

**OPTIMIZATION PROCESS OF BINAHONG EXTRACTION AND
CHARACTERIZATION STUDY**

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ABSTRACT

Binahong is *Anredera cordifolia* (Ten) Steenis is including ones a medicinal plant and being used as herbal medicine. These compounds include saponin and polyphenols. They involve a lot of activities, one of these is the antimicrobial activity that will be used to treat infectious diseases by microorganism agents, resistance by microorganisms produce. However, the compound of Binahong plant is not well documented and scientific evidence is limited to establish as medicinal plant. The research proposed to know optimization process of Binahong extraction and characterization study. The samples are leaves, tuber, stems, flower and local capsule of Binahong carried out optimization processing of Total Phenol Content (TPC) by using UV Vis-Spectrophotometer and tetracycline activity (TCA) by bioassay. The optimization extraction of processes was evaluated with the aid of Design Expert version 6.0.8 using response surface methodology (RSM). The characterization of TPC and TFC by using UV Vis-Spectrophotometer, the antibiotic activity analysed by bioassay, the bioactive compounds from a volatile oil compound analysed by using GCMS, and accumulation of heavy metals on Binahong plant analysed by Inductively Coupled Plasma Mass Spectrometer (ICPMS). The results of TPC in leaves were optimum temperature at 65°C and concentration between 45 mg/l to 62.5 mg/l, in actual factor time 17.50 minutes. While the point optimum of tetracycline activity (TCA) from tuber, at temperature 72.50 °C for time 32.50 minute. The phenolic and flavonoid compound were higher in flower extract, then leaves and tuber. For the local capsule of Binahong was higher than leaves and tuber. The result of compounds analysed by using GC-MS were 68 kind of compounds. The heavy metals result were found Mg (38.80 ± 0.10) ppm; Cu (1.542 ± 0.01) ppm; Se (0.023 ± 0.002) ppm and Zn (7.310 ± 0.02) ppm, but in tuber was Mg (10.10 ± 0.02) ppm, Cu (0.652 ± 0.01) ppm, Se ($<1.636 \pm 0.003$) ppm and Zn: 2.729 ± 0.01 ppm. However, from contamination of heavy metal such as in the leaves, As (0.008 ± 0.01) ppm, Cd (0.003 ± 0.02) ppm, Pb (0.0629 ± 0.03) ppm and Cr: (0.098 ± 0.01) ppm. While from a tuber was: As ($< 0.017 \pm 0.01$) ppm, Cd ($< 0.015 \pm 0.03$) ppm, Pb (0.0091 ± 0.02) ppm and Cr 0.036 ± 0.03 ppm. The Binahong plant as medicinal herbal has the high antioxidant activity such as total phenolic, flavonoid and saponin compound. The plant is also contained group of fatty acid, phytosterol, tocopherol, polyphenols, phytol and antibiotic activity. The Binahong plant was safe for humans consumption and has a lot of benefit for human health.

ABSTRAK

Binahong adalah *Anredera cordifolia* (Ten) Steenis adalah termasuk orang-orang yang tumbuhan ubatan dan digunakan sebagai ubat herba. Sebatian-sebatian ini termasuk saponin dan polifenol. Mereka melibatkan banyak aktiviti, salah satunya ialah aktiviti antimikrobial yang akan digunakan untuk merawat penyakit berjangkit oleh ejen-ejen mikroorganisma, rintangan oleh mikroorganisma menghasilkan. Walau bagaimanapun, sebatian tumbuhan Binahong tidak didokumenkan dengan baik dan bukti saintifik adalah terhad untuk menubuhkan sebagai tumbuhan ubatan. Penyelidikan yang dicadangkan untuk mengetahui proses pengoptimuman Binahong pengekstrakan dan kajian pencirian. Sampel daun, ubi, batang, bunga dan kapsul tempatan Binahong dijalankan pemprosesan pengoptimuman Kandungan Total Fenol (TPC) dengan menggunakan aktiviti Dicuiri UV-Spectrophotometer dan tetracycline (TCA) oleh bioassay. Pengekstrakan pengoptimuman proses dinilai dengan bantuan Pakar Rekabentuk versi 6.0.8 menggunakan kaedah respons permukaan (RSM). Pencirian TPC dan TFC dengan menggunakan UV Dicuiri-Spectrophotometer, aktiviti antibiotik yang dianalisis oleh bioassay, sebatian bioaktif daripada sebatian minyak yang tidak menentu yang dianalisis dengan menggunakan GCMS, dan pengumpulan logam berat ke atas loji Binahong dianalisis oleh induktif Digandingkan Plasma spektrometer massa (ICPMS). Keputusan TPC di daun suhu optimum di 65OC dan penumpuan antara 45 mg / l kepada 62.5 mg / l, dalam masa faktor sebenar 17,50 minit. Walaupun titik optimum aktiviti tetracycline (TCA) dari ubi, pada suhu 72,50 OC untuk minit masa 32,50. Sebatian fenolik dan flavonoid adalah lebih tinggi dalam ekstrak bunga, daun dan ubi. Bagi kapsul tempatan Binahong adalah lebih tinggi daripada daun dan ubi. Hasil sebatian yang dianalisis dengan menggunakan GC-MS 68 jenis sebatian. Hasil logam berat didapati Mg ($38,80 \pm 0.10$) ppm; Cu ($1,542 \pm 0.01$) ppm; Se ($0,023 \pm 0,002$) ppm dan Zn ($7,310 \pm 0.02$) ppm, tetapi ubi Mg ($10,10 \pm 0,02$) ppm, Cu ($0,652 \pm 0.01$) ppm, Se: ($<1,636 \pm 0,003$) ppm dan Zn: ($2,729 \pm 0.01$) ppm. Walau bagaimanapun, daripada pencemaran logam berat seperti di daun, Sebagai: ($0,008 \pm 0.01$) ppm, Cd: ($0,003 \pm 0.02$) ppm, Pb: ($0,0629 \pm 0.03$) ppm dan Cr: ($0,098 \pm 0.01$) ppm. Manakala dari ubi: ($<0,017 \pm 0.01$) ppm, Cd: ($<0,015 \pm 0.03$) ppm, Pb: ($0,0091 \pm 0.02$) ppm dan Cr: ($0,036 \pm 0,03$) ppm. Binahong tumbuhan sebagai ubat-ubatan herba mempunyai aktiviti antioksidan yang tinggi seperti jumlah fenolik, flavonoid dan kompaun saponin. Tumbuhan juga mengandungi kumpulan asid lemak, phytosterol, tokoferol, polyphenols, aktiviti phytol dan antibiotik. Kilang Binahong adalah selamat untuk kegunaan manusia dan telah banyak memberi manfaat kepada kesihatan manusia

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LIST OF ABBREVIATIONS

ATP.	Adeno three phosphates
AG's	Amino glycoside group
α	Alpha
AOAC	Association Office Analytical Chemistry
ATCC	American Trade Chemical
CCD	Central Composite Design
CCRD	Central Composite Rotatable Design
DPPH	2,2-diphenyl-1-picril hydrazil
FAO	Food Agriculture Organization
FRIM	Food Research Investigation Malaysia
GAE	Gallic acid Equivalent
GC-MS	Gas Chromatography Mass Spectrophotometer
ICPMS	Inductively Coupled Plasma Mass Spectroscopy
IFRA	International Fragrance Association
JECFA	Joint Expert Committee on Food Additive
KM	Kanamycin
LDL	Low Density Lipoprotein
ML's	Macrolides group
NCMP	National Committee on Medicinal Plants
OTC	Oxytetracycline
PCA	Potential Component Analytical
PC's	Penicillin group
ppm	part per millions
ppb	part per billions
QE	Quercetin Equivalent
RSM	Response Surface Methodology

SNI	Standar Nasional Indonesia
TC's	Tetracycline group
TCA	Tetracycline activity
TPC	Total Phenolic Compound
TFC	Total Flavonoid Compound
TS	Tylosine
UV	Ultra violet
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Anredera cordifolia (Ten) Steenis or Binahong from the family of Basellaceae, is one among from medicinal plants growing well for a long time in Indonesia, especially in Java island. Binahong is a common name in Indonesia, yet this plant is not popular, before. The plant has been known to have extraordinary properties healing and for thousands of years it has been consumed by people from China, Korea, and Taiwan. Due to its properties herb medicine industry, this plant is great importance for the health of the individuals and communities. Its leaves, stems, flowers, rhizomes and tubers are being used for therapeutic purposes and its active principles in medicinal plants play a strategic role in photochemical. These properties are very important to their potential pharmacological effects. Binahong (*Anredera cordifolia* (Ten) Steenis) importance lies in their components in photochemical such as alkaloids, phenolic, polyphenols, flavonoids, saponin, triterpenoid and steroid compounds which are produced from all parts. Their definite physiological action on the human approach in neutraceutical and drug research lends promotes the great importance to health. Herbal medicines using Binahong are widely being used in conventional as well as alternative. Medical practices in many countries, including both developed and developing countries have yet to develop it up to a level to be served as a substitute. However, incorporation of the traditional medicine into the health industry without systematic investigation and,

standardization may cause problems, such as inaccurate dosage, lack of proof of safety, efficacy and interaction risk if used with modern drugs. Standardization and formulation of traditional herbs into modern phytopharmaceuticals shall provide the solution to most of these problems with traditional medicine. Binahong is commonly known in traditional medicine, of which the leaves and tubers are derived from China. When any family members are ill or in the recovery period after picking surgery, it is recommended to just grab, boiled a few leaves and then drink the boiled water. For those who are lazy or busy, and have not time to boil the leaves, there are other options, they can take the pills or capsule claim to contain Binahong leaf extract. Why *Anredera cordifolia* (Ten) Steenis leaves and tuber are also named Binahong, a name which originate from the language of Canton. Binahong is very good in healing wounds and also inflammation. Relating to a true story was a Vietnam War, when a Vietcong soldier was severely wounded, and had to eat the leaves Binahong. The soldier wounds suddenly began to become fresh and healthy. Since then, Binahong become a compulsory food in Vietnam. The leaves and tuber of Binahong contain antioxidant and antimicrobial/antibiotic functions. The gel from the leaves and tuber could help in the recovery of the wound. The objective of this study is an optimization process of Binahong extraction and characterization study from the bioactive compounds, by way of extraction of the leaves and tubers of the medicinal plant, that is Binahong (*Anredera cordifolia* (Ten) Steenis), to important parameters of the leave of concentration for the optimum level.

Many researchers have studied about the bioactivity in Binahong (*Anredera cordifolia* (Ten) Steenis) as antimicrobial activity, but there is no study about the groups of antibiotic activity functions from bioactive compound of this plant. The study here to find out the function of antibiotic activity. Function such in penicillin activity (PCA) and tetracycline activity (TCA), of which are in broad spectrum, have the power, almost the same as broad spectrum activity from some microorganism. The

first study about the groups of antibiotic functions in Binahong plant has not been researched yet. The second study here is about the accumulation of heavy metal in Binahong plant. The third in the study here, many researchers have been studying about some bioactive compounds in Binahong plant, but they have not studied about the optimization of the bioactive compound such as total phenolic content (TPC). The last part of the study to identify bioactive compounds from the Binahong plant, of which many compounds could be identified and used as a source of bioactive for traditional medicine, health food and which would be beneficial for human beings. Binahong plant can treat many diseases. The topic research has not been published yet and it is not well documented.

1.2 PROBLEM STATEMENT

The research of Binahong plant (*Anredera cordifolia* (Ten) Steenis), has not been published about this advantage of the optimization process of Binahong extraction and characterization study. While of bioactive compound extract in Binahong plant is important, because the society from Javanese, Indonesia trusted, Binahong plant is miracle of plant can be treat of several diseases and also make the body health. The several diseases like diabetes mellitus, hypertension, hemorrhoids, tuberculosis, rheumatism, excess uric acid, asthma, infection kidney, postpartum recovery, healing wound and post-circumcision operation, also for the recovery of wound from post cesarean surgery, colitis, diarrhea, gastritis and also cancer, but there are only on testimonies, (Manoi, 2009; Rosmalawati, 2010 and Sukandar, 2010). Besides that, the problem is the imported Binahong capsules available in Indonesia market, are very expensive, but the local capsules of Binahong have been produced and certified by Majelis Ulama Indonesia (MUI) for halal, but without completely researched and certified by Health Department.

The research on the identification of Binahong leaves and tubers extract of in term of its bioactive compound analysis of the phytochemical constituent from this plant, have not been ever researched in Malaysia. The rationale for this novel application has been researched from the optimization process of total phenol content, antibiotic activities, the characterize study of bioactive compounds, accumulation of heavy metal in Binahong plant.

The research and publication about the level of heavy metal toxicity in Binahong leaves and tubers extract, to elevate the quality of the medicinal plant used as herbal medicine is very important. The quality of traditional medicine from Binahong is an important role for the safety and health of its consumers. Often the analysis involved using the Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), which has clear advantages in its multi elements characteristic, and speed of analysis. While the analysis of heavy metal in Binahong has not been researched and published yet. Identification of heavy metal accumulation in leaves and tuber are important in the role of safety for human consumption according to the limited standard set by the World Health Organization (WHO) and the Malaysian National Pharmaceutical Control Bureau.

1.3 OBJECTIVE OF THE RESEARCH

This study was undertaken in the optimization process of Binahong (*Anredera cordifolia* (Ten) Steenis) extraction and characterization study from the bioactive compound. It embarks on the following objectives.

- 1 The phytochemical constituent from Binahong leaf and tuber extraction to optimize processing

2. The characterization study bioactive compound of medicinal plant from Binahong plant extract.
3. The characterization study of analysis total phenol content, total flavonoid content, screening of saponin groups and activity of antibiotic from Binahong extraction.
4. The characterization study used as a polar solvent for volatile compound in Binahong extraction to find out many kind of bioactive compounds.
5. The characterization study of heavy metal accumulation analysis in medicinal plant of Binahong extraction.

1.4 RESEARCH SCOPE

This study focus on the content of the optimization process of Binahong (*Anredera cordifolia* (Ten) Steenis) leaves and tubers extraction, included total phenolic content, total flavonoid content, antibiotic activity analysis, identification of bioactive compound. The research scopes are as follows:

1. to prove the phytochemical constituent process optimization and characterization in Binahong, especially to carry out screening of bioactive compound to indicate polyphenols, flavonoid, saponin, triterpenoid/ steroid, and alkaloid,
2. to prove by analysis all bioactive compounds from metabolite in phytochemical, from Binahong plant using GC-MS,
3. to find out the level of total phenolic content in Binahong leaves and tubers in ethanol extraction of prepared under different concentrations, temperatures and

time. This study is to find out of the optimum point of temperature, time and concentration of TPC in leaves and tubers. Beside that in term of the TPC and TFC characterization studies,

4. to determinate the antibiotic activity in Binahong plant with deferential times being carried out during screening on the levels of these activities and effects. The microbial spore culture being used were *Bacillus subtilis*, *Mycobacterium lotus*, *Bacillus cereus* and *Bacillus stereothermophilus* and the standard representation of antibiotic function being used are Na-PC (Penicillin); KM-SO₄ (Kanamycin); OTC-HCl (Tetracycline) and TS-tartrat (Tylosine), also to characterize and to optimize of tuber extraction, to find out optimum point for the effect of activities. The analysis was done by using Bioassay,
5. to identify the toxicity level of heavy metals in Binahong leaves and tubers extraction by analysis using the ICP-MS and,
6. to analyse the data, by performing the multiple linear regression analyses using the Design-Expert (Version 6.0.8), statistical SPSS (Version 15.0) and from statistical version 13.4.03 of XLSTAT 2011.

1.5 SIGNIFICANT OF THE RESEARCH

The optimization process of Binahong extract (*Anredera cordifolia*(Ten) Steenis) and characteristic study are very important in order to verify this plant application as herbal medicine to treat diabetes mellitus, hypertension, hemorrhoids, tuberculosis, rheumatism, uric acid, asthma, infection kidney, healing wound and for the recovery of wound from the post Caesarean surgery, colitis, diarrhea, gastritis and cancer. Thus, in

this research, extraction of bioactive compound would be done using polar and non polar solvent from Binahong leaves and tubers.

The study of the accumulation of heavy metals and toxic levels in Binahong plant especially from the leaves and tuber extract is very important for the quality of the medicinal plant to be used as herbal medicine and human consumption, in term of safety and health, under limited permission of standard by the World Health Organization (WHO). The metals element analysis of this plant, are being done using the Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), because it has clear advantages in its multi elements characteristic and speed of analysis. Due to the study about all bioactive compounds from metabolite in the phytochemical of Binahong, this compound also important for the role of the human health, as it could help in the recovery of many diseases. Thus in this research, the determined of phytochemical as secondary metabolites by first screening and then to identifying of this compound using GC-MS. It has the advantages of specific detections of much kind of compounds in this plant with the polar solvent extractions. The polyphenols and TPC are being included in the TFC of this plant, they also have important roles as an antioxidant and antimicrobial activities, in collaboration with secondary metabolite compound would have pharmaceutical effects on human diseases. The optimization of the TPC will be done in different concentrations, time and temperatures obtain the optimum level as the analysis would be performed by spectrophotometer. The active compound in antibiotic, with the function like the gram positive and negative described as tetracycline and penicillin activity from this bioactive compound of Binahong (*Anredera cordifolia* (Ten) Steenis), is more effective from the tuber with ethanol solvent in process optimization also will be done.

From all parts of Binahong plant will be used an herbal supplement, herbal medicine, and herbal ointment, because Binahong plant is the source of higher

antioxidant, the extract of leaves and tuber well susceptible of microorganism inhibition of antibiotic function, and also used of prepared hormone from phytosterol groups. Saponin terpenoid as a natural source of herbal supplement, while a flavonoid compound is being employed in medicine from Binahong plant for traditional medicine and to treat many diseases. However from all parts of Binahong plant being developed to produce the herbal ointment and a capsule / tablet of herbal supplement for the pharmaceutical industry. The society can use of the benefit of Binahong for the health, alternative treatment and herbal supplement and also safety to consumed with low price.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The traditional medicine from the source of natural product from one of medicinal plants such as Binahong (*Anredera cordifolia* (Ten) Steenis) is important in the future of medicine and a supplement by way of caplets and capsules to be used for human health and to treat any diseases (Manoi, 2009). Phytochemical constituents in this plant are great of importance to the health in developing countries. These compounds include saponin, triterpenoid, steroid, phenolic and flavonoid compounds (Edeoga *et al.*, 2005). The compound involves a lot of activities; one of these is the antibiotic activity that will be used to treat infectious diseases by microorganism agents, resistance by microorganisms produce. Infection is the prime cause of diseases in the world especially in tropical areas. Treatment of infectious diseases using antibiotics causes many bacterial resistances, which would demonstrate that Binahong compound has antibacterial activity of the extract of its leaves and tubers (Setiaji, 2009). The leaves and tubers compound, have a potential to make clean and could be first in the collagen form, there is the protein content to aid recovery in post operation, apply them directly to the dry wound after surgery, to reduce the burning pain during healing (Puryanto, 2009). The other benefit from this compound in Binahong is from saponin, phenolic and flavonoid compounds used as antioxidant activity, anti inflammation and as analgesic. Saponin could be used to lowering cholesterol level because it has the