In Vitro Effect of Alfa Mangostin on Multiresistant Uropathogenic Escherichia Coli

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Objective: This study examined the effect of α-mangostin on the growth of multiresistant E. coli.

Methods: α-mangostin treatment of E. coli uropathogenic bacteria was administered in vitro, using 14 levels of concentration: 14; 28.13; 56.25; 112.5; 225; 450 μg/mL with 4 times repetition at each concentration. The antibacterial activity of α-mangostin was determined by evaluating bacterial growth at each concentration using the indirect method by sample absorbance reading. The Samples of uropathogenic E. coli treated with various doses of α-mangostin were incubated for 18-20 hours and then subjected to the absorbance reading using a UV-Vis spectrophotometer.

Results: Minimum inhibitory concentration (MIC) in this study was 450 μg/mL. Based on linear regression (STATA 13.1) relationship between α-mangostin concentrations and bacterial growth inhibition activity showed 0.0001 <0.05 showing that all concentrations of α-mangostin were incubated for 18-20 hours and then subjected to the absorbance reading using a UV-Vis spectrophotometer λ 625 nm.

Conclusion: α-mangostin has not been effective to inhibit the growth of multiresistant uropathogenic E. coli due to a relatively high MIC (450 mcg/mL). A Potentially relevant activity in the clinical setting will occur if the value of the MIC of a substance in vitro <100 μg/mL. Even the pharmaceutical industry prefers the development of antibiotics with in vitro MIC value of ≤ 2 μg/mL.

Keywords: MDR, antibiotic, Garcinia mangostana L.

INTRODUCTION

The most common extraintestinal infections caused by the bacteria of Escherichia coli (E. coli) has been urinary tract infection (UTI). This disease occurs at least 25-35% adult women during their life (Sukandar, 2009). Meanwhile, the incidence of multi-drug resistance of E. coli has become a global problem (Alhashash et al., 2013).

The incidence of multi-drug-resistant uropathogenic Escherichia coli has been reported in Nepal (Baral et al., 2012) representing 38.2% total isolates of patient with UTI. Moreover, 55.2% uropathogenic E. coli was detected to produce Extended Spectrum Beta Lactamase (ESBL). Another study stated that the most...
common E. coli resistance was to co-trimoxazole (86%), amoxicillin (76%), class of cephalosporins (53.75% - 70%), and followed by the fluoroquinolone group (52.5% - 57.5%) (Ihsan et al., 2014). Meanwhile, broad-spectrum antibiotics such as amoxicillin clavulanate, fluoroquinolone, and cephalosporins have been avoided due to the risk of resistance. In Indonesia, the most common resistance in uropathogen E. coli is resistance to ampicillin (91.9%), followed by ciprofloxacin (83.7%) and cefixime (67.6%). The resistance is caused by the irrational use of antibiotics (Khalilullah et al., 2011).

The multiresistant uropathogenic E. Coli incidence requires us to look for alternative treatment. Despite efforts to optimize the parameters of the available pharmacokinetics/pharmacodynamics of each has been done, but optimizing the parameters PK/PD is not completely reduce the incidence of antibiotic resistance (Gloede et al., 2010). Other efforts that can be done is infection therapy using herbal medicine or chemical isolates from herbal ingredients. Mangosteen (Garcinia mangostana L.) Are found in Indonesia, some active isolates were tested possess antibacterial activity. Mangosteen extracts are reported to have pharmacological activity as anti-inflammatory, cytotoxic agents, antitumor, allergy, antioxidant, antiviral, and antibacterial. Primary isolates α–mangostin showed a fairly high cytotoxic activity against bacteria (Al Massarani et al., 2013). The antibacterial activity of α–mangostin is bactericidal with the mechanism of action undermines the integrity of bacterial cytoplasmic membrane so that the components of intracellular bacteria out (Koh et al., 2013).

Several studies have demonstrated the effectiveness of alpha-mangostin against Gram + especially Staphylococcus aureus, Leptospira and Mycobacterium tuberculosis (Seesom et al., 2013). Another study of Bhat and Daihan (2013) showed a moderate susceptibility of bacteria to xanthones in the extract of Garcinia mangostana L. is Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis and Escherichia coli. But among the four strains tested, Streptococcus pyogenes was the most susceptible to the extract of Garcinia mangostana L. The antibacterial activity of α–mangostin against multidrug resistant uropathogenic E. coli has not been proven. Therefore, this study aimed to determine antibacterial activity of α–mangostin against multiresistant drug uropathogenic E. coli in vitro.

METHODS

A. Population and sample research

The study population was uropathogenic E. coli isolated from patients with UTIs in Muhammadiyah Roemani hospital of Semarang. The multidrug resistant uropathogenic E. coli samples were confirmed with a series of biochemical tests.

B. Uropathogenic biochemical test of multidrug Resistant uropathogenic E. coli

The isolates obtained from UTI patients were grown in MacConkey media containing Eosin Methylene Blue (EMB), before gram staining. A series

Multidrug resistat uropathogenic E. coli with density of $5 \times 10^3$ CFU/mL

Sample absorption reading on spectrophotometry $\lambda$ 625 nm

Every test group was incubated at 37°C for 18 – 2 hours

Analysis of the correlation between the concentration of alpha mangostin and the growth of uropathogenic E. coli, analysis regresion analysis using STATA 13.1
of biochemical tests were performed to identify the isolate. The series of biochemical test materials included IMViC (Indol test tube, Methyl red, Voges proskaur, and Citrate). Having identified uropathogenic E. coli, the pattern of resistance were determined using media Mueller Hinton Agar (MHA, Merck). The Antibiotics used to assess uropathogenic resistance patterns of E. coli were amoxicillin, erythromycin, cotrimoxazole and ciprofloxacin. The multidrug Uropathogenic resistet E. coli were then used to test α–mangostin active substance (purity ≥ 98%, Sigma-Aldrich) that was dissolved in methanol solution(Merck).

C. Test the effectiveness of α–mangostin against E. coli MDR uropathogenic E. coli

The density of the bacterial suspension for determining the effectiveness of alpha mangostin refers to the standard as described by Matuschek et al. (2013) suggesting a minimum density of the bacterial suspension used in the test of minimum inhibitory concentration (5 x 10^5 CFU/mL).

The effectiveness of alpha-mangostin against E. coli bacteria uropatogen was evaluated with broth dilution method using a series of specific levels of alpha-mangostin in broth or liquid media (MHB) (Figure 1). The level range was as described by Al Massarani et al. (2013), who reported that alpha activity mangostin dimetilsulfoxide diluted with dimethylsulfoxide (DMSO) for E. coli in the form of IC50 values of ν>200 µM (equivalent to 82.09 mg/mL). In addition to the use of a serial level, one tube without alpha mangostin (positive control) was used as a control of bacterial growth and a negative control tube (sterile bacterial media and alfa mangostin without bacteria) as a sterility control.

Figure 1. Schematic flow of treatment A, B, C, D, E, and F in each group of E. coli bacteria using different concentrations of alpha-mangostin. (A) 450.0 µg/mL, (B) 225.0 µg/mL, (C) 112.5 µg/mL, (D) 56.2 µg/mL, (E) 23.1 µg/mL, dan (E) 11.5 µg/mL, for control K (+) alpha mangostin containing 6.75 µg/and MHB media were used. Each treatment was tested with 4 repetitions.

The antibacterial activity of alpha mangostin was measured by observing the growth or death of bacteria at each concentration. The growth or death of bacteria were tested using the indirect method, absorbance readings using UV-Vis λ 625 nm (wavelength readings McFarland standard). Samples or uropathogen of E. coli treated with various doses of alpha-mangostin were incubated for 18-20 hours.

D. Statistical analysis

Absorbance in each treatment group was compared with that of positive and negative control group. To analyze the relationship between the concentration of alpha mangostin and the growth of uropathogenic E. coli, regression analysis test was performed using STATA 13.1.

RESULTS

a. Biochemistry test of Multidrug resistant Uropathogenic of E. coli

Uropatogen E. coli is a Gram-negative growing in the agar media of Mac Conkey. E. coli is a strong lactose fermenter, so that the production of acid produced is enough to precipitate bile salts. Colonies produced by E. coli on MacConkey agar medium was pink with a halo effect around the colony. For bacteria that can not perform lactose fermentation, the Gram bacteria colony - growing in MacConkey media will be transparent or yellowish (Allen, 2013).

The second identification test using EMB agar medium, colonies of purple E. coli and have a metallic green color luminescence characteristics. Agar media of EMB was used to distinguish E. coli lactose fermentant from Enterobacter aerogenes (Oxoid, 2010). biochemical tests, ie indol test, methyl red, proskaur vogens, and citrate were done for confirmation. The results of biochemical tests on samples of the uropathogenic E. coli isolates of patients with UTI is shown in table 1:

Table 1. Results of biochemical test on the samples of uropathogenic E. Coli

<table>
<thead>
<tr>
<th>No</th>
<th>Type of test</th>
<th>result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Uji Indol</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Uji Methyl red</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Uji Voges Proskaur</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Uji Citrate</td>
<td>-</td>
</tr>
</tbody>
</table>

To get Multidrug resistant uropathogenic E. coli, the bacteria confirmed by chemical were subjected to sensitivity test to assess the patterns of resistance. Multiresistant bacteria are bacteria resistant to three or more antibiotics. Samples of uropathogenic E. coli used uropatogen included antibiotics multiresistant bacteria. The uropatogenic samples of E. coli were resistant to amoxicillin, erythromycin and cotrimoxazole (Satari and Mieke, 2012). The Interpretation of sensitivity test results is presented in table 2 below:

b. Minimum Inhibitory Assay Results of α-Mangostin against Uropathogenic E. coli

Minimal inhibitory concentration assessment
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was performed by broth dilution method using a Mueller Hinton Broth (MHB) media. Mangostin alpha concentration range 14-450 µg/mL, the absorption after the treatment on uropathogenic E.coli is shown as follows;

<table>
<thead>
<tr>
<th>No</th>
<th>Antibiotics</th>
<th>Diameter results (mm)</th>
<th>Interpretation</th>
<th>Sensitive (mm)</th>
<th>Intermediate (mm)</th>
<th>Resistant (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>amoxicillin 25 µg</td>
<td>8</td>
<td>Resistant</td>
<td>≥17</td>
<td>14-16</td>
<td>≤13</td>
</tr>
<tr>
<td>2</td>
<td>erythromycin 15 µg</td>
<td>9</td>
<td>Resistant</td>
<td>≥23</td>
<td>14-22</td>
<td>≤13</td>
</tr>
<tr>
<td>3</td>
<td>cotrimoxazole 25 µg</td>
<td>10</td>
<td>Resistant</td>
<td>≥16</td>
<td>11-15</td>
<td>≤10</td>
</tr>
<tr>
<td>4</td>
<td>Siprofloxacain 5 µg</td>
<td>35</td>
<td>Sensitive</td>
<td>≥21</td>
<td>16-20</td>
<td>≤15</td>
</tr>
</tbody>
</table>

Table 3. Mean ± SD difference (decrease/increase) in absorbance after treatment α - mangostin on uropathogen E. coli in MHB media

<table>
<thead>
<tr>
<th>No</th>
<th>Concentration (µg/mL)</th>
<th>Absorption*</th>
<th>Mean Absorptions ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>450</td>
<td>0.293</td>
<td>0.288 ± 0.022</td>
</tr>
<tr>
<td>2</td>
<td>225</td>
<td>0.242</td>
<td>0.196 ± 0.039</td>
</tr>
<tr>
<td>3</td>
<td>112.5</td>
<td>0.172</td>
<td>0.117 ± 0.068</td>
</tr>
<tr>
<td>4</td>
<td>56.25</td>
<td>0.196</td>
<td>0.179 ± 0.017</td>
</tr>
</tbody>
</table>

* The absorbance was determined from the absorbance value of the treatment after incubation minus the absorbance value of the treatment prior to incubation. The brackets () shows a negative value, meaning that there was a decrease in treatment absorbance (decrease in the number of bacteria), whereas increased absorbance means there is an increase in the number of bacteria after incubation.

The results in Table 3 showed that the concentration of α-mangostin 450 µg /ml showed a decrease in the number of bacteria syndicated from a decrease in absorbance of the solution. For -mangostin α concentrations of 14-225 mg/mL there was an increased absorbance or an increase in the number of bacteria after incubation. It can be concluded that the minimal inhibitory concentration of α-mangostin against uropathogenic - E. coli was 450 µg /mL. Furthermore, α-mangostin at concentrations of 14-225 µg/mL showed no bacterial growth inhibition against uropathogenic E. coli.

The control group was used as control of the media and control the growth of uropathogenic E. coli. the control positive group showed bacterial growth, whereas negative control showed bacterial growth. Results of negative controls showed that α-mangostin and media used was sterile. Positive control results indicated that uropathogenic E. coli can grow on the medium.

c. Statistical analysis

The statistical analysis of linear regression was applied to determine the effectiveness of a dose of α-mangostin against E. coli. the tests were carried out by finding the relationship between the value of the dose of α-mangostin and the decrease/increase in absorbance after incubation. The analysis was performed using STATA 13.1 and the results is shown in Figure 2:

The lowest absorbance value was found at concentrations of 450 µg/mL. The coefficient of determination ((R2) was 0.6115 = 61.15%. This indicates that the sample absorbance variable variation can be explained by variable concentrations of α-
mangostin was 61.15%. The equation obtained by the regression line was $y = -0.0009x + 0.1987$. The value of $y$ is predicted log of the number of bacterial colonies of uropathogenic E. coli (UPEC), while the value of $x$ is the concentration of $\alpha$-mangostin. F test value 0.0001 <0.05 stated that all concentrations of $\alpha$-mangostin simultaneously have a significant effect on the absorbance, in other words they have an effect on the growth of uropathogenic E. coli.

DISCUSSION

the treatment with a differet levels of concentration of $\alpha$-mangostin, showed that the minimum inhibitory concentration of $\alpha$-mangostin by evaluating the turbidity in the test tube after incubation for 18-20 hours and at 370 C could not be done. This is due to the fact that turbidity can also be caused by $\alpha$-mangostin, not only due to the presence of bacteria. The minimum inhibitory concentration were determined by calculating the difference in absorbance before and after 18-20 hours incubatio at 37°C. The Minimum inhibitory cocentration againts uropathogenic E. coli was established at 450 µg/ml. This showed a negative result or decreased absorbance of the treated solution compared to absorbance prior to incubation. In $\alpha$-mangostin with a concentration 14 to 225 µg/mL showed an increase i absorbance after incubation incicating no bacterial growth inhibition againts uropathogeic E. coli.

Alfa xanthon mangostin is derivataives compounds found in the peel of the mangosteen fruit (Garcinia mangostana L) and has the IUPAC name 1,3,6-trihydroxy-7-methoxy-2,8-bis (methyl-2-butenyl) -9H-xanten- 9-on (MY Ibrahim et al., 2014). Xanthon is the main compound of Garcinia mangostana L., the widely distributed plant in Indonesia. This plant belongs to the family Clusiaceae. The optimum temperature for Garcinia mangostana L. is 25-35°C (Brunner and Payan, 2011). Some of the pharmacological activities associated with $\alpha$-mangostin include antioxidants, anti-inflammatory, anticancer, hypo-allergenic, antiviral, antibacterial, antifungal, and antimalarial activity. Other Xanthones such as gamma mangostin and xanthon derivatives has bee show to have no antibacterial activity (Jindarat, 2014).

The minimum inhibitory levels were obtained by the study conducted by Zou et al. (2013) which showig that the $\alpha$-mangostin less effective in killing gram-negative bacteria with the minimum inhibitory concentration >200 µg/mL. Uropathogenic Escherichia coli belongs to the gram-negative bacteria with outer cell membrane composed of lipopolysaccharide. This lipopolysaccharide serves as a non permeable barrier that is not permeable to hydrophobic molecules. Test
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In this study, the determination of the activity of \( \alpha \)-mangostin was conducted to merely evaluate the value of the minimum inhibitory concentration. Determination of minimal bactericidal activity was not conducted. Some literature state that potentially relevant activity in the clinical setting will occur if the value of MCI < 100 \( \mu \)g/mL. Even the pharmaceutical industry prefers the development of antibiotics with \( \leq 2 \) \( \mu \)g/mL. Minimum bactericidal activity is greater than MIC value, because the KHM value obtained in this study was 450 \( \mu \)g/mL (or greater than 100 \( \mu \)g/mL), then the MIC was not determined (Sanchez and Kouznetsov, 2010; Levison and Levison, 2009).

The antibacterial activity of alpha mangostin, especially against Gram negative bacteria requires further studied. The study of synergism with some antibiotics also did not show effective results. The combination of alpha mangostin with ampicillin, penicillin G, tetracycline and streptomycin did not showed effectiveness against Gram - (E. coli) and Gram + (S. aureus) (NHS Ibrahim et al., 2014). The crude extract activity of either the ethanol or water fraction of Garcinia mangostana Linn was also tested on several pathogenic bacteria. The results of these studies suggested that the required concentration of MIC and MBC is quite large (4000 mg/mL to extract water and 2000 mg/mL ethanol extract), to be able to inhibit and kill bacteria E. coli (RV Geetha et al., 2011).

CONCLUSION
\( \alpha \)-mangostin is not effective to inhibit the growth multi drug resistat uropahogenic E. coli multiresistant
uropatogen due to a relatively high MIC (450 mcg/mLz). The antibacterial activity of alpha mangostin, especially its activity against Gram negative requires further studied. The study of synergism with some antibiotics also did not show effective results.

**ACKNOWLEDGMENT**

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**CONFLICTS INTEREST**

There is no conflict of interest with any party, including the pharmaceutical industry.

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