ARTESMININ CONTENT ON ARTEMISIA ANNUA L. TREATED BY GLORIOSA SUPERBA SEEDS’ WATER EXTRACT

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ABSTRACT

Objective: The aim of this study was to determine the artesminin content on Artemisia annua L. treated by water extract of Gloriosa superba seeds.

Methods: G. superba seeds obtained naturally on Krakal Beach, Gunung Kidul, Yogyakarta. Determination of artesminin content in leaf extract of A. annua L. was done using TLC-densitometric method with n-hexane:ethyl acetate (4:1) as mobile phase.

Result: The result showed that artesminin content in plant treatment of G. superba seed water extract was higher (9.78 µg/µl ±3.21)–16.60 µg/µl ±1.39] compared to control plants (6.39 µg/µl ±1.49). The concentration water extract of G. superba seed affected the level of artesminin in the treatment plant. On the other hand, the soaking of A. annua L. sprouts using the water extract of G. superba seed did not affect the level of artesminin content.

Conclusion: Artesminin content in treatment plant by G. superba seed water extract treatment was higher compared to control plants.

Keywords: Artemisia annua L., Gloriosa superba, Natural colchicin

INTRODUCTION

Malaria is caused by the protozoan Plasmodium parasite, which is spread by the Anopheles mosquito. Nowadays, malaria parasite has already developed chloroquine resistance, and this resistant form is now widespread throughout the world. Artemisia annua L. is a potential Chinese medicinal plant that produces an active compound known as artesminin in malaria medication to replace quinine that has been resistant to Plasmodium falciparum. A. annua showed very remarkable antifungal activity [1]. Today, medicines produced from artesminin derivatives are applied as a first line chemotherapy due to their high antimalarial efficacy and low toxicity [2].

Artemisinin content accumulates in the glandular trichomes, an organ found only in leaves, stems, and flowers [3]. Cultivation targets are directed at enhanced levels of artesminin and high leaf production. Despite its wide distribution across the world, A. annua content of artesminin varies greatly among herbs from different places. Improving the yield and the artesminin content is the main objective for breeding this herb. Increased the number of chromosomes in medicinal plants is needed in order to increase its secondary metabolite level which make this medicine easily available and affordable [4]. Artificial polyploidy is a technique to increase the chromosome quantity in plant. Polyploidy can be made artificially with chemicals such as colchicine because most of these substances are easily soluble in water and effectively induce polyploidy [5]. Polyploidy induction of A. annua is able to increase artesminin production [6,7]. A. annua tetraploid produces artesminin 6 times of diploid plants [8]. A. annua polyploidy tends to have a higher content of metabolite (artesminin) than diploid plants [6,9,10]. A. annua is a good candidate of new source in the development of new antimalarial drugs [11].

Gloriosa superba is a herbaceous plant that grows propagate and naturally around Krakal Beach, Gunung Kidul, Yogyakarta. Colchicine and Gloricosine are the alkaloids contained in Gloriosa superba that used in medicinal applications for treatment of gout and rheumatism. Colchicine content in G. superba as reported was ranged from 0.15% to 0.25% in the seeds [12]. The content of alkaloid colchicine compounds in G. superba plant can be used as a potential polyploidy mutagen [13]. G. superba is a good source of colchicine used in plant breeding studies to produce polyploidy [14].

G. superba seed water extract was used as polyploidy mutagen on A. annua sprouts. The aims of this research were to determine the artesminin content on A. annua treated by water extract of G. superba seeds.

METHODS

Extraction of G. superba seeds

Extraction of G. superba seeds was using maceration method by water solvent (1:1). The water that used in this extraction was distilled water (aqadest). Analysis of colchicine content of G. superba seed extract was conducted by thin-layer chromatography (TLC)-densitometry method. Water extracts of the seeds were used as polyploidy mutagen of A. annua sprouts.

A. annua sprouts soaking

A. annua sprouts used were 1–2 weeks old. The treatment variables in this study were the concentration of water extracts of G. superba seeds (0%, 25%, 50%, 75%, and 100%) and the soaking time of A. annua sprouts (0 min, 30 min, 60 min, and 90 min). Design of treatment in this research is presented in Table 1.
The concentration of colchicine on the water extract of *G. superba* seed used as *A. annua* sprout soaking mutagen was 12.84 μg/μl (±2.88). The lowest levels of artemisinin in this study were control leaf samples of 6.39 μg/μl (±1.40). The highest artemisinin content in treated plant of 16.60 μg/μl (±1.39) was found in the ED leaf sample (100%, 90 s), while the lowest artemisinin content in treated plant of 9.78 μg/μl (±3.21) was found in the BA leaf sample (25%, 0 s).

Analysis of artemisinin content on the soaking time of *A. annua* sprouts was not significantly different with a significance value of α≤0.05, while artemisinin content analysis on concentration treatment of water extract of *G. superba* seeds was significantly different with significance value α≤0.05. In this study, artemisinin content was influenced by the concentration treatment of *G. superba* seed water extract. Duncan test for concentration treatments was performed as a further test. The results showed that the concentration of *G. superba* seed water extract of 0% was significantly different on concentrations of 25%, 50%, 75%, and 100%.

**DISCUSSION**

Artemisinin content on leaf extract of *A. annua* that soaked with water extract of *G. superba* ranged from 6.39 μg/μl (±1.39) to 16.60 μg/μl (±3.21). There are higher than the artemisinin content in leaf extract of *G. superba* ranged from 9.78 μg/μl (±3.21)-16.60 μg/μl (±5.52). Artemisinin content on leaf extract of *A. annua* that soaked with water extract of *G. superba* ranged from 9.78 μg/μl (±3.21)-16.60 μg/μl (±5.52). Artemisinin content on leaf extract of *A. annua* was significantly different on concentrations of 25%, 50%, 75%, and 100%.

Artemisinin analysis was performed on all leaf extract samples using KLT-densitometry method. Data analysis included the data levels of colchicine in the seed water extract of *G. superba, A. annua* morphological observation, observation of *A. annua*, and level of artemisinin in *A. annua*. Chromosome observation of *A. annua*, stomata observation of *A. annua* and artemisinin content observation in *A. annua*. Observation and analysis data of chromosome and stomata *A. annua* using Raster Image Viewer and Opti-Lab. The research data were analyzed statistically with SPSS 16.0 using factorial ANOVA test and ANOVA General linear model. The data analysis also is a descriptive study with Microsoft office. Excel using tables and graphs. Standard artemisinin used for comparison is obtained from B2P2TOOT, Tawangmangu, Karanganyar. The dried extract and standard artemisinin were precoated by silica gel F<sub>254</sub> aluminum plate (E-Merck grade) as a narrow band with 1 cm width at constant rate using CAMAG Linomat. The sample on the silica plate was eluted on a mixture of n-hexane solution:ethyl acetate (4:1) as the mobile phase. The elution of *A. annua* leaf extract was observed under ultraviolet (UV) light. The Rf values and the color of the compound indicated determine the artemisinin compounds observed in UV light. Densitometric scanning was performed using a CAMAG TLC Scanner with CATS 4 software to observe the artemisinin area at selected wavelengths. The levels of artemisinin contained in *A. annua* leaf dry extracts were calculated from the area of the same wavelength as standard artemisinin.

**RESULT**

**Table 3: Comparison of artemisinin content in control and treated plant**

| A. annua | Code | Artemisinin | Description of treatment |
|---------|------|-------------|-------------------------|
| Control Treatment | aa   | 6.39 μg/μl (±1.40) | (0%, 0 s) |
| Higher Treatment | ed   | 16.60 μg/μl (±1.39) | (100%, 90 s) |
| Lowest Treatment | ab   | 7.38 μg/μl (±4.82) | (0%, 30 s) |

**Extraction of *A. annua* L leaves**

*A. annua* leaf sample was dried under the sun for 3 days. The dried samples are smoothen with mortar. A total of 0.1 g of fine leaf samples were extracted with 5 ml of methanol and then filtered. The extraction was repeated 3 times, and the extraction results were placed on the Petri dish. The extract was evaporated using a fan until dry.

**Analysis of artemisinin content**

Artemisinin analysis was performed on all leaf extract samples using KLT-densitometry method. Data analysis included the data levels of colchicine in the seed water extract of *G. superba, A. annua* morphological observation, observation of *A. annua*, and level of artemisinin in *A. annua*. Chromosome observation of *A. annua*, stomata observation of *A. annua* and artemisinin content observation in *A. annua*. Observation and analysis data of chromosome and stomata *A. annua* using Raster Image Viewer and Opti-Lab. The research data were analyzed statistically with SPSS 16.0 using factorial ANOVA test and ANOVA General linear model. The data analysis also is a descriptive study with Microsoft office. Excel using tables and graphs. Standard artemisinin used for comparison is obtained from B2P2TOOT, Tawangmangu, Karanganyar. The dried extract and standard artemisinin were precoated by silica gel F<sub>254</sub> aluminum plate (E-Merck grade) as a narrow band with 1 cm width at constant rate using CAMAG Linomat. The sample on the silica
present in the leaves can be obtained in a high concentration (Table 3) [16].

CONCLUSION

Based on the result, it shown that A. annua treated by water extract of G. superba of 12.84 µg/µl (±2.88) content of colchicine influences artemisinin content. A. annua on control treatment has artemisinin levels of 6.39 µg/µl (±1.40). The highest content of artemisinin in leaf of A. annua treated by water extract of G. superba was 16.60 µg/µl (±1.39), while lowest artemisinin content treated by water extract of G. superba seeds was 9.78 µg/µl (±3.21). The concentration of G. superba seeds water extract affects the level of artemisinin, while the soaking time of A. annua does not affect the level of artemisinin.

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AUTHORS' CONTRIBUTION

Sri Indah conducted the experiment and prepared the manuscript. Dr. Ari Susilowati designed the experiment and Dr. Yuli Widyastuti contributed in the experimental part of the work. Prof Ahmad Yunus designed and conducted the experiment and finalization of the manuscript.

CONFLICTS OF INTEREST

All authors confirm that this article content has no conflict of interest.

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