acid and protein synthesis and bacterial metabolism.[26] Therefore, antibiotic resistance in the bacterial isolates did not significantly impact the antibacterial potential of single-bulb garlic, aligning with research that has shown sensitivity of multidrug-resistant (MDR) *E. coli* to garlic extract.[28]

Despite containing various antibacterial compounds, including flavonoids, saponins, organosulfur compounds, alkaloids, and tannins,[29] the single-bulb garlic extract did not produce inhibition zones, which may be due to the insufficient concentrations of these compounds in the extract. In contrast, testing with 75% and 100% single-bulb black garlic extract produced moderate-size inhibition zones, indicating antibacterial activity against clinical UPEC isolates. The 75% single-bulb black garlic extract showed the most significant antibacterial activity The slightly larger inhibition zones observed at 75% compared to

100% could be attributed to slower diffusion rates and higher viscosity at the higher concentration, which may hinder the diffusion process in the media and reduce the size of the inhibition zone.[30]

During the fermentation process of black garlic, the concentrations of several secondary metabolites, such as flavonoids, phenolics, alkaloids, tannins, and saponins, increase. This aligns with previous research by Anzaz et al[29] which identified the presence of phenolics in single-bulb black garlic but not in its raw form. Choi et al[31] also reported significant increases in flavonoid and phenolic content during black garlic fermentation, with flavonoid levels increasing 5-6 times and phenolic levels increasing 3-4 times.[31] Flavonoids and phenolics are known for their antibacterial mechanisms by inhibiting nucleic acid synthesis and damaging bacterial cell membranes.[26,32]

Single-bulb black garlic also retains organosulfur compounds, such as allicin and SAC, from the original single-bulb garlic, with SAC content increasing by 4-8 times during fermentation. The elevated SAC levels enhance the antibacterial properties of the extract, working synergistically with allicin to inhibit nucleic acid, cell wall, and protein synthesis in bacteria.[6,24]

Tannins, saponins, and alkaloids also increase the fermentation process.[8,34] These compounds are known to damage the function of cell membranes in bacteria. Moreover, alkaloids have the capacity to inhibit nucleic acid synthesis, while saponins are known to inhibit protein synthesis in bacteria.[26,35] The elevated concentration of these secondary metabolites in single-bulb black garlic likely enhances its antibacterial activity compared to single-bulb garlic, as evidenced by the inhibition zones observed at 75% and 100% concentrations of the black garlic extract, whereas the single-bulb garlic extract showed no antibacterial activity against clinical UPEC isolates.

In this study, nitrofurantoin was used as a positive control due to its established effectiveness as a first-line therapy for UTIs caused by UPEC.[5] The positive control confirmed that nitrofurantoin is highly effective in inhibiting the growth of UPEC. Conversely, the negative control, 1% DMSO did not show any inhibition zones, suggesting that the solvent used in this study did not affect the growth of the UPEC. DMSO is a solvent capable of dissolving both polar and non-polar compounds, and it is characterized by its low toxicity and lack of bactericidal properties.[36] This was corroborated by studies showing that DMSO at low concentrations (2.5%, 2%, 1.5%, 1%, and 0.5%) did not have antimicrobial activity against *E. coli*.[37]

A limitation of this study is the inability to control the growth conditions of the single-bulb garlic used, including soil pH, humidity, and environmental temperature. These factors can significantly influence the concentration of antibacterial compounds within the garlic.

Conclusion

The research findings conclude that single-bulb black garlic extract exhibits antibacterial activity against clinical isolates of UPEC at a concentration of 75% and 100% with a moderate zone of inhibition diameter of 7.28 mm and 7.24 mm, respectively. Therefore, the effective concentration of single-bulb black garlic extracts to inhibit the growth of clinical isolates of UPEC is 75%. In contrast, the ethanol extract of single-bulb garlic showed no antibacterial activity against UPEC at any tested concentration.

Conflict of Interest

There is no conflict of interest.

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