10.5005/jp-journals-10015-1453

ORIGINAL RESEARCH

Quantity Differences of Secondary Metabolites (Saponins, Tannins, and Flavonoids) from Binahong Plant Extract (*Anredera cordifolia* (Ten.) Steenis) treated and untreated with Colchicines that play a Role in Wound Healing

[1]Olivia A Hanafiah, [2]Diana S Hanafiah, [3]Eva S Bayu, [4]Trimurni Abidin, [5]Syafrudin Ilyas
[6]Marline Nainggolan, [7]Endang Syamsudin

ABSTRACT

Aim: The aim of this study is to compare whether there are differences in the value content of secondary metabolites (saponins, tannins, and flavonoids) between the group treated with Binahong leaves (administration of colchicines) and the group treated with Binahong leaves without the application of colchicines.

Materials and methods: The design of this study is an experimental research laboratory study. This research was done in four different places in which colchicines were applied to improve the quality of Binahong, conducted in the USU Agricultural Faculty's Greenhouse, whereas no application of colchicines was done in the Desa Simpang Pergendangan Kelurahan Tiga Binanga Kabupaten Karo harvesting Binahong plant. The pharmacy faculty of USU was chosen as a place to obtain the extract and the Laboratory of the Research Institute for Spices and Medicinal Plants (Balitro) was where the phytochemical analysis was conducted.

Results: The results showed that there were differences in the descriptive values of secondary metabolites in the Binahong leaf extract, such as saponins, tannins, and flavonoids, wherein the group with colchicines was higher than groups without colchicines, but there was no statistical differences between the groups treated with colchicines and those not treated with colchicines (value $p > 0.05$).

Conclusion: Administration of colchicines can improve the content of secondary metabolites in Binahong leaf extract and the plant morphology without affecting the content of the plant extract.

Clinical significance: Secondary metabolites in herbal plants, such as saponins, flavonoids, and tannins are able to speed up the healing process. One of the plants that have all three of these active compounds is Binahong (*Anredera cordifolia*). Binahong leaf extract can stimulate the process of fibroblasts and collagen formation that will accelerate the process of wound healing. Colchicine as a reagent for the mutation is able to affect the number of leaves and morphological characteristics of the Binahong plant.

Keywords: Binahong leaf extract, Colchicine, Secondary metabolites, Wound healing.

How to cite this article: Hanafiah OA, Hanafiah DS, Bayu ES, Abidin T, Ilyas S, Nainggolan M, Syamsudin E. Quantity Differences of Secondary Metabolites (Saponins, Tannins, and Flavonoids) from Binahong Plant Extract (*Anredera Cordifolia* (Ten.) Steenis) treated and untreated with Colchicines that play a Role in Wound Healing. World J Dent 2017;8(4):296-299.

Source of support: The research was funded by Direktorat Riset dan Pengabdian Masyarakat Direktorat Jenderal Penguatan Riset dan Pengembangan Kementerian Riset, Teknologi, dan Pendidikan Tinggi, sesuai dengan Surat Perjanjian Penugasan Pelaksanaan Program Penelitian, nomor: 017/SP2H/LT/DRPM/2/2016, tanggal 17 February 2016.

Conflict of interest: None

[1]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Sumatera Utara, Medan, Indonesia

[2,3]Agroecotechnology Studies Program, Faculty of Agriculture University of Sumatera Utara, Medan, Indonesia

[4]Department of Conservative Dentistry, Faculty of Dentistry University of Sumatera Utara, Medan, Indonesia

[5]Department of Biology, Faculty of Mathematics and Science University of Sumatera Utara, Medan, Indonesia

[6]Department of Pharmacology, Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Sumatera Utara Medan, Indonesia

[7]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Padjajaran, Indonesia

Corresponding Author: Olivia A Hanafiah, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Sumatera Utara, Medan, Indonesia, e-mail: olivia_avriyanti@yahoo.com

INTRODUCTION

Anredera cordifolia (Ten.) steenis also known as Binahong is a family of Basellaceae, which is a medicinal plant that has been growing very well since long. In Indonesia, the Binahong plant is still uncommon but in Vietnam, this plant has a high demand and is often used as a vegetable in Taiwan. In China and Taiwan, this plant is known to have tremendous benefits and has been consumed more than a thousand years ago. Almost all parts of the Binahong plant, such as tubers, stems, and leaves can be used in herbal therapy.[1] Binahong leaf extract can stimulate fibroblasts and collagen formation, which accelerates the process of wound healing.[2,3]

Optimal Binahong growth and productivity are determined by the type of plant and the contents of the secondary metabolites. A little genetic variation may

cause difficulty in obtaining a good quality Binahong plant. Therefore, it is necessary to increase the genetic variation of the Binahong plant. The genetic diversity can be improved through induction of mutation on other genetic characteristics, such as plant morphology, leaf, flower form, and color of the flowers.[2]

Deoxyribonucleic acid (DNA) is the primary component of genes that are the main target of mutagens, to cause mutational changes in the plant regulating genes which can be inherited. The mutation will eventually form a new genetic diversity. Colchicine is one of the reagents for the mutation that cause the occurrence of polyploids. This compound can hinder the formation of thread-like spindle in cell division so that the number of chromosomes in each cell becomes two folds or polyploidization process occurs.[4] The concentration of colchicines used as a polyploidy inducer compounds varied depending on the type of plant.[5]

The research of Bayu et al[4] found that administration of colchicines with a concentration of 0.050% significantly affects the amount of leaves and wet weight of the canopy, as well as the morphological characters of better Binahong plants.

Along with the development of modern medicine, herbal ingredients become an alternative replacement for chemical medications. Herbal medicines had been developed and used for healing of wounds for decades in the world of medicine. The active substances contained in the plants and herbs help speed up the healing process of wounds including saponins, flavonoids, tannins, and alkaloids.[1] One of the plants that contain active compounds is the Binahong plant.

The process of wound healing is a dynamic and complex process that involves several phases continuously overlapping and programmed from one phase to another phase. Wound healing phase is divided into the hemostasis phase, inflammatory phase, proliferative phase, and remodeling phase. There are many factors that can affect the wound healing process. A variety of growth factors, such as platelet-derived growth factor, fibroblast-derived growth factor, transforming growth factors (TGFs) α and β, and vascular endothelial growth factor (VEGF), which plays a role in wound healing process by inducing proliferation of various cells, such as fibroblasts, endothelial cells, and epithelial cells.[6]

The content of Binahong leaf extract on wound healing is as follows:

- *Saponins*: Saponins play a role in influencing the activity and synthesis of TGF-β1 and modifications of the receptors TGF-β1 and TGF-β2 in fibroblasts. This will stimulate the synthesis of fibronectin. This process is an important in the formation of collagen matrix during the remodeling phase. Thus, Binahong is expected to enhance the wound healing process. Saponins have also acted as agents for angiogenesis by regulating VEGF that increases the mitogenic activity of endothelial cells in the formation of blood vessel during the proliferative phase.[2]
- *Tannins and alkaloids*: Tannins and alkaloids have antioxidants and antimicrobial properties that aid the wound healing process by preventing and keeping the wound area from being damaged by free radicals and inhibit the growth of pathogenic bacteria in the wound.[7]
- *Flavonoids*: Flavonoids have the potential as antioxidant, antibacterial, antiaging, antileukemic, and vasodilator activity. The antimicrobial effect of flavonoids is by inhibiting the synthesis of DNA, proteins, and lipids of bacteria. Flavonoids inhibit the function of the cytoplasmic membrane by forming complex compounds against extracellular proteins that interfere with the integrity of bacterial cell membrane. Flavonoids also have a tendency to bind to bacterial proteins and are capable of inhibiting the enzyme activity involved in the metabolic processes of bacteria.[8,9]

The purpose of this research is to improve the quality of Binahong in plant morphology and the number of secondary metabolites that play a role in wound healing.

MATERIALS AND METHODS

The design of this study is an experimental research laboratory. This research took place at four different places in which colchicines was applied to improve the quality of Binahong conducted in the USU Agriculture Faculty's Greenhouse, without application of colchicines is done in Desa Simpang Pergendangan Kelurahan Tiga Binanga Kabupaten Karo harvesting Binahong plant, and the USU Faculty of Pharmacy as a place to make the extract as well as the Laboratory of the Research Institute for Spices and Medicinal Plants (Balitro) for phytochemical analysis.

After the Binahong plant had fully grown, the leaves were then picked as a sample. Binahong leaves were derived from the Binahong plant by colchicines (grown in USU Agricultural Faculty greenhouses) and without the administration of colchicines (planted by farmers in the Desa Simpang Pergendangan Kelurahan Tiga Binanga Kabupaten Karo). The leaves are then picked and washed under running water to remove sand and dirt, drained, and weighed and noted as its wet weight. Binahong leaves are made into extracts with the following steps:

- Preparation of powder simplicia of binahong leaf
  The initial process of making the extract is dried simplicia pulverizing stages. Simplicia powder is made with specific equipment to a certain degree of fineness. Binahong leaves are taken and weighed as much as 10 kg, then washed and dried with temperature of 40°C, and later weighed in as a dry weight of 400 gm.

Furthermore, the leaves are crushed and simplicia Binahong leaves are obtained.

- *Soaking simplicia with liquid solvent (maceration)*: Liquid solvent used in the extraction process is a good solvent (optimal) due to its nutritious or active compounds; thus, the compound can be separated from the material or other compounds, and its extract only contains most of the content of the desired compounds. A total of 400 gm of simplicia Binahong leaves was placed in a closed vessel and soaked with 80% ethanol that has been distilled for 5 days at room temperature. After 5 days, the liquid is removed and filtered with filter paper, then simplicia soaked again with 80% ethanol that has been distilled for 2 days and a final filtering process was conducted.
- *Concentration/evaporation*: Concentration means that an increasing number of partial solutes (dissolved compounds) by evaporation is used to evaporate the solvent until the extract becomes dry and thick/dense. The tools used in this process are vacuum rotary evaporator and waterbath.
- *Examination*: Testing of flavonoids and tannins was done using a spectrophotometry testing method and saponins were tested using a thin-layer chromatographic scanner method. The data obtained were analyzed statistically with data analysis, descriptive analysis, and continued with the bivariate analysis of correlation between case groups.

RESULTS

The results of phytochemical analysis test on the content of Binahong leaf extract's secondary metabolites and quantitative analysis of the differences in the content of Binahong leaf extract's secondary metabolites, such as saponins, tannins, and flavonoids are shown in Tables 1 and 2.

The purpose of this analysis is to determine any differences between the secondary metabolites of the Binahong leaf extract (saponins, tannins, and flavonoids) in the Binahong group giving colchicines compared with the Binahong group without colchicines measured three times. Table 2 shows an increase in the value of saponins, tannins, and flavonoids in Binahong added with colchicines.

Table 1: Tally sheet containing secondary metabolites of Binahong leaf extract

The active ingredient of secondary metabolites of Binahong	*Test result*
Saponins	+
Tannins	+
Triterpenoids	+
Alkaloids	+
Flavonoids	+
Phenolics	+
Steroids	+
Glycosides	+

The plot is based on the descriptive analysis of the secondary metabolites, where it shows that the group with colchicines had the effect of increased content of saponins, tannins, and flavonoids in the leaves of binahong that was compared with the group without colchicines.

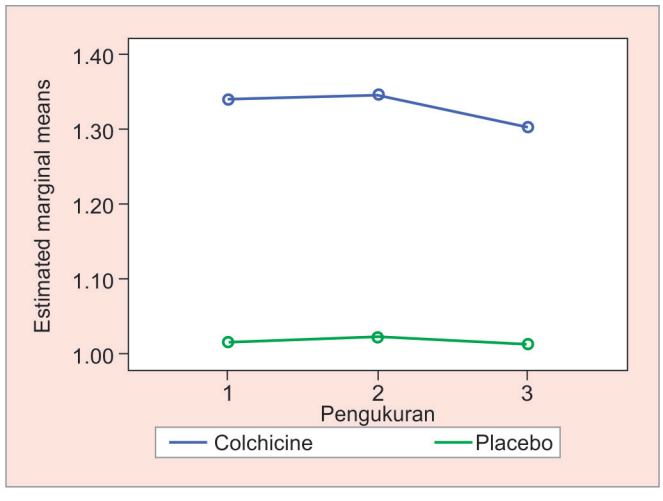
Statistical Analysis

Statistical analysis was done by bivariate correlation between the two groups.

Graph 1, Tables 3 and 4 show that the p-value was 0.295, meaning that there is no statistical difference between colchicine and the placebo groups ($p > 0.05$)

DISCUSSION

Binahong is one of the medicinal plants with the potential to be developed into raw materials for the pharmaceutical



Graph 1: Colchicines increase the secondary metabolites of binahong

Table 2: Analysis of quantitative differences in the content of secondary metabolites of Binahong leaf extract

The active ingredient of secondary metabolites of Binahong	*Colchicine group (%)*								
	Colchicines (+)				*Colchicines (−)*				
	Test 1	*Test 2*	*Test 3*	*∑ test*	*Test 1*	*Test 2*	*Test 3*	*∑ test*	*Testing methods*
Saponins	1.09	1.09	1.06	1.08	1.08	1.07	1.09	1.08	TLC scanner
Tannins	2.02	2.01	1.96	1.99	1.56	1.61	1.58	1.58	Spectrophotmetry
Flavonoids	0.91	0.94	0.89	0.91	0.41	0.39	0.37	0.39	Spectrophotmetry

TLC: Thin-layer chromatography

Table 3: Descriptive Analysis between colchicine and placebo

	Mean ± standard deviation	*n*
Colchicine	1.3267 ± 0.5872	3
Placebo	10200 ± 0.60225	3

Table 4: Correlation Analysis between colchicine and placebo

	Colchicine	*Placebo*
Colchicine		
Pearson correlation	1	0.895
Significant (two-tailed)		0.295
N	3	3
Placebo		
Pearson correlation	0.895	1
Significant (two-tailed)	0.295	
N	3	3

industry. About 20% of the Binahong raw materials are obtained from cultivation, while the rest was obtained from wild plants or found in the forest.[1-4]

From this research, it can be concluded that binahong leaf extract has chemical compounds, such as saponins, tannins, alkaloids, triterpenoid, flavonoid, phenolics, steroids, and glycosides. Thus, it has similarities to the research conducted by Kaur et al[2] and Yuliani et al.[3] Research conducted by Astuti et al[1] shows that saponin, terpenoid, and steroid compounds are only found on the stems and leaves of the plant, whereas alkaloid compounds can be found on the leaves, stems, and tubers of plants.

Saponin is known to have an influence in stimulating proliferation of fibroblasts and collagen formation, which plays a role in the process of wound healing.[2,3] According to Paju et al,[10] saponins have the ability to act as a cleanser and possesses antiseptic properties which serve to kill and prevent the growth of microorganisms that arise in the wound, so that the wound does not experience severe infections.

The study showed that the ethanol extract of Binahong leaves is able to heal wounds at a concentration of 20% on marmots.[2,3] Flavonoids of plants are capable of reducing or even eliminating inflammation through a few mechanisms, such as binding free radicals directly, through the inhibition of nitric oxide induction, decrease in leukocyte adhesion in the blood vessels, prevention of neutrophil degranulation, and interaction with other enzyme systems.[7] Flavonoids can also cause damage to the arrangement and change in the bacterial cell wall permeability mechanism.[10]

Tannin helps the healing process by preventing and protecting the wound area from damage by free radicals and inhibits the growth of pathogenic bacteria in the wound.[7]

Optimal Binahong plant growth and productivity are determined by the quality of planting material used and the amount of secondary metabolites found in the plant itself. One of the plant breeding programs that can be used to obtain cultivars or varieties of superior breeding techniques is by mutation. The use of the mutation technique in plant breeding programs is to obtain polyploids.[4]

Researchers found that the administration of colchicine during cultivation of Binahong has the effect of increasing the content of substances in the leaves of Binahong. In this study, there were no significant differences in the amount of secondary metabolite in Binahong leaf extract, namely, saponins, tannins, and flavonoids, with colchicine and without colchicine.

In conclusion, the administration of colchicines is able to improve the quality value of the Binahong plant morphology and secondary metabolites content. Besides, it can also be used as one of the ingredients for wound healing.

ACKNOWLEDGMENT

The authors acknowledge the rector of the University of Sumatera Utara.

REFERENCES

1. Astuti SM, Sakinah AMM, Andayani BMR, Risch A. Determination of saponin compound from *Anredera Cordifolia* (Ten) stennis plant (binahong) to potential treatment for several diseases. J Agric Sci 2011 Dec;3(4):224-232.
2. Kaur G, Utami NV, Usman HA. Effect of topical application of binahong (*Anredera cordifolia* (Ten.) steenis leaf paste in wound healing process in mice. Althea Med J 2014 Jun;1(1):6-11.
3. Yuliani SH, Fudholi A, Pramono S, Marchaban. The effect of formula to physical properties of wound healing gel of ethanolic extract of binahong (*Anredera cordifolia* (Ten.) steenis). Int J Pharm Sci Res 2012 Nov;3(11):4254-4259.
4. Bayu ES, Hanafiah DS, Hanafiah OA, Mahyuni R. The effect of colchicine on morphology and number of chromosome of binahong plant [*Anredera cordifolia* (Ten.) Steenis]. Proceeding of the International Symposium on Agricultural and Biosystem Engineering. Indonesia: Departemen Teknik Pertanian Fakultas Teknologi Pertanian Universitas Gadjah Mada; 2016. p. 1-5.
5. Fajrina A, Idris M, Mansyurdin, Surya NM. Penggandaan kromosom dan pertumbuhan somaklonal andalas (*Morus macroura* Miq. Var *Macroura*) yang diperlakukan dengan kolkhisin. J Bio Unand 2012 Sep;1(1):23-26.
6. Guo S, DiPietro LA. Factors affecting wound healing. J Dent Res 2010 Mar;89(3):219-229.
7. Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 2001 Oct;74(4):418-425.
8. Agyare C, Dwobeng AS, Agyepong N, Boakye YD, Mensah KB, Ayande PG, Adarkwa-Yiadom M. Antimicrobial, antioxidant, and wound healing properties of *Kigelia africana* (Lam.) Beneth. and *Strophanthus hispidus* DC. Adv Pharmacol Sci 2013 Mar;2013:692613.
9. Sharma DK. Pharmacological properties of flavonoids including flavonolignans-integration of petrocrops with drug development from plants. J Sci Ind Res 2006 Jun;65(6):477-484.
10. Paju N, Paulina VY, Kojong N. Test efektivitas salep ekstrak daun binahong (*Anredera cordifolia* (Ten.) Steenis) pada kelinci (*Oryctolagus cuniculus*) yang terinfeksi bakteri *Staphylococcus aureus.* Pharmacon J Ilmiah Farm 2013 Feb;2(1);8-9.