Colchicine concentration of *Gloriosa superba* seed at different locations and seasons

F Rahmawati¹, Sugiyarto² and A Yunus³

¹ Bioscience, Graduate Program, Universitas Sebelas Maret, Surakarta, Indonesia
² Faculty of Mathematic and Natural Science, Universitas Sebelas Maret, Surakarta, Indonesia
³ Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia

E-mail: yunus.uns7@yahoo.com

**Abstract.** Colchicine is an alkaloid compound from *Gloriosa superba* which has potential to be developed as a mutagenic agent for plant breeding. The concentration of colchicine produced by plants is affected by different environmental conditions. This research used samples which were obtained from two locations: Sukoharjo, Central Java (collected on December 2016 until January 2017) and Gunungkidul, Yogyakarta (collected on May 2017). The colchicine concentration in *G. superba* seeds need to be quantified to ensure the application dose. Solvent used for extracting colchicine from *G. superba* seed is ethanol 70%. Thin Layer Chromatography-densitometry using Chloroform:diethylamin (9:1) as a mobile phase was used to determine colchicine concentration before validated using selectivity, accuracy, precision, and linearity parameter. The results showed that the colchicine concentration in *G. superba* seeds from Sukoharjo was 30.81 mg/ml and from Gunungkidul was 57.92 mg/ml when measured with densitometer on maximum wavelength 371 nm.

1. Introduction

Colchicine is a main alkaloid compound of *Gloriosa superba* which is potential to be developed as a mutagenic agent for plant breeding in agriculture. Colchicine as anti-mitotic agents has a mode of action by thwarting the polymerization of tubulin protein into microtubules by binding to the β-tubulin subunit of microtubules. Due to the failure of this tubulin polymerization, complete microtubules will pull the duplicate chromosome to the cell pole and the process of cell division is aborted. As a result, cell division remains in the metaphase and the duplicate chromosome remains in the metaphase plane (metaphase plate or division plate) and cell division is canceled but the number of chromosomes has doubled.

Colchicine are mutagenic compounds that do not affect the DNA sequences, but only change the number of chromosomes in the genome set. It will produce polyploid plants when applied to the plant. Colchicine as a cell toxin shows its effect on plant cell division. In a nucleus division, colchicine can prevent the formation of plasma spindles so that chromatidal separation in the anaphase doesn’t occur and leads to chromosome doubling without the formation of cell walls. The process of mitosis that has undergone this modification is called C-mitosis [1].

Colchicine is present in almost every part of the *G. superba* plant. Around 0.1-0.8% of the of colchicine content were presented in the tubers. It can be used in plant breeding to induce the occurrence of polyploid plants [2].
The results of a study stated that the immersion in *G. superba* bulb extract solution had a significant effect on cell division (mitotic index) of onion bulb [3]. It indicates that effective colchicine compound was used to induce mutation (polyploid). It is further explained that this compound can stop cell division (antimitosis), i.e. by inhibiting the formation of spindles so that the cell cannot be drawn to the opposite pole and the chromosomes spread in the cell, the formation of new cell membrane is inhibited and eventually forming cells with increasing number of chromosomes or polyploidy.

2. Methods
Samples of *G. superba* seed are obtained from two locations: Sukoharjo, Central Java on December 2016 - January 2017 and Gunungkidul Yogyakarta on May 2017. Samples were extracted using solvent ethanol 70% [4].

Colchicine analysis of *G. superba* extract were done with Thin Layer Chromatography (TLC) method [5]. Before validated using selectivity, accuracy, precision, and linearity parameter, Thin Layer Cromatography-densitometry using Chloroform:diethylamin (9:1) as a mobile phase was used to determine colchicine concentration with maximum wave length 371 nm.

3. Result and discussion
*Gloriosa superba* seeds were extracted using alcohol 70%. Colchicine (C_{22}H_{25}NO_{6}) is a water-soluble alkaloid (1:22), easily dissolved in alcohol or chloroform [4]. The extract was bottled on a silica plate, then eluted using eluent chloroform:diethylamine (9: 1). The colchicine compound exhibits the same RF, as shown in Figure 1.

![Figure 1](image1.png)

**Figure 1.** The result of standard elution of colchicine and extract of *G. superba*.

The elution results were sprayed using fainiline sulphate to prove that the compound obtained was colchicine with the appearance of the same color between the standard and the sample extracts (Figure 2).

![Figure 2](image2.png)

**Figure 2.** The labelling of *colchicine* to the order of appearance
The eluted silica plate was read using a densitometer to see the precision of wave similarity and absorption between standard colchicine and *G. superba* extract samples (Figure 3).

![Figure 3. Spectral profile](image_url)

The levels of plant colchicine produced can be affected by different environmental conditions. To ensure the application dose, it is necessary to determine the colchicine concentration in *G. Superba* seeds. The determination of colchicine concentration was estimated by the linear function (Figure 4.), based on sample uptake on the curve area (Table 1).

![Figure 4. Linearity](image_url)

| Code | AUC     | Colchicine concentration (ug / ul) | Average   |
|------|---------|------------------------------------|-----------|
| A    | 25382.3 | 32.05116                           | 30.80984  |
|      | 23522   | 29.32425                           |           |
|      | 25824.8 | 32.69979                           |           |
|      | 23412.8 | 29.16417                           |           |
| B    | 17870.9 | 63.12181                           | 57.92436  |
|      | 15311.7 | 51.86763                           |           |
|      | 17818.6 | 62.89182                           |           |
|      | 14793.2 | 49.58751                           |           |
|      | 17650.6 | 62.15303                           |           |
The difference of secondary metabolite concentration is strongly influenced by the environment. Plant growth and metabolism is influenced by changes in environmental temperature, elevation, soil factor, water and fertilizer [6].

4. Conclusion
The results of colchicine concentration in *G. superba* seed from Gunungkidul was 57.92 mg ml\(^{-1}\) and from Sukoharjo was 30.81 mg ml\(^{-1}\). It can be concluded that *G. superba* seeds from Gunung Kidul at dry season has higher colchicine concentration than *G. superba* from Sukoharjo at rainy season.

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