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The Analysis of Binahong Leaves Potential (*Anredera cordifolia*) as an Alternative Treatment of Anticataractogenesis

Feriyani Feriyani[1,2]*, Darmawi Darmawi[3,4], Ummu Balqis[5], Rodiah Rahmawaty Lubis[6]

[1]Graduate School of Mathematics and Applied Sciences, Universitas Syiah Kuala, Banda Aceh, Aceh, Indonesia; [2]Faculty of Medicine Universitas Abulyatama; [3]Laboratory of Research, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Aceh, Indonesia; [4]Laboratory of Microbiology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Aceh, Indonesia; [5]Laboratory of Pathology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Aceh, Indonesia; [6]Department of Ophthalmology, Faculty of Medicine, Universitas Sumatera Utara, Indonesia

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***Correspondence:** Feriyani, Department of Chemistry, Faculty of Mathematics and Applied Sciences, Universitas Syiah Kuala, Aceh, Indonesia.
E-mail: feriyani@albuyatama.ac.id
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Abstract

BACKGROUND: Cataract is a condition where the retina cannot capture images completely and causes blindness due to an imbalance of reactive oxygen species (ROS). These free radicals can be suppressed using antioxidants from several plants, one of which is binahong leaves.

AIM: The purpose of this study is to analyze the potential of binahong leaves as an initial screening that can play a role in inhibiting the process of cataractogenesis.

METHODS: By extracting binahong leaves with 60% ethanol, the extracts were tested for phytochemistry using the X technique and GC–MS using the Perkin-Elmer GC Clarus 500. The experimental process and all laboratory analyses were fully carried out from June to December 2019 at the Laboratory of Food Analysis and Yield Agriculture, Department of Agricultural Product Technology, Syiah Kuala University, Banda Aceh.

RESULTS: Analysis of binahong leaf potential showed that binahong leaves contain flavonoids, steroids, phenols, alcoholics, terpenoids, and saponins. While the GC–MS analysis results showed that binahong leaves are rich in phytol and contain fatty acids.

CONCLUSION: Binahong leaves have the potential to be anticataractogenesis because they contain compounds rich in antioxidants.

INTRODUCTION

A cataract is a condition of framing of the lens of the eye so that it reduces the amount of light entering the eye so that it can cause blindness [1]. The World Health Organization (WHO) data show that the number of people with severe visual impairment or blindness reaches 235 million, with 67 million of them caused by cataracts that are not surgery [2], [3] Cataracts can be caused by many factors such as oxidative stress, UV radiation, calcium levels in the lens, and complications of diabetes mellitus (diabetic cataracts) [4], [5], [6]. Effective treatment of cataracts to date is cataract removal surgery, but the surgical process can still cause complications of the disease [7]. Side effects caused by high operating costs often make people switch to traditional medicine. Prevention of cataracts with drugs and herbs is a good strategy, but there are still a few drugs that have been proven to be good for consumption. Herbal plants are known to be rich in bioactive compounds that have the potential to be natural and safe inhibitors because they have few or no side effects [8]. Some herbs have been tested to have the potential to reduce the cataract process because they contain secondary metabolites such as flavonoids, terpenes, fatty acids, phenols, and others [9], [10]. One of the herbal plants that have high antioxidant potential is the leaves of binahong (*Anredera cordifolia*). Binahong leaves in some countries are known as madeira vine, potato vine, fat leaf, Folha Gorda, or lamb's tail vine considered to be creepers that grow in coastal areas or rain forests [11]. This plant is also believed to be rich in flavonoids, where flavonoids will increase the activity of antioxidant enzymes (superoxide dismutase and catalase) which can then prevent subsequent selenite-induced cataractogenesis [12], [13]. Binahong leaves increase free radical scavenging activity very high >250 (1572.9 ± 192.0) [14]. Binahong leaves contain antioxidants reaching 40.27% or 4.25 mmol/100 g (fresh) and 3.68 mmol/100 g (dry) [15]. The binahong plant (*A. cordifolia* (Ten.) Stennis) is one of the species of the Basellaceae family which is widely used in medicine in the field of human health and also as an antimicrobial plant pathogen [16]. Binahong leaves are known to contain oleanolic acid. Binahong leaf is an alternative source of antioxidants that can be developed further

as a natural ingredient for cataract treatment. Looking at the magnitude of the potential of binahong leaves as an antioxidant, the purpose of this study is to analyze the content of secondary metabolites and compounds contained in binahong leaves as natural ingredients for cataract treatment.

METHODS

Location and time

The study was conducted from June to December 2019 at the Laboratory of Food and Agricultural Product Analysis (APHP) Program in Agricultural Product Technology, Syiah Kuala University, Indonesia.

Material preparation and binahong leaf extract

Binahong leaves collected as much as 2 kg, then wash thoroughly and drain. Followed by drying and cut into small pieces. Then, maceration with 70% ethanol for 3 days and concentrated using a vacuum rotary evaporator so that it becomes a gel.

Phytochemical test

Phytochemical test of active compound content with reagent test from the ethanol extract of binahong leaves. Furthermore, it was dissolved with solvents, and alkaloid, polyphenol, tannin, saponin, steroid, and triterpenoid tests were carried out.

Alkaloid test

Ethanol extract of 1 g of binahong leaves was added 0.5% HCl. The solution is divided into two tubes, tube 1 is added 2–3 drops of Dragendorff's reagent and tube 2 is added 2–3 drops of Mayer. If orange deposits are formed in tube 1 and white deposits in tube 2 indicate the presence of alkaloids.

Flavonoid test

The ethanol extract of binahong leaves is put into a test tube then dissolved in 1–2 mL methanol. then Mg metal and 4–5 drops of concentrated HCl are a red or orange-colored solution which is formed to show the presence of flavonoids.

Poliferase test

The ethanol extract of 200 mg binahong leaves was dissolved in 10 ml of water then heated for 10 min and filtered. The filtrate was dropped with FeCl3 for 3 drops, then a change was observed the color. Positive results of polyphenols if the formation of a black-green or dark blue-colored solution, then the material contains polyphenols.

Tannin test

The 1 g binahong leaf ethanol extract was added with 20 mL aqua dest. Then, it was cooled for 30 min, added 5 drops of 10% NaCl solution, an cooled and filtered. The filtrate was divided into two as a control and the parts were tested by adding 3 drops of FeCl3. Then, do the comparison, if there is a black-blue color indicates the presence of hydrolyzed tannins and brownish-green color indicates the presence of condensed tannins.

Saponin test

The ethanol extract of 0.5 g binahong leaves is added with 10 m hot water and cooled. After being chilled for 10 s, if the froth formed stable for 10 min as high as 1–10 cm and after adding 1 drop of 2 N HCl the foam does not disappear then it shows the presence of saponin compounds.

Steroid and triterpenoid test

The 1 g binahong leaf ethanol extract was extracted with n-hexane until it was colorless, then the extract residue was added with 10 mL of chloroform and stirred for 5 min. Taking the chloroform layer using a pipette and adding sodium sulfate anhydrous and then filtered and divided into two parts. The first filtrate (as a control) if there is any remaining filtrate then 3 drops of acetate anhydride are added in 1 drop of concentrated H_2SO_4, and color changes occur with the control. If it is formed blue-green or purple-red indicates a steroid or triterpenoid compound.

GC–MS analysis

GC–MS analysis of *Phellodendron chinense* ethanol extract was carried out using the Perkin-Elmer GC Clarus 500 system which consisted of AOC-20i auto-sampler and gas chromatograph connected with mass spectrometer (GC–MS) equipped with Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused the capillary column (30 × 0.25 μm ID × 0.25 μm df). For GC–MS detection, the electron ionization system is operated in an electron impact model with 70 eV ionization energy. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 μl was used (split ratio 10:1). The injector temperature is maintained at 250°C, the ion source temperature is 200°C, the oven temperature is

programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with an isothermal 9 min at 280°C. The mass spectrum is taken at 70 eV; scanning interval 0.5 s and fragments from 45 to 450 Da. The solvent delay is 0–2 min, and the total GC/MS operating time is 36 min. The relative percentage of each component is calculated by comparing the average peak area with the total area. The mass detector used in this analysis is Turbo-Mass Gold Perkin-Elmer, and the software adopted to handle the mass spectrum and chromatogram is Turbo-Mass ver-5.2 Identification of a Compound. Interpretation of GC–MS is carried out using a database of X. Interpretation of the GC–MS mass spectrum is carried out using the National Institute of Standards and Technology (NIST) database which has more than 62,000 patterns. The spectrum of unknown components is compared with a spectrum of components that are known to be stored in the NIST library. The name, molecular weight, and structure of the test material components are confirmed.

RESULTS

Binahong leaf profile

In China, Korea, and Taiwan, the binahong plant has been known to cure diseases and has been consumed for more than hundreds of years [17]. This plant is commonly used to treat wounds, smooth the skin, eliminate body aches, increase stamina, as well as antioxidants. This plant grows easily in the lowlands and highlands. Binahong leaves containing dry flavonoids based on the results of the study amounted to 11.23 mg/kg and binahong leaves containing fresh flavonoids amounted to 7.81 mg/kg. The types of flavonoids obtained from the isolation and identification of fresh powder and dried powder of ethanol extract of binahong leaves are vitexin and acid. Binahong plant material for extracts was obtained from the area around the Banda Aceh area. Plant extracts are prepared by blending fresh leaves in sterile distilled water using a blender until smooth. The extract produced is directly used for testing phytochemical results from Binahong leaves.

Phytochemical Results of Binahong (A. cordifolia) leaves

The leaves of binahong (*A. cordifolia*) which had been dissolved in 70% ethanol were analyzed phytochemically using gas chromatography–mass spectrometry (GC–MS). Phytochemical analysis results showed that binahong leaves contained all active components but only alkaloids with Metode Mayer showed negative results (Table 1).

Table 1: Phytochemical results of binahong (*Anredera cordifolia*) leaves

No.	Compounds	Information
1	Flavonoids	+
2	Saponin	+
3	Terpenoid	+
4	Tannins/phenolics	+
5	Steroid	+
6	Alkaloid	
	Mayer	-
	Wagner	+
	Dragendorff's	+

GC–MS analysis

GC–MS analysis aims to determine the content of chemical compounds. GC–MS results were taken five compounds that have the highest peak or dominant.

The results of the GC–MS analysis indicate the presence of compounds that can prevent the process of cataractogenesis. Table 2 shows the leaves of binahong (*A. cordifolia*) contain phytol (35.68%), Cis-cis, cis-7,10,13-hexadecatrienal (9.94%), 2,3-dihydroxy propyl palmitate (6, 6, and 27%), 2-ethyl butyric acid, monododecyl ester (5.86%), and hexadecanoic acid (1.61%–5.12%). Binahong leaves also contain squalene, hexadecanoic acid, methyl ester, 3 (2H)-selenophene, 2-(dihydro-4,4 dimethyl-3-oxo selenophene-2 (3H)–ylidene-0-dihydro-4,4-dimethyl, neophytadiene, and 9-octadecanoid acid with a smaller percentage than other ingredients.

Table 2: Compound content of binahong (*Anredera cordifolia*) leaves

No.	Compounds	Chemical formula	RT	Contents (%)
1	Phytol	$C_{20}H_{40}O$	28.934	35,68
2	Cis-cis,cis-7,10,13-hexadecatrienal	$C_{16}H_{28}O$	31,685	9.94
3	2,3-dihydroxypropyl palmitat	$C_{19}H_{38}O_4$	30,844	6,27
4	2-Ethylbutyric acid, monododecyl ester	$C_6H_{12}O_2$	30,927	5,86
5	Hexadecanoic acid	$C_{16}H_{32}O_2$	30,410	5,12
6	Squalene	$C_{30}H_{50}$	32,168	4,23
7	Hexadecanoic acid	$C_{16}H_{32}O_2$	30,596	4,07
8	Hexadecanoic acid	$C_{16}H_{32}O_2$	30,168	3.79
9	Hexadecanoic acid	$C_{16}H_{32}O_2$	30,444	3.68
10	Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	28,762	3,35
11	Hexadecanoic acid	$C_{16}H_{32}O_2$	30,237	2,42
12	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	27.493	2,40
13	3 (2H)-selenophene, 2-(dihydro-4,4 dimethyl-3-oxo selenophene-2(3H)-ylidene 0-dihydro-4,4-dimethyl	$C_6H_{10}O_2$	40,704	2,34
14	Hexadecanoic acid	$C_{16}H_{32}O_2$	29,996	2,20
15	Neophytadiene	$C_{20}H_{38}$	26,514	2,16
16	Hexadecanoic acid	$C_{16}H_{32}O_2$	30,044	1,61
17	9-octadecanoid acid	$C_{18}H_{34}O_2$	29,837	1,31

*Source: PubChem

Discussion

The test results show that binahong leaves contain all active components but only alkaloids with Metode Mayer. All of these contents can be potential as antioxidants or reactive oxygen species (ROS) scavenger, aldose reductase inhibitors, antiglycation agents, and apoptotic inhibitors of lens epithelial cells. Binahong leaves contain antioxidant compounds such as flavonoids, carotenoids, ascorbic acid, tocopherol, caffeine, and pyruvate. Compounds that can play a role in aldose reductase inhibitors are ellagic acid, quercetin,

epicatechin, procyanidin, cyanidin-3-O-β-glucoside, peonidin-3-O-β-glucoside, and others, while those that act as antigenic are polyphenols, phenolic acids, flavonoids, terpenes, carotenoids, and polyunsaturated fatty acids. The same content can also be found on the leaves of binahong (*A. cordifolia*). Analysis of compounds from the leaves of binahong (*A. cordifolia*) using GC–MS showed that there are several active compounds that can act as anti-cataracts. The most compounds contained in the leaves of binahong (*A. cordifolia*) are terpenoid groups, both triterpenoids, and diterpenoids such as phytol, neophytadiene, and squalene. The presence of terpenoids inhibits the catalysis of glucose reduction into sorbitol and converts it to fructose due to the enzyme sorbitol dehydrogenase. This condition causes inhibition of glucose metabolism, thereby preventing cataractogenesis [18]. In addition, the triterpene content is known to inhibit glycation and suppress ROS and repair the vascular injury by inhibiting the activation of the RAGE (NADPH oxidase-NF-α-B) transduction pathway for RN transduction [19].

Phytol ($C_{20}H_{40}O$) is a diterpenoid compound with a molecular weight of 296.5 g/mol where hexadec-2-en-1-ol is replaced by methyl groups at positions 3, 7, 11, and 15 (Figure 1). Phytol is able to reduce the production of free radicals, and this activity can be attributed to their structural features because phytol is branched chain unsaturated alcohol and its antioxidant properties may be related to the hydroxyl group (OH) present in its molecule. Perhaps, phytol, by reacting with free radicals, donates hydrogen atoms with unpaired electrons (H·), transforming free radicals into less reactive species. Phytol is able to remove hydroxyl groups. [20]. Phytol and its derivatives can play a role as metabolism-modulation, cytotoxic, antioxidant, autophagy and apoptosis inducing, antinociceptive, anti-inflammatory, immune modulation, and antimicrobial effects [21]. High antioxidant content can inhibit the process of cataractogenesis in the lens of the eye [22]. The ability as an antioxidant from the leaves of binahong, it can be assumed that binahong leaf plants can balance the imbalance that occurs in the lens of cataract sufferers. Keratogenesis inhibition pathways that occur are through radical inhibitory pathways free by substituting phytol with ROS. This antioxidant will change the oxidative atmosphere into oxidation. This condition is in accordance with the resume presented by those who explain antioxidants will play a role by changing the ROS through three stages, namely, taking it directly, stabilizing the oxidation-reduction reaction, and as an oxidizing substrate. Oxidative stress has also been recognized as an important mediator of apoptosis in lens epithelial cells and plays an important role in the pathogenesis of cataracts.

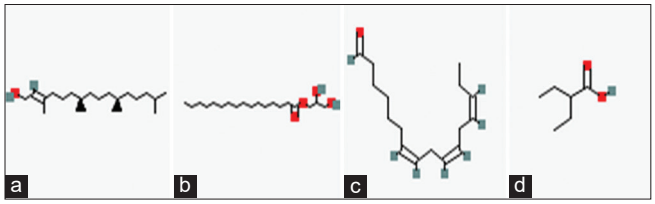

Figure 1: (a) Phytol, (b) Cis-cis, cis-7,10,13-hexadecatrienal, (c) 2-Ethyl butyric acid, monododecyl esters, and (d) hexadecanoic acid

Conclusion

Based on the analysis of binahong leaves, the result shows that binahong leaves can an alternative to prevent cataractogenesis because it is rich in antioxidants. Binahong leaves work through several inhibitory pathways such as ROS scavenger, aldose reductase inhibitors, antiglycating agents, and apoptotic inhibitors of lens epithelial cells. Very high amounts of phytol can overlap the oxygen balance of the lens, thereby preventing the occurrence of cataractogenesis.

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