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Analysis of Gossypol content on roots, seeds, and leaves of cotton plant (*Gossypium Hirsutum* L.) cv. Kanesia 12

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Abstract
Plants have long been known to produce secondary metabolites that are useful for medicines, insecticides, etc. Gossypol is one of the secondary metabolites produced by cotton plants (*Gossypium hirsutum* L.). Gossypol plays a role in self-protection for plants, but it also has the potential a medicine. Gossypol is the main pigment found in lisigen glands scattered in some organs and tissues of cotton plants. The content of gossypol in each part of the cotton plant is different. This study aimed to analyze the content of gossypol compounds in three plant parts of *Gossypium hirsutum* L cv. Kannesia 12, including seeds, roots, and leaves. Gossypol was extracted using petroleum ether. The levels of gossypol were determined using the High-Performance Liquid Chromatography (HPLC) assay. The results showed all parts of *Gossypim hirustum* L. cv. Kanesia 12 contained gossypol at different levels. The highest gossypol content was found in the seeds (25.75µg/g), followed by the roots with 8.00µg/g, and the least gossypol content in leaves was 1.05µg/g.

Keywords: Gossypol, Root, Seed, Leaves, *Gossypium hirsutum*.

1. Introduction

Plants have long been known to produce compounds that can be used for medicines, insecticides, dyes, and others. These compounds are secondary metabolites synthesized by plants through secondary metabolic pathways. Primary metabolites such as carbohydrates, proteins, and fats are the basic ingredients to form secondary metabolites. For plants themselves, secondary metabolites play a role in self-defense against unfavorable conditions, either physically, chemically, or biologically [1].

One of the secondary metabolites plants produce is gossypol, a group of plants from the cotton genus (*Gossypium* spp.). Gossypol compounds are yellow pigments that are permanently present in cotton plants. Gossypol is naturally produced by pigment glands that are scattered in all parts of the cotton plant [1,2]. Stipanovic *et al*. [3] stated that gossypol naturally occurs in the seeds, leaves, and roots of the cotton plant (*Gossypium*). In addition, Wagner *et al.* [4] also stated that gossypol is a dimeric sesquiterpene that was first found in cotton seeds. However, gossypol is also found in various tissues of cotton plants. Besides being a constitutive pigment in cotton plants, gossypol is also a phytoalexin, a compound produced by plants in response to unfavorable environmental conditions, either physical, chemical, or biological. Thus, gossypol plays a role in self-protection from pests and pathogen attacks. Zhou *et al.* [5] stated that the presence of gossypol can increase plant defense responses against herbivorous insects and pathogens. Hagenbucher *et al*. [6] and Tian *et al*. [7] also made a similar statement, which states that gossypol plays an important defense mechanism against herbivores and pathogens. Mellon *et al*. [8] have proven that gossypol is an effective natural antimicrobial against various cotton fungal pathogens.

Like other secondary metabolites, in addition to functioning as self-protection for the plants that produce it, gossypol has been known to have the potential to be used as a drug. Zayani *et al*. [9] showed that giving cottonseed extract to mice could reduce the number of mouse embryos. A similar study was also conducted by Sumarmin & Zayani [10], who reported that cotton seed extract affected the reproductive performance of female mice by reducing the number of corpus luteum and increasing fetal mortality. In addition, Zhao *et al*. [11] stated that gossypol could be used as a compound for anti-cancer and contraception for men. Thus, cottonseed extract has the potential to be used as a contraceptive.

Gossypol is the main pigment found in lisigen glands which are scattered in some organs and tissues of cotton plants. These glands are especially abundant in seed embryos, young carpels, young leaves, old roots, and the bark of stems. According to Li *et al*. [12], gossypol is stored in dark pigment glands (not exposed to light) and is only produced by plants of the *Gossypium* species group. This study aimed to analyze the content of gossypol in the roots, seeds, and leaves of the cotton plant *Gossypium hirsutum* L. cv. Kanesia 12.

2. Materials and methods

2.1 Materials

The materials used in this study were: seeds, roots, and leaves of the cotton plant (*Gossypium hirstum* L.) cv. Kanesia 12, petroleum ether, methanol, acetone, phosphoric acid, Whatman filter paper no.1, syringe filter 0.45m, aquadest, and standard gossypol (Sigma, USA).

2.2 Sample Extraction

The extraction method was carried out following a modified Schmidt & Wells [13] method. All parts of the cotton plant (seeds, roots, leaves) were dried and finely ground. A total of 1 gram of powder from each organ was extracted with 5ml of petroleum ether, stirred and then centrifuged at 1500rpm for 15min. The supernatant was discarded, and the pellet was dried overnight, then extracted with acetone and filtered with Whatman paper no.1. The extract obtained was then evaporated in a water bath at a temperature of 45-50^{o}C. The obtained residue was then dissolved in 1 ml of methanol, and the extract was ready for analysis.

2.3 Gossypol Content Analysis

The gossypol content in the samples was determined by the High-Performance Liquid Chromatography (HPLC) method, carried out at the Pharmacy laboratory of Airlangga University. The steps were as follows: The extract obtained at the extraction stage was filtered through a 0.45m syringe filter and then injected into the Slum-pack CLC-ODS column (0.15m). x 6.0mm) with methanol and water as the mobile phase in a ratio of 90:10 and the addition of 0.1% phosphoric acid with a flow rate of 1 ml/minute. The elution results were observed at 230nm. For comparison, standard gossypol (25, 60, 110, and 200ppm) was injected into the same column [13].

3. Results and Discussion

The results showed all samples, roots, seeds, and leaves of the cotton plant *Gossypium hirsutum* L. cv. Kanesia 12, contains gossypol (Figure 1). Zhang *et al*. [14] stated that gossypol is a yellow dimeric sesquiterpene compound produced by the cotton plant and its related species. In addition, Karishma *et al.* [15] also indicated that gossypol is a phenolic compound in the form of a yellow pigment found in various parts of the cotton plant. Gadelha *et al*. [1] and Wedegaertner & Rathore [2] stated that gossypol is naturally produced by pigment glands which are scattered in all parts of the cotton plant. In addition, Stipanovic *et al*. [3] also stated that gossypol naturally occurs in the seeds, leaves, and roots of the cotton plant (*Gossypium*).

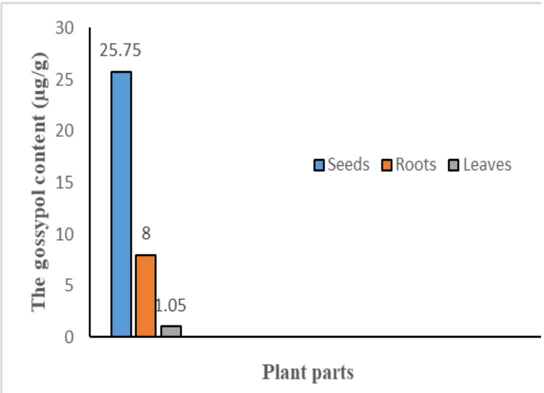

Figure 3.1: Gossypol content (µg/g) in each part of the cotton plant (*Gossypium hirsutum* L.) cv. Kanesia 12

Gossypol content in the three plant parts (seeds, roots, and leaves) was different (Figure 3.1). seeds have the highest levels of gossypol, which is 25.75µg/g, followed by roots at 8.00µg/g, and the leaves are the least, which is 1.05µg/g. The results of this study are in accordance with Tian *et al*. [16] that there is considerable variation in the content of Gossypol and related sesquiterpenoids between tissues and stages of development of *Gossypium* species. The results of this study are also in accordance with the statement of Cai *et al*. [17], who stated that in most cultivars, pericarp and mature seeds had a relatively high content of gossypol. In contrast, leaves had a moderate content, and young tissues such as hypocotyl had a lower content. Differences in the content of gossypol in each plant part have also been reported by Bolek *et al*. [18], who reported that there were significant differences in the content of gossypol in stems, ball walls, leaves, and seeds of cotton plants. In addition to plant parts, the stage of plant development is also a factor determining the amount or minimum content of gossypol, for example, young leaves usually have a higher sesquiterpenoid content (including gossypol) than older leaves [16]. Zhao *et al*. [11] have reported that cotyledon seeds are the main source of gossypol at the germination stage. However, after this stage, gossypol mainly comes from roots. In addition, root culture in vitro has a high ability to synthesize gossypol.

In addition to being different in each plant organ, Karishma *et al*. [15] have proven that the gossypol content in each cotton plant is also different. Abdelrahman *et al.* [19] have also reported that the gossypol content in Seini-1 cottonseed, genetically engineered cotton, has a lower gossypol content than hamid, which is traditional cotton. Likewise, the gossypol content in cotton plants *Gossypium barbadense* is higher than in *Gossypium hirsutum*.

According to Zhao *et al*. [11], gossypol is mainly synthesized in the roots, then transported to the aboveground parts of the plant and deposited in the brown pigment glands in the epidermal tissue of the roots, stems, leaves, and seeds of cotton plants. The plant parts above the soil surface also can synthesize gossypol but do not cause significant gossypol content in these parts [11].

Cotton plants produce gossypol and other terpenoid compounds that fight herbivorous and pathogenic attacks. Gossypol is a constitutive pigment produced by the cotton plant. In addition, gossypol is also a phytoalexin, a compound produced by plants in response to unfavorable conditions physically, chemically, and biologically. Cotton plants respond to pest attacks by producing glands and/or terpenoid compounds [20]. Furthermore, Rathore *et al*. [20] reported that 7 days after the cotton plants were damaged (insects attack), there was a significant increase in the production of gossypol, Hemigossypolone (HGQ), and heliosides, compared to plants that were not damaged. This proves that cotton plants' gossypol and other terpenoid compounds are important in self-defense mechanisms. General research results strongly suggest that glandless cotton is highly susceptible to insect pests and mammalian herbivores [20]. Some of the evidence above further strengthens that gossypol and other secondary metabolites in cotton play a role in determining plant resistance.

4. Conclusion

This study concluded that all parts of the cotton plant (*Gossypium hirsutum* L.) cv. Kanesia 12 gossypol was at different levels. The seeds contain the highest levels of gossypol (25.75µg/g), followed by roots (8.00µg/g) and leaves (1.05µg/g).

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