

## Determination colchicine content in aquadest-extracted *Gloriosa superba* seed from Sukoharjo and Gunung Kidul

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**Abstract.** Colchicine is a toxic alkaloid compound from the *Colchicum autumnale* plant. Colchicine can be used in agriculture as anti-mitosis in the procurement of plants with polyploid cells. *Gloriosa superba* is an herb contains colchicine as a mutagen (polyploid) potential in its parts, especially in the seeds. The aims of this research were to determine colchicine content from aquadest-extracted *Gloriosa superba* seed obtained from Sukoharjo and Gunung Kidul. *Gloriosa superba* seed samples were obtained naturally in January - April 2017. Samples were divided into immature seed samples from Mulur, Sukoharjo, Central Java and old seed samples from Krakal Beach, Gunung Kidul, Yogyakarta. Extraction seeds of *Gloriosa superba* using the maceration method with aquadest solvent (1: 1). Determination content of colchicine extract using TLC-Densitometry method. The results indicated that colchicine content of old seed *Gloriosa superba* samples 37,6 µg/µl (± 6,63) higher than colchicine content of immature seed *Gloriosa superba* samples 12,84 µg/µl (± 2,88)

### 1. Introduction

*Gloriosa superba* is an herbaceous vine plant found in the yard of Mulur, Sukoharjo and hill around Krakal Beach, Gunung Kidul. Usefulness and benefits of this plant is not widely known by the surrounding population. This plant is usually used as animal feed by the local people and allowed to grow on the sidelines of other plants. The abundance of this plant is found wildy and widely in Mulur, Sukoharjo and Krakal Beach, Gunung Kidul. *Gloriosa superba* L. is a perennial climber and is used as an ayurvedic medicinal herb to cure diseases in various parts of Africa and Southeast Asia. The plant was under threatened category due to its imprudent harvesting from wild as it is extensively used by medicinal industries for its colchicine content [1]. The high colchicine content accompanied by prospects of good availability from both wild and cultivated sources make the seeds of *Gloriosa superba* a potential commercial source of colchicine and colchicoside [2]. The colchicines content has been estimated to be 0.6% in seeds [3] and colchicosides 0.8%. The seeds are the best source of colchicines as their content is 2–5 times higher than in tubers. Thiocolchicoside (TCC), a semi-synthetic derivative of naturally occurring colchicoside from the seeds of *Gloriosa superba* [4] and *Colchicum autumnale*. It is chemically 2-demethoxy-2-glucosidoxythiocolchicine with a chemical



formula  $C_{27}H_{33}NO_{10}S$ . TCC has been used clinically for humans as a centrally acting muscle relaxant agent for the treatment of orthopedic, traumatic and rheumatological disorders [5]. This compound also has anti-inflammatory and analgesic effects [6]. It is an analogue of colchicines, since they share the same benzo(alpha) heptalenic moiety [7]. The aim of this study was determine of colchicine content in aquadest-extracted of *Gloriosa superba* seeds obtained from Sukoharjo and Gunung Kidul.

## 2. Methods

The method used in this research is the sampling method. *Gloriosa superba* is a wild plant located in Mulur, Sukoharjo and Krakal Beach, Gunung Kidul. Sampling conducted in January-April 2017. Samples obtained in the form of seeds *Gloriosa superba* consisting of immature seeds and old seeds obtained at a different place. *Gloriosa superba* immature seed samples were obtained from Mulur, Sukoharjo, while the old seed sample was obtained from Krakal Beach, Gunung Kidul. Both seed samples immature and old seed were dried using oven at 70°C for 24 hours. The extraction of both *Gloriosa superba* seeds using the maceration method with aquadest solvent (1:1). Determination content of colchicine on aquadest-extracted of *Gloriosa superba* seeds based on parameters of selectivity or specificity, accuracy, precision and linearity using TLC-Densitometry method [8]. Both samples of seed extract on silica plate was eluted using phase chloroform: diethylamine (9:1). The elution results were observed under UV light to detect the presence of a colchicine compound in a sample indicated by color and Rf value. The silica plate after eluted then sprayed using vanillin sulphate to see the color change of the compound. Colchicine area at selected wavelengths in the analysis using a densitometer.

## 3. Result and Discussion

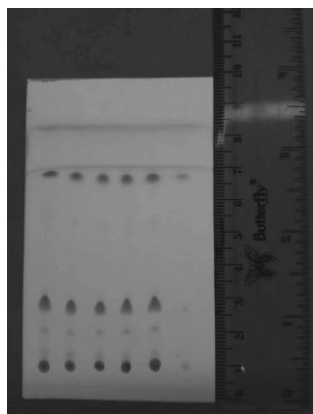
*Gloriosa superba* seeds sample in this study consisted of immature seed samples obtained from Mulur, Sukoharjo and old seed samples obtained from Krakal Beach, Gunung Kidul. *Gloriosa superba* seed samples were dried using oven at 70°C for 24 hours. Both samples of dried seeds were extracted using the same method of maceration method with aquadest solvent (1:1) for 24 hours. The extraction result was aquadest-extracted of immature seed and old seed of *Gloriosa superba*. Aquadest-extracted seeds using TLC-Densitometry method for determination of colchicine content. Results of colchicine content in immature seeds and old seeds sample were presented in Table 1.

**Table 1.** Colchicine Content of *Gloriosa superba*

Samples	Colchicine Content
Immature seeds	12.84 µg/µl (± 2.88)
Old Seeds	37.65 µg/µl (± 6.63)

### 3.1. Immature Seeds Sample of *Gloriosa superba*

Immature seeds of *Gloriosa superba* were obtained wildly around Mulur's yard, Sukoharjo. This plant is a wild plant that grows around the houses and residents in the gardens. Immature seeds of *Gloriosa superba* was conducted in January-February 2017 during the rainy season. Immature seeds drying using oven with temperature 70°C for 24 hours. Extraction of immature seed of *Gloriosa superba* using the method of maceration with water solvent (1:1) for 24 hours with 3 times repetition. The extraction result is aquadest-extracted immature seed which then analyzed using TLC-Densitometry to presence, determine and calculate the colchicine content contained in immature seed extract.

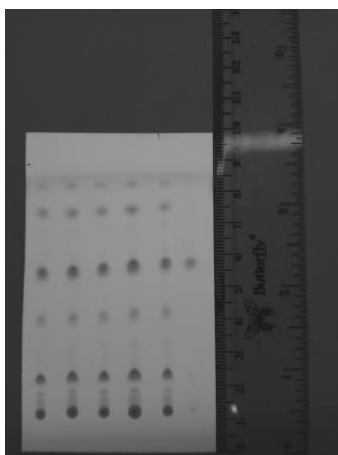


**Figure 1.** TLC Results of *Gloriosa superba* Immature Seeds

Aquadeast-extracted immature seeds of *Gloriosa superba* eluted on solution chloroform:diethylamine (9:1). The elution results were then seen using UV light (Figure 1). The Rf values and the colors between the samples of the eluted compound appear to be the same as the standard colchicine (right). Detection of colchicine compounds was also done by spraying vanillin sulphate to determine the color change of elution compounds formed on silica plates. The spray results show no change in color after being sprayed. This shows that the compound in the extract is colchicine because it has the same color and Rf value as the standard colchicine (right). Determination of levels of colchicine on immature seeds extract using densitometric method. Colchicine content in immature seeds extract is 12.84  $\mu\text{g}/\mu\text{l}$  ( $\pm 2.88$ ).

### 3.2. Old Seeds Sample of *Gloriosa superba*

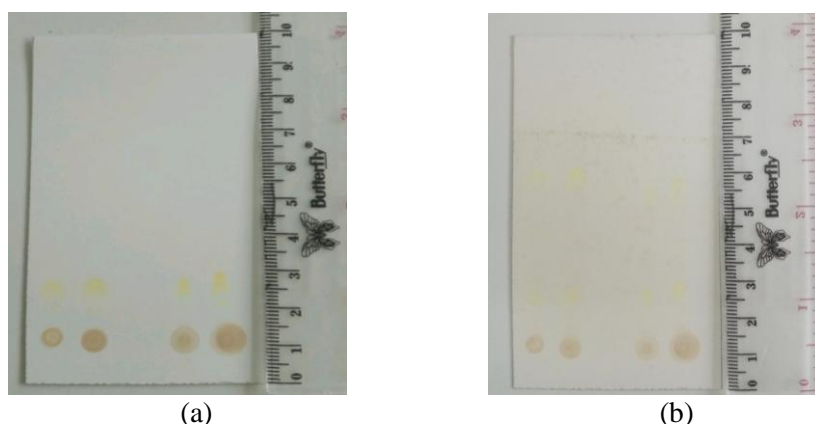
*Gloriosa superba* old seeds sample obtained in March-April 2017 at Krakal Beach, Gunung Kidul. This plant grows wildly and vine over the coastal hills. Old seed sampling is done around the hills of the Krakal Beach entering the summer. *Gloriosa superba* around the Krakal beach usually begins to grow seeds and flowering at the beginning of the rainy season, old after 2-3 months and after that it will dry and then die. The old seeds of *Gloriosa superba* are ripe brown and start to dry. Seed samples were dried using oven at 70°C for 24 hours. The dried samples was extracted using maceration method with aquadeast solvent (1:1) for 24 hours with 3 replications. Determination levels of colchicine contained in the extract of old seeds using the TLC-Densitometry method.



**Figure 2.** TLC Results of *Gloriosa superba* Old Seeds

The aquadeast-extracted *Gloriosa superba* old seeds was analyzed by using TLC method to determine the presence or absence of colchicine compounds in the extract. The extract that has been

on silica plate then elution using solution chloroform:diethylamine (9:1). Elution results are more clearly seen using UV light to know the compounds that are formed. The  $R_f$  value and the color of the formed compound look the same as the standard colchicine. Spraying silica plate elution using vanillin sulphate is also done to see clearly the color of extract samples and standard colchicine are formed. The results of the aquadest-extracted *Gloriosa superba* old seeds using TLC and spraying vanillin sulphate showed that the compound formed is a colchicine compound with yellow color and the same  $R_f$  value between samples with standard colchicine. Determination of the value of colchicine content in the aquadest-extracted samples of *Gloriosa superba* old seeds using densitometry method. The results showed the colchicine levels in aquadest-extracted old seeds was  $37.65 \mu\text{g}/\mu\text{l}$  ( $\pm 6.63$ ).



**Figure 3.** (a) TLC result, (b) TLC result after spraying with vanillin sulphate

The research that has been done on determination levels of colchicine contained in the aquadest-extracted immature seed and old seed of *Gloriosa superba* showed different grade values. The colchicine level in immature seeds aquadest-extracted was ( $12.84 \mu\text{g}/\mu\text{l}$  ( $\pm 2.88$ )) lower than in old seed aquadest-extracted ( $37.65 \mu\text{g}/\mu\text{l}$  ( $\pm 6.63$ )). This was possible that there are factor seed maturity, sampling time and sampling area. Other that may affect is the environmental factor where it grows from both plants. *Gloriosa superba* that grows and develops in Mulur, Sukoharjo is in the yard of the residents and around the garden of the residents. The plant grows creeping under the shade of large trees are not experiencing water shortage. *Gloriosa superba* plant that grows and develops on Krakal Beach, Gunung Kidul is located in the hills around the coast and creeps among other coastal vegetation. The plants grow wildly with the conditions of the hot beach environment and are on a hill that is possible away from the water source. The growth plants of the two different *Gloriosa superba* can be observed in the morphology of leaves, flowers, stems, and seeds. The plants grown in Mulur have greener leaves, striking flowers, short stems and large seeds. In plants grown on the Krakal Beach has a slightly yellowish leaves, smaller flowers, high and large creeping stems, and smaller seeds. In different growing conditions it is possible that the content and level of colchicine in the seeds in the two plants is different. Colchicine is a secondary metabolite compound produced by plants. In severe environmental conditions, secondary metabolites may be higher than plants grown in nutrient-rich environments.

#### 4. Conclusion

Colchicine content on *Gloriosa superba* aquadest-extracted obtained from two different places has different colchicine values. Based on the research that has been done, the factor of time, place and environmental condition of *Gloriosa superba* seed sampling affect the value of colchicine content even though using the same method of extraction. The old seed sample has a higher content of colchicine than the immature seed sample of *Gloriosa superba* plant. Further research can be done by sampling old seeds and immature seeds at once in both places to compare clearly the content of colchicine.

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### References

- [1] Jana S, and G S Shekhawat. 2010. Critical review on medicinally potent plant species : *Gloriosa superba*. *Journal Elsevier, Fitoterapia* **82** 293-301
- [2] Sivakumar G, Krishnamurthy KV. *Gloriosa superba* L. — a very useful medicinal plant. In: Singh VK, Govil JN, Hashmi S, Singh G, editors. *Recent Progress in Medicinal Plants*, Vol. 7. Ethnomedicine and Pharmacognosy, Part II. Texas: Series Sci Tech Pub, Texas, USA; 2002. p. 465–82
- [3] Sarin Y K, Jamwal P S, Gupta R K, Atal C K 1974 Colchicine from seeds of *G. superba*. *Curr. Sci.* **43** 87–90
- [4] Carta M, Murru L, Botta P, Talani G, Sechi G P, De Riu P L 2006 The muscle relaxant thiocolchicoside in an antagonist of GABAA receptor function in the central nervous system. *Neuro Pharmacol* **51** 805–15
- [5] Sandouk P, Bouvier d'Y voire M, Chretien P, Tillement J P, Scherrmann M 1994 Single-dose *bioavailability* of oral and intramuscular thiocolchicoside in healthy volunteers. *Biopharm. Drug Disp.* **15** 87–92
- [6] Giavina-Bianchi P, Giavina-Bianchi M, Tanno LK, Ensina L F C, Motta A A, Kalil J 2009 Epileptic seizure after treatment with thiocolchicoside. *Ther. Clin. Risk. Manage.* **5** 635–7
- [7] Janbroers J M 1987 Review of the toxicology, pharmacodynamics and pharmacokinetics of *thiocolchicoside*, a GABA-agonist muscle relaxant with anti-inflammatory and analgesic action *Acta Ther.* **13** 221–50
- [8] Hilmi A, Sudjarwo, and Asri D 2013 Validasi *Metode* Kromatografi Lapis Tipis-Densitometri untuk Penetapan Kadar Kolkisin Dalam Infus Daun Kembang Sungsang (*Gloriosa superba* Linn.). Departemen Kimia Farmasi, Fakultas Farmasi, Universitas Airlangga. *Jurnal Berkala Ilmiah Kimia Farmasi* **2** 2