

Antibacterial Activity of Combination of Chloramphenicol and Ethanolic Extract of Garden Balsam (*Impatiens balsamina*) Leaves Against *Escherichia coli* and *Shigella sonnei*

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Abstract—Chloramphenicol, a broad spectrum antibiotic, is used to treat several bacterial infections. It has several side effects such as headache, rash, diarrhea, nausea, vomiting, bone marrow suppression, and aplastic anemia. Combining antibiotic with another antibacterial agent may decrease antibiotic dose and hence the side effects. This study aimed to investigate the antibacterial activity of chloramphenicol in combination with ethanolic extract of garden balsam (*Impatiens balsamina*) leaves against *Escherichia coli* and *Shigella sonnei*. Chloramphenicol (30 µg), extract (2500 µg), combination of chloramphenicol and extract (22.5 µg+625 µg and 15 µg+1250 µg), dimethylsulfoxide, and water for injection were tested for antibacterial activity against *Escherichia coli* and *Shigella sonnei* using well diffusion method. Data was analyzed using Mann-Whitney test. The results showed that only chloramphenicol (22.5 µg) combined with extract (625 µg) has inhibition zone diameter, which was not significantly different from chloramphenicol alone (30 µg) when it tested against *E. coli*. These results indicated that combination of the antibiotic in lower concentration and extract can achieve the same antibacterial activity as antibiotic alone in higher concentration.

Keywords—antibacterial; chloramphenicol; *Impatiens balsamina*; combination

I. INTRODUCTION

Chloramphenicol is an effective antibacterial agent and has wide spectrum against Gram negative and Gram positive bacteria, rickettsia, mycoplasma, chlamydia, spirochetes, and anaerobic bacteria. The antibiotic has several adverse reactions such as bone marrow suppression, irreversible aplastic anemia, gray baby syndrome of neonates, and hemolytic anemia. Because of the adverse reactions, chloramphenicol use is limited for several

indications and used as alternative medication for serious infections [1]. The toxicity of antibiotic can be reduced by combining the antibiotic with other antimicrobial agents [2] because it may reduce the dose of toxic medicines [3].

Combination of antibiotics with plant extracts may increase their antibacterial activity. Chloramphenicol in combination with ethanolic extract of bark of *Ziziphus mucronata* showed higher antibacterial activity against *Enterococcus faecalis* ATCC 29212 and *Shigella flexneri* KZN compared with antibiotic alone [4]. Reference [5] demonstrated that combination of chloramphenicol and ethanolic extract of *Mellisa officinalis* leaves against *Bacillus subtilis*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* has wider inhibition zones than that of chloramphenicol alone. Increase in antibacterial activity was also observed in combination of chloramphenicol and methanolic extract of bark of *Acacia merrisii* against *Bacillus subtilis* KZN [6]. If antibacterial activity of chloramphenicol in combination with plant extracts is higher than that of chloramphenicol alone, it is possible to reduce antibiotic concentration and combine it with extract to achieve antibacterial activity of antibiotic alone in higher dose. One of plant extracts that can be used in combination with antibiotics is ethanolic extract of garden balsam (*Impatiens balsamina*).

Several researches showed that ethanolic extract of garden balsam leaves has antibacterial activity against several bacteria. Research findings of [7] revealed that ethanolic extract of garden balsam leaves has antibacterial activity against several Gram positive and Gram negative bacteria. Another researcher found that the growth of *Bacillus subtilis* and *Escherichia coli* were

inhibited by ethanolic extract of garden balsam leaves [8]. Reference [9] demonstrated that the extract also has antibacterial activity against *Streptococcus pyogenes* and *Shigella sonnei*. The objective of this study was to investigate antibacterial activity of combination of chloramphenicol and ethanolic extract of garden balsam leaves against *Escherichia coli* and *Shigella sonnei*.

II. MATERIALS AND METHODS

A. Plant Materials Collection

Garden balsam were collected from Selo, Boyolali, Central Java. The plant was authenticated at Faculty of Biology, Universitas Muhammadiyah Surakarta. Leaves were separated from any other parts of the plants.

B. Preparation of Extract

Leaves were washed under running water, dried, and pulverized. About 750 gram of pulverized leaves were macerated in 70% ethanol for 3 days. The extract was filtered through filter paper and solvent was evaporated using rotary evaporator (Heidolph). Extract was further concentrated using water bath (Memmert).

C. Tested Bacteria

Escherichia coli and *Shigella sonnei* were purchased from Sebelas Maret University and Balai Laboratorium Kesehatan Yogyakarta, respectively. Bacteria were streak on Mueller Hinton Agar and incubated at 37°C for 24 hours. Three to five colonies from 24 hours old culture were inoculated in 5 mL of brain heart infusion and then incubated at 37°C for 2-6 hours with shaking. The turbidity of bacterial cultures was adjusted to obtain turbidity of 0.5 McFarland standard (Remel™) by adding sterile saline. This bacterial suspension was used in antibacterial assay.

D. Antibacterial Assay

About 150 µL of bacterial suspension was transferred on Mueller Hinton agar and spread evenly. The cultures were left at room temperature for 15 minutes. Six wells with diameter of 8 mm were cut out of agar using sterile cork borer and 10 µL of chloramphenicol (3 mg/mL), 25% extract in dimethylsulfoxide (DMSO), chloramphenicol : extract = 3:1, chloramphenicol : extract = 1:1, water for injection (WFI), and DMSO were transferred into each well. Cultures were incubated at 37°C for 24 hours. Diameter of inhibition zone were measured in mm.

E. Statistical Analysis

Data was analyzed using Mann-Whitney test.

III. RESULT AND DISCUSSION

Chloramphenicol is one of antibiotics that inhibit protein synthesis by binding to the 50S subunit of 70S ribosome. In developing countries, chloramphenicol has an important role for treating life-threatening infections. The antibiotic causes several dose-related and not dose-related adverse effects [1]. Synergism as a result of combination two antibiotics has advantage of possibility to use toxic antibiotic in lower dose [3]. It is possible that synergistic activity may be obtained by combining antibiotic with plant extract that has antibacterial properties.

In this study, chloramphenicol was combined with ethanolic extract of garden balsam leaves and tested against *Escherichia coli* and *Shigella sonnei*. Ethanolic extract of garden balsam leaves with load of 2500 µg inhibited the growth of *Escherichia coli* [8] and *Shigella sonnei* [9]. Reference [7] also reported antimicrobial activities of ethanolic extract of garden balsam leaves against Gram negative and Gram positive bacteria as well as *Candida albicans*.

Antibacterial activity tests were carried out to investigate antibacterial activity of chloramphenicol alone and chloramphenicol in combination with garden balsam leaves extract. The results (Table 1) showed that all solution has antibacterial activity except WFI and DMSO (negative controls). Data of antibacterial assay were analyzed statistically.

Table 1. antibacterial activity of chloramphenicol and its combination with garden balsam leaves extract

No	Treatments	Diameter of inhibition zones (average ± SD mm)	
		<i>Escherichia coli</i>	<i>Shigella sonnei</i>
1	WFI	8 ± 0	8 ± 0
2	DMSO	8 ± 0	8 ± 0
3	Chloramphenicol (30 µg)	25 ± 0	22.3 ± 0.6
4	Extract (2500 µg)	14.7 ± 0.6	13.7 ± 1.5
5	Chloramphenicol + extract with ratio of 1:1 (22.5 µg + 625 µg)	24 ± 1	17.3 ± 0.6
6	Chloramphenicol + extract with ratio of 3:1 (15 µg + 1250 µg)	21 ± 0	15 ± 1

*diameter of inhibition zones includes well diameter of 8 mm

Data analysis of antibacterial assay against *Escherichia coli* showed that extract alone has the lowest antibacterial activity meanwhile antibacterial activity of chloramphenicol alone was higher than that of extract alone and combination chloramphenicol and extract with ratio of 1:1 (15 µg + 1250 µg). Combination of the

antibiotic and extract with ratio of 3:1 (22.5 µg + 625 µg) has higher antibacterial activity than that of chloramphenicol combined with extract with ratio of 1:1. There was no significantly different between diameter of inhibition zone of chloramphenicol alone and combination of chloramphenicol and extract with ratio of 3 : 1. It means that chloramphenicol alone and combination of chloramphenicol and extract with ratio of 3 : 1 have the same antibacterial activity.

Results of antibacterial test against *Shigella sonnei* revealed that chloramphenicol alone has the highest antibacterial activity. Extract alone has lower antibacterial activity than chloramphenicol alone and combination of the antibiotic and extract with ratio of 3:1 but has the same activity as combination of the chloramphenicol and extract with ratio of 1:1. The combination of the chloramphenicol and extract with ratio of 3:1 has higher antibacterial activity than that of chloramphenicol combined with extract with ratio of 1:1.

Among combinations, only combination of chloramphenicol and extract with ratio of 3:1 (22.5 µg + 625 µg) has the same antibacterial activity against *Escherichia coli* as chloramphenicol alone (30 µg). Lower concentration of chloramphenicol in combination with plant extract achieved the same activity as chloramphenicol in higher concentration. This result indicated that the combination of antibiotic in lower concentration may result in higher activity. Different antibacterial mechanism action of chloramphenicol and secondary metabolites in extract may contribute to the enhanced activity.

Garden balsam leaves contains several secondary metabolites that may contribute to its antibacterial activity. Anthrones, phenolics, alkaloids, antraquinones, coumarines, triterpenoids, and flavonoids were detected in ethanolic extract of garden balsam leaves [8] [9]. Research findings of [10] demonstrated that 3 main naphthoquinones in garden balsam leaves extract ie. lawsone, lawsone methyl ether, and methylene-3-3'-bilawsone have antimicrobial activity against bacteria and fungi. Isolated 1,4-naphthoquinone from leaves of garden balsam has antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* [11]. Antimicrobial effects of quinones may be due to its capability to make irreversible complex with nucleophilic amino acids in proteins that lead to protein inactivation and loss of protein function. There are several probable target of quinones in the microbial cell ie. membrane-bound enzymes, cell wall polypeptides, and surface-exposed adhesins [12]. A new series of 1,4-naphthoquinones has been proven to have antibacterial activity and they inhibited bacterial

growth (bacteriostatic) instead of killing the bacteria [13].

Combination of antibiotics may result in several effects. As postulated by Jawetz and Gunnison [14], combination of a bacteriostatic antibiotic and another bacteriostatic antibiotic may lead to additive effect, a bactericidal antibiotic combined with another bactericidal antibiotic may result in synergism, a bacteriostatic antibiotic in combination with another bactericidal antibiotic may be antagonistic. In the current study, chloramphenicol that is bacteriostatic was combined with ethanolic extract of garden balsam leaves which contains naphthoquinones. Naphthoquinones acted as bacteriostatic [13]. It means that it was two bacteriostatic interactions between chloramphenicol and the extract. The interactions result in additive effect. This may explain the same antibacterial activity between combination of chloramphenicol and extract with ratio of 3:1 (22.5 µg + 625 µg) and chloramphenicol alone (30 µg).

IV. CONCLUSION

This research suggested the possibility to combine antibiotic with plant extract in order to reduce antibiotic dose and hence the adverse effects.

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