

THE TRITERPENOID SAPONIN FROM BINAHONG [*Anredera cordifolia* (Ten) Steenis] TO POTENTIAL USING AS ANTIDIABETIC ACTIVITY IN ANIMAL LABORATORY

Sri Murni Astuti^{1,2}, A. M. Mimi Sakinah¹, Awalludin Risch^{1,2}

Faculty of Chemical and Natural Resources Engineering (Bio-Process), Universiti Malaysia Pahang, 26300 Gambang, Kuantan, Pahang, Malaysia

Abstract

Binahong plant or Anredera cordifolia (Ten) Steenis is one of the medicinal plants having mimetic of insulin. This plant growing well in Indonesia in the high and low lands. The Binahong from Chinese's land and native from South America. Tubers of Binahong purchased from farm in Jakarta, Indonesia. One part of tubers cultivated in polybag plastic, in Gambang, Pahang, Malaysia. After six months, harvested and used in the research. Binahong rich in the bioactive compound as saponin, triterpenoid, steroid, alkaloid, oleanolic acid, poly phenol, flavonoid and tocopherol. The triterpenoid saponin extraction used ultrasonic assisted. While the total saponin can be determined by analytical method and UV Spectrophotometer. The triterpenoid saponin analysis using by colorimeter method. And for the isolation by TLC and HPLC. Due to for toxicity analysis of heavy metal by ICP-MS. The Triterpenoid saponin which exhibits widely hypoglycemic/antidiabetic and that oleanolic acid is significantly enhanced insulin secretion at basal and stimulatory glucose concentration in blood control, with target organ is the pancreatic β -cell. The last for experiment by animal laboratory used for clinical test symptom. The result of heavy metal in Binahong is under limited permissible of standard. The Binahong plant, including one of the antidiabetic medicinal plants having insulin mimetic. Leaves and tuber extraction to potential used as antidiabetic source from plant. The triterpenoid saponin as bioactive compound can treat diabetes mellitus by antidiabetic drug with safety to human consumed.

Keyword : *Binahong plant, extraction, triterpenoid saponin, antidiabetic, clinical test*

²National Veterinary Drug Assay and Laboratory, Bogor. Jln. Raya Pembangunan, 16340 Gunung Sindur, Bogor, West Java, Indonesia
E-mail: srilmurni.astuti@yahoo.com

INTRODUCTION

Binahong is *Anredera cordifolia* (Ten) Steen's plant is one of the medicinal plants, which easily grow on the high and low lands. This plant is growing as a creeper and reaches five feet in length. It is characterized by soft trunk, cylinder shaped, single, and short-stemmed, leaves with shape like a heart, grows very well in Paraguay and southern Brazil (Starr *et al*, 2003). Indonesian peoples known *Anredera cordifolia* is Binahong, this plant growing well and fast a long time ago in Indonesia, and the peoples in java Indonesia, cultivated in the way to the garden, makes the triumphal arch. (Manoi, 2009). It has been several of biological activities that can be used treatment of many diseases such as diabetes mellitus, stroke, and infection of kidney (Sukandar *et al*, 2010 and Rosmalawati. *et al* 2010). In Taiwan Binahong or *Anredera, cordifolia* used as vegetables (Mao-Te *et al*, 2007). And also this plant known to have extraordinary healing, it has consumed over thousand years by the nation of China, Korea and Taiwan (Manoi, 2009).

Diabetes mellitus is one of the common endocrine metabolic disorders acquiring around 2.8% in the world's population and is anticipated to cross 5.4% by the year 2025. The diabetic has caused significant morbidity and mortality due to micro vascular as retinopathy, neuropathy and nephropathy and macro vascular heart attack, stroke and peripheral vascular diseases, make to complication (Patel *et al*, 2011). Also the diabetes is a chronic disorder in metabolism of carbohydrates, protein and fat due to absolute of relative deficiency of insulin resistance (Edwin *et al*, 2008). Insulin is a hormone produced by the pancreas to control blood sugar. Diabetes can be caused by too little insulin, and resistance to insulin, or both. According to the World Health Organization (WHO), diabetes causes nearly 5% of deaths worldwide, and expected to rise by 50% in the next 10 years (WHO, 2010).

The WHO has estimated that Indonesia ranks fourth in terms of diabetes sufferers throughout the world. In 2000, that are 8.4 million Indonesians suffered from diabetes, the

figure is expected to increase to 21.3 million in 2030, said Professor Sidartawan Soegondo, metabolic endocrine and diabetes consultant at University of Indonesia's medical faculty. Indonesia. Otherwise with a population of more than 230 million, had the fourth biggest number of diabetes sufferers after China, India and the United States (Xinhua, 2007). While in Malaysia's survey by Universiti Kebangsaan Malaysia, Medical Centre (UKMMC) according to Prof Nor Azmi Kamarudin, the head of diabetes & endocrinology, there are 1.8 million of Malaysians were diagnosed with diabetes in 2010, a significant increase from 1.4 million in 2006. Diabetes was responsible for more than 23,800 Malaysian people's deaths in 2010. The World Health Organization (WHO) has estimated that in the year 2030. Malaysia would have a total of 2.48 million people with diabetes cases. (New Strait Times, 2010)

The saponins are glycosilated compound, which are widely distributed throughout the plant kingdom and be divided into three major groups a triterpenoid, a steroid or a steroidal glycoalkaloid (Figen, 2005). This including in a class of chemical compound, one of many secondary metabolites found in natural sources, with saponin found, in particular, abundance in various plant species (Hosttetman and Marston, 1995). Saponins are glycosides of triterpens, steroids, and sometimes alkaloids, which occur primarily, but not exclusively in plants (Kareru *et al*, 2008). The saponins can therefore be classified as steroidal, triterpenoidal or alkaloid depending on the nature of the aglycone. The aglycone part of a saponin referred to as a sapogenin, while the glycone parts of the saponins are generally oligosaccharides (Natori *et al.*, 1981).

Triterpenoid saponin has found from the leaves and tuber of this Binahong plant (Manoi, 2009). According to the research of Astuti *et al* (2011), from all part of Binahong plant has a compound the saponin, triterpenoid/steroid and saponin crude substances from leaves (28.14 ± 0.22 %), and tubers (43.15 ± 0.10 % from mg/g (dry weight material). In fact, saponins in the

rhizome of plant species are used for diabetes in folk medicine (Jeong and Jim Wong, 2005). The saponin has a function of stimulation insulin secretion in pancreatic β -cells in a concentration dependent manner (Amy *et al*, 2011). Pancreatic α amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to maltose and finally glucose (Sudha *et al*, 2011).

More than 400 plant species having hypoglycemic activity have been available in literature, searching for new antidiabetic drugs from natural plants is still interesting, because contain substances which safe effect on diabetes mellitus. The classification on natural product review is terpenoid, alkaloids, flavonoids, phenolic and some other categories have shown antidiabetic potential through the insulinomimetic activity of the plant extract. The plant derived of active principles representing the different type of biological activity, among this alkaloid, glycosides, polysaccharides, peptidoglycans, guanidine, steroid, carbohydrates, glycopeptides, terpenoid, amino acids and inorganic ions have demonstrated activity, including treatment of diabetes (Grover *et al*, 2002). According to Astuti *et al* (2011), Binahong plant has a bioactive compound from secondary metabolic such as saponin, triterpenoid, steroid, flavonoid, phenolic and alkaloid from extraction of this plant.

Binahong plant used to treat diabetes mellitus and safety to be consumed.

Binahong plant or *Anredera cordifolia* (Ten) Steenis is one of the antidiabetic medicinal plant having insulin mimetic. Plants have always been a very good source of drugs, and many of the currently available drugs have been derived directly or indirectly from them (Patel *et al*, 2012). The Etnobotanical information suggested that about 800, plants may possess antidiabetic potential, among all of them have been reported to be beneficial for treatment type 2 of diabetes (Ponnusamy *et al*, 2011 and Jung *et al*, 2006). While about 1200, plants are used for traditional

medicine for their alleged hypoglycemic activity (Kesari *et al*, 2007). Hypoglycemic or antidiabetic activity, but hyperglycemic or diabetes mellitus is caused by the inherited or acquired deficiency in production of insulin. The classifications on natural product review are terpenoid, alkaloids, flavonoids, phenolic and some other categories have shown antidiabetic potential through the insulinomimetic activity of the plant extract. Isolation from this plant material has shown significant insulin mimetic activity along with antidiabetic potential. While the flavonoid and polyphenols as well as sugar derivatives and to be effective due to some other extra pancreatic mechanism (Patel *et al*, 2012). Binahong plant has a bioactive compound from secondary metabolic such as saponin triterpenoid, steroid, flavonoid, phenolic and alkaloid from the plant extraction. Binahong plant has been reported to be beneficial for treatment of diabetes mellitus (Manoi, 2009; Rosmalawati, 2010 and Sukandar, 2010). The phytoconstituents with antidiabetic activity that comes under the category of polysaccharides, peptides, alkaloids, glycopeptides, triterpenoid, amino acids, steroids, xanthone, flavonoids, lipids, phenolic, coumarins, iridoids, alkyl disulfides, inorganic ions and guanidine's are reported to have antidiabetic activity (Gover *et al*, 2002 and Pulkot *et al*, 2006). 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 in 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 element's characteristic, and speed of analysis. Identification of heavy-metal accumulation in leaves and tubers are important as 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. The total concentration from heavy metals accumulated in leaves of Binahong plant with concentrations of heavy metals in

plant tissues of Binahong are ppm (mg/kg). These samples collected from Binahong plant cultivated used polybag plastic. As shown in Table. I

limited permissible of standard from Europe Food Safety Authority, 2009; Codex Alimentarius Committee, 1996; Malaysian Food Regulation, 1985; Ministry of Health, Malaysia,

Table I Heavy metal toxicity (ppm) from Leaves of Binahong Cultivated in polybag plastic.

No	Sample	Elements in ppm	Averages
1	Leaves	Plumbum (Pb)	1.445 ± 0.03
2	Leaves	Cadmium (Cd)	0.003 ± 0.02
3	Leaves	Chromium (Cr)	0.198 ± 0.01
4	Leaves	Copper (Cu)	1.542 ± 0.01
5	Leaves	Arsenic (As)	0.008 ± 0.01

Source: (Astuti *et al*, 2011.b)

The last accumulated of heavy metals in tubers of Binahong plant concentration's elements in plant tissues of Binahong are ppm (mg/kg), as shown in Table II.

2010; Indonesian standard food regulation(SNI) for food regulation 7387, 2009, from W.H.O and USFDA, 1990. For the heavy-metal guidelines

Table II Heavy metal toxicity (ppm) from tubers of Binahong cultivated in polybag plastic

No	Sample	Elements in ppm	Averages
1	Tuber	Plumbum (Pb)	0.0091± 0.02
2	Tuber	Cadmium (Cd)	< 0, 015± 0.03
3	Tuber	Chromium (Cr)	0.0036± 0.03
4	Tuber	Cooper (Cu)	0.0652± 0.01
5	Tuber	Arsenic (As)	< 0.017± 0.01

Source: (Astuti *et al*, 2011.b)

The heavy metal from leaves and tuber of Binahong source from micro element essential and non essential, all the data described under

used food and pharmaceutical in ppb and ppm, showed above the table III..

Table III Heavy metal guidelines used food and pharmaceutical in ppm/ppb

No	Pb	Cd	Cr	Cu	As	Source
1	5.0	0.3	0.01	-	2.0	Pb;Cd,As,: Kelvin et al, 2009;Cr:Kumar et al, 2009
2	2.0	1.0	-	200	<0.02	Pb,Cd : FAO, 2002;Cu: Food Standards Committee, 1950; As: Ministry of Agriculture, 1982
3	2.0	3.0	-	-	1.0	Pb, Cd, As (ppb): SNI-7387,2009
4	10.0	0.3	-	-	5.0	Pb,Cd,As (ppb) :Ministry of health Malaysia,2010

1. Saponin compound

Saponin compound in Binahong plant is dominating. Saponin comprises a large family of structurally diverse compound containing a steroidal or triterpenoid aglycone linked to one more or more oligosaccharide moieties (Ceyhun and Artik, 2010). The aglycone non Saccharide portion of the saponin molecule is called the genin or sapogenin. Depending on the types at genin present, the saponin can be divided into three major classes (Hosttman and Marston, 1995). Saponins are generally known as non volatile, a surface-active compound that is widely distributed in nature, occurring primarily in the plant kingdom (Olezek, 2002; Hosttman and Marston, 1995). The isolated of saponins have shown variety of activities such as antitumor, cholesterol lowering, immune potentiating, anticancer, antioxidants (Blumert and Liu, 2003) and to presser lower risk of implicated in coronary heart diseases (Achinrwhu, 1983),

percentage crude yield of saponins of the Binahong plant, showed that leaves and tubers large amount of saponins crude from Binahong plant, from mg/g material dried sample. The crude can be seen such as a potential source of useful drug shown in table IV..

Table. IV. Determination of saponins investigated in Crude of Binahong plant (Analytical method)

No	Sample	Saponin (%)	Remark
1	Leaves	28.14 ± 0.22	mg/g in dry weight
2	Stems	3.65 ± 0.11	mg/g in dry weight
3	Tuber	43.15 ± 0.10	mg/g in dry weight

Saponin crude from 20 mg dried sample Source (Astuti *et al*, 2011)

$$\text{SAPONIN} = \text{SUGAR (glycone)} + \text{SAPOGENIN (aglycone)}$$

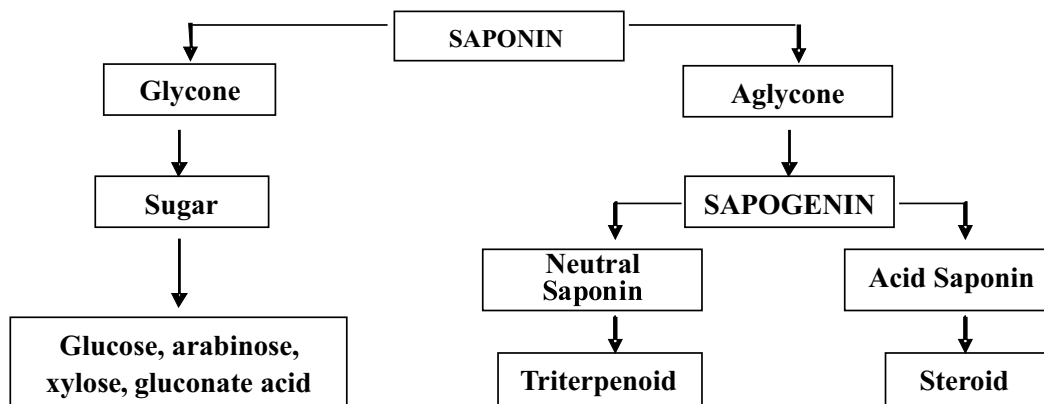


Figure 1 Scheme of group saponin glycosides (Duke, 1992; Kato, 1995)

Determination of saponin crude from leaves and tubers of Binahong plant, described that saponin in tubers is the high compound then leaves and stem. The saponin crude used analytical method source from Edeoga *et al* (2005) and result by Astuti *et al*, (2011). The saponin compound from Binahong plant, in the research of quantitative estimation of the

2. Triterpenoid saponin

The saponin compound in Binahong is saponin triterpenoid from the leaves and tuber of this plant (Manoi, 2009). Triterpenoid is a component of plants that have a smell and can be isolated from vegetable and essential oil and the structure of terpenoid compound from variety of secondary reactions, such as hydrogen,

oxidation, and reduction. While steroids are the sterols, bile acids, sex hormones, hormone adenocorticoid, aglycone, cardiac, this group determined R1, R2 and R3 (side chains) to the basic framework of carbon (Lenny, 2006.). Triterpenoid has also been reported to possess antifungal properties (Olezek, 1996; Nita, 2009; Yanuar, 2009). The classification of terpenoid determinate of unit chains of compound to general biosyntheses arrangement from terpenoid, the group of terpenoid mechanism one of a triterpenoid, the groups are 4000 content and haven isolation about 40 compounds (Lenny, 2006). The terpenoid referred to as isoprenoid are class of natural product and related compound formally derived from the C-5 isoprene unit. Terpenoid is deferent sizes, and composition is found among all classes of living thing and is the large group of naturally (De Las Herras *et al*, 2003). In recent years, naturally-occurring compounds including saponins have been reported to inhibit the effect of diverse environmental mutagens and carcinogens (Konoshima, 1996). The triterpenoid saponins are triterpens which belong to the group of saponin compounds, which exhibit widely hypoglycemic/antidiabetic, antimicrobial, anti-inflammatory, antibiotic, hemolytic analgesic, anthelmintic and cytotoxic activity (Bandaranayake, 2002). Triterpenoid saponin has characterized as oleanane acid aglycone with an oligosaccharide moiety at C-3 with or without sugar moiety at C-28 are the predominant chemical constituent from the Chinese plant. In search for new antidiabetic constituent from Traditional Chinese Medicine (TCM), that found the total saponin from this species of plant from Chinese's land. (Linlin *et al*, 2012). The two triterpens of saponin are ursolic acid and oleanolic acid as major constituent, the other study of triterpenoid saponin reported that oleanolic acid is significantly enhanced insulin secretion at basal and stimulatory glucose concentration. Chronic treatment with oleanolic acid of triterpenoid saponin to increase of total cellular insulin protein and mRNA (Teodoro *et al*, 2008). Although from the Binahong, plant can find oleanolic acid, there is the group of

triterpenoid saponin of the antioxidant activity. Terpenoid saponin is Eugene's oil from leaves and tuber of Binahong plant (Liu, 1995 and Rachmawati, 2008). The triterpenoid saponin into ethanol solvent from the dried sample by ultrasonic extraction was found to be the most efficient method for the extraction of triterpenoid saponin from the leave's sample. The ultrasonic method employed provides high extraction efficiency in short time and less solvent consumption. Otherwise, ultrasonic is an alternative extraction technique for fast extraction of triterpenoid saponin from leaves on the plant (Firdaus *et al*, 2010)

3. Steroid saponin

Saponin steroid as sapogenin compound is also found in genus of Anredera, which is in Binahong plant. This is very useful in pharmaceutical industries as a natural source of steroidal hormones. Dysgenic is the steroid level in plant found in few higher plant species and has gained increasing interest in its medicinal properties recently (Liu *et al.*, 2005; Bishnu and Wiesman, 2005). A diasogenin can also be absorbed through the gut, and it plays an important role as the control of cholesterol metabolism (Roman *et al*, 1995). It has also been reported that it possesses estrogenic effects (Aradhana *et al.*, 1992). The isolation and identification of two steroid glycosides and spirostanol with structure compound from atomic C₂₅ and C₂₇, also from sapogenin steroid spirostan and three glycone. (Bogoriani, 2008). Other ways from the research of Binahong plant derivate of steroid as sitosterol, stigma sterol, and chondrilasterol and dihydro spina sterol to determination by Gas Chromatography Mass spectrophotometer (GC-MS).

4. Glycone (Glycosides in saponin)

Glycosides in saponin which contains one sugar chain triterpens glycosides that is a bioactive compound constituent of therapeutic interest. In plants is it pentacyclic triterpenoid group being known as aciaticoside (De Lucia *et al.*, 1997). The saponins are naturally occurring

surface-active glycosides. Due to the saponin in Binahong plant (*Anredera cordifolia* (Ten) Steenis), that as glycosides content.

Diabetes mellitus

Most of the food we eat is broken down into simple sugar called glucose. This glucose is the main source of fuel to get energy for the body. After digestion, the glucose reaches of the blood stream which available to the body cells to utilize for energy (Edwin *et al*, 2008). Diabetes mellitus is a chronic disorder of metabolic in the endocrine system, there are carbohydrates, proteins and fat due to absolute or relative deficiency of insulin secretion with varying degree of insulin resistance (Barar *et al*, 2000). The diabetes its complications constitute a major health problem in modern societies. While the characterized by hyperglycemia in the postprandial and fasting state, and in serve form is accompanied by ketosis and protein wasting (Zanatta *et al*, 2007). The targeting postprandial hyperglycemia may be difficult with conventional diabetes therapy and in this regard, the availability of á glycosidase inhibitor is help full (Babu *et al*, 2003). The classification of diabetes mellitus in type 1 (Insulin-dependent diabetes), it is prevalent in 10% of diabetic patients, islet á cell destruction usually leads to absolute insulin deficiency (Ranjan *et al*, 2002). Type 1 of diabetes is usually diagnosed in childhood. In this disease, the body makes little or no insulin. Daily injections of insulin are needed. But in Type 2 diabetes (Non insulin-dependent diabetes), accounts for more than 80% of cases worldwide. It is a heterogeneous type, ranging from insulin resistance to insulin deficiency (Lokesh *et al*, 2006), also the 2 type of diabetes is a multifactorial disease with both a genetic component and an important non genetic component (Torben, 2002).

1. Insulin product

Insulin is a hormone secreted by the pancreas to transport glucose from blood into the body cell and control the blood sugar. If the

pancreas does not produce enough insulin or the produced insulin does not work properly, the glucose cannot enter the body cells. Then the glucose still stays in the blood cell, which makes the blood-sugar level high (Edwin *et al*, 2008). The insulin resistance mainly happens in cell membrane where glucose is not transported to the cell for oxidation (Alam *et al*, 2003).

Screening, determination and isolation of triterpenoid saponin compound

1. Preparation of sample

The leaves and tubers were washed and dried by oven with temperature 60°C and times 24 hours. And then ground by blender to be powder before extraction. The solution for extraction used ethanol for ultrasonic extraction.

2. Ultrasonicextraction assisted.

Extractions were carrying out in an ultrasonic bath; working frequency was set in 20 to 60 kHz. Four grams of sample extracted with 100 ml of ethanol 95% (v/v) in conical flask (100 volumes) and kept for 15-minute sonification time and temperature at 60°C. After the extraction, filtered and evaporated to dryness using rotary evaporator.

3. Terpenoid Test (Screening)

5 ml of water extract from plant was mixed in 2 ml of chloroform, and concentrated 3 ml of H₂SO₄, and was carefully added to form a layer. A reddish-brown coloration of the inter face was formed to show positive results from the presence of terpenoid (Edeoga *et al*, 2005)

4. Steroids Test (Screening)

0.5 grams of crude powder were dissolved in 5 ml of methanol. One ml of the extract was treated with 0.5 ml of acetic acid anhydride and cooled 3 minutes in the refrigerator. And then mixed with 0.5 ml of chloroform. After that added one ml for concentrated sulphuric acid, be carefully by means of a pipette. At the

separations level of the two liquids was formed, as the indication of the presence of steroids (Kola wok *et al*, 2006; Majaw and Moirangthem, 2009).

5. Determination of total saponin in crude (analytical method)

The samples were ground and 20 gram of each was put into conical flask and 100 ml of aqueous ethanol were added. The samples were heated over hot in-water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re extracts with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C temperature was transferred into 250 ml separatory funnels and 20 ml of diethyl ether were added shaken vigorously. The aqueous layer was recovered while the ether layer was dischard. The purification process was repeated. 60. ml of n-butanol was added. The combined n-butanol extract were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to constant weight, and the saponins' content was calculated. (Edeoga *et al*, 2005).

6. Determination of total triterpenoid saponin by colorimetric

Volume of 0.2 ml of extract was treated by using colorimetric method measured at 550 nm used spectrophotometer. The standard curve was done preparing stock solution using oleanolic acid (604g/L) standard. The solution was diluted into seven different volumes. This method described by Xiang *et al* (2001)

7. Qualitative separation of Saponin's compound by TLC

Two grams of powdered from leaves stem, and tubers of plant were extracted with 10 ml 70% Ethanol by refluxing for 10 minute. The filtrate is condensed enriched with saturated n-butanol and thoroughly mixed. The butanol was retained condensed and used for

chromatography. The saponins were separated using chloroform, glacial acetic acid, methanol and water (64: 34:12:8), solvent mixture. The color and values of these spots were recorded by exposing chromatogram to the iodine vapors (Wagner *et al*, 1996).

8. Analysis of saponins by HPLC (Agilent)

The sample preparation, weight of the dried sample 5 gram and extraction by soxhlet extractor using 50 ml ethyl ether for 4 hour and filter after cooling down and discard the liquid phase then dry the residues. And then put the residue in flask (250 ml volume) and add 50 ml methanol and extract for 2 hour. Repeat and mix the extract. Due to recover the methanol under vaccum and add 20 ml of water to residue dissolve. Extract using 15 ml of water saturated n-Butanol 3 times then mix the extract and evaporate under vaccum. The last dissolve the residue with up to 5 ml methanol and filter this final sample with a 0.45µm membrane filter before injecting into HPLC.

. HPLC condition. Mobile phase 35% water, 65% methanol. Flow rate 1ml/min for 4.6 mm x 150 mm, 5 µm. 0.4 ml/min for 3.0 mm x 50 mm, 1.8 µm. Inject volumes: 5 µl for 4.6 mm x 150 mm, 5 µm and 2 µl for 3.0 mm x 50 mm, 1.8 µm. Column : Zorbax Eclipse Plus C.18, 4.6 mm x 150 mm, 5 µm and 3.0 mm x 50 mm, 1.8 µm. UV 210 nm; TCC temperature 30°C ; ELSD temp 40°C. The compound was separated well on the Zorbax Eclipse Plus C.18 column (4.6 x 150mm, 5 µm) by Rongjie, (2009).

Analysis Heavy Metal for toxicity by ICP-MS

1. Reagents and Apparatus

Inductively Coupled Plasma Mass Spectroscopy (ICPMS) unit, microwave, volumetric flask, nitrate acid (HNO₃), peroxisidase (H₂O₂).

2. Analytical method

The powder samples were digested and the analyses for heavy-metal concentration

following the published methodologies. The mean, of each sample were calculated and with linear regress.

3. Principal of ICPMS method

The principle behind this method is the measurement of ions produced by a radiofrequency inductively coupled plasma. Species originating in a liquid are nebulizer and the resulting aerosol is transported by argon gas into the plasma torch. The basic instrumental components of the ICP-MS are ions produced by high temperatures are entrained into the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced at the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier pensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix (van der Wiel, 2003).

4. The Procedure test

Place a TFM vessel on the balance plate, tare it and weigh of the sample. Introduce the TFM vessel into the HTC safety shield. Add the acid; if part on the sample stays on the inner wall of TFM vessel, wet it by adding acids drop by drop, then gently swirl the solution to homogenize the sample with the acids. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench. Insert the segment into the microwave cavity and connect the temperature sensor. Run the microwave program to completion. Cool the rotor by air or water until the solution reaches room temperature. Open the vessel and transfer the solution to a marked flask. 0.5 grams sample added with 7 ml HNO₃ and 2 ml H₂O₂ digested in microwave digester. Then, mark up with 2% HNO₃ in volumetric flask and analyzed with ICPMS equipment (AOAC, 1990)

Study on hypoglycemic response in normal rats

Animal laboratory used mouse or rats. For male rat weight 150 – 250 gram but male of mouse weight 60 gram. The standard of temperature condition: 23 – 25 °C, humidity and dark light cycle (light from 6.am – 6.pm). Tap water was available ad libitum.

The hypoglycemic effect was evaluated by force oral feeding of plant extract in 24 normal rats, previously fasted for hour and randomly divided into four groups (n=6/per group) The group 1 – 4, treated with aqueous extract of saponin triterpenoid from Binahong plant with different doses. For the control received distilled water.

CONCLUSION

The Binahong plant is one of the antidiabetic medicinal plants having insulin mimetic with the leaves, and tubers extract to be potential as herbal medicine for treatment of diabetes mellitus source from plant. Triterpenoid saponin has the target organ is the pancreatic β -cell to stimulation of insulin produce for control glucose in blood. While the triterpenoid saponin also is ones of the bioactive compound from Binahong to potential used as antidiabetic drug with safety to human consumed according to the limited standard permissible. Due to the triterpenoid saponin which exhibited widely hypoglycemic/antidiabetic and also the oleanolic acid is significantly enhanced insulin secretion at basal and stimulatory glucose concentration. However, the leaves and tubers' sample extraction is important for started to the research experiment, from the medicinal plant, including the Binahong plant the most efficient method for the extraction of triterpenoid saponin from this sample using by ultrasonic extraction assisted. The last for experiment using animal laboratory (rats) to test of antidiabetic for symptom of clinical test. The further research is continued of analysis the bioactive compound from Binahong plant has a research development for new drug from this plant.

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