The Secondary Metabolite and Antibiotic Activity of Calabash Leaf and Fruit (C*rescentia Cujete* L) Ethanol Extract

Patricius Kianto Atmodjo, Boy Rahardja Sidharta
Biotechnology Faculty, Atma Jaya Yogyakarta University, Jl. Babarsari No.44, Yogyakarta, Indonesia
kianto.atmodjo@uajy.ac.id

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Abstract **—** Antibiotic resistance is a big problem in medication, food security, and human development goals. Indonesia has a very huge diversity in term of medicinal plants, which are proven to cure many illnesses by their ancestors, including Calabash. However, research on the bioactive substances from the leaves and fruit of calabash as antibiotic [both antibacterial and antifungal] are dearth. Antibiotic activity test of the substances found has never been done yet, including the comparison to antibiotic ampicillin. The objectives of the research are: a] to determine bioactive substances found in the leaves and fruit of Calabash which have antibiotic activities, b] to reveal the antibiotic activity of leaves and fruit extract of calabash to two [2]] pathogenic bacteria and two [2] pathogenic fungus, c] to determine minimum inhibitory concentration [MIC] of calabash's leaves and fruit extract to four [4] pathogenic microbes. The methods applied in this research are as follows. The bioactive substances were extracted using maceration method with ethanol as the solvent. The extracts were then analyzed using GC-MS equipment and tested to four [4] pathogenic microbes in agar diffusion method to determine the inhibition zones of the extracts. Later, the extract was also used to determine the MIC of the extracts by dilution technique. This present research found that fruit and leaves extract have many bioactive substances such as saponin, flavonoid, terpenoid and alkaloid that have low-medium antibacterial and antifungal activity, with minimum concentration of inhibition 50% for bacteria and 75% for fungus., which later can be developed into products or antibacterial and antifungal drugs that may give benefits to Indonesian people and the world.

Keywords **—** Calabash [*Crescentia cujete* L], antibiotic, ethanol extract, phytochemistry

Abstrak**—** Resistensi antibiotik merupakan masalah besar dalam pengobatan, ketahanan pangan, dan tujuan pembangunan manusia. Indonesia memiliki keanekaragaman tumbuhan obat yang sangat besar, yang telah terbukti dapat menyembuhkan banyak penyakit oleh nenek moyangnya, termasuk Calabash. Namun penelitian tentang kandungan zat bioaktif dari daun dan buah labu kuning sebagai antimikroba [baik antibakteri maupun antijamur] masih sedikit. Uji aktivitas antimikroba dari zat yang ditemukan belum pernah dilakukan, termasuk perbandingan dengan antibiotik ampisilin. Tujuan penelitian ini adalah: a] untuk mengetahui zat bioaktif yang terdapat pada daun dan buah labu kuning yang memiliki aktivitas antimikroba, b] untuk mengungkap aktivitas antimikroba ekstrak daun dan buah labu kuning terhadap dua [2]] bakteri patogen dan dua bakteri patogen. [2] jamur patogen, c] menentukan konsentrasi hambat minimum [KHM] daun dan ekstrak buah labu kuning terhadap 4 [empat] mikroba patogen. Metode yang diterapkan dalam penelitian ini adalah sebagai berikut. Zat bioaktif diekstraksi menggunakan metode maserasi dengan etanol sebagai pelarut. Ekstrak kemudian dianalisis menggunakan peralatan GC-MS dan diuji terhadap 4 [empat] mikroba patogen dengan metode difusi agar untuk mengetahui zona hambat ekstrak. Kemudian, ekstrak juga digunakan untuk menentukan KHM ekstrak dengan teknik pengenceran. Penelitian ini menemukan bahwa ekstrak buah dan daun memiliki banyak zat bioaktif seperti saponin, flavonoid, terpenoid dan alkaloid yang memiliki aktivitas antibakteri dan antijamur rendah-menengah. dengan konsentrasi daya hambat minimal 50% untuk bakteri dan 75% untuk jamur, yang nantinya dapat dikembangkan menjadi produk atau obat antibakteri dan antijamur yang dapat memberikan manfaat bagi masyarakat Indonesia dan dunia.

Kata Kunci**—** Berenuk, *[Crescentia cujete L],* antibiotic, ekstrak etanol, fitokimia

INTRODUCTION

Disease and damage to living things by microbial activity are very high. On the other hand, the use of drugs to kill microbes is decreasing in its killing power due to the presence of microbial resistance, which is increasing recently, prompting health actors to seek "new" antibiotics. Antibiotic resistance poses a major threat to health, food safety, and development problems; and can affect anyone, both human and livestock and cultivated plants; occurs naturally and the use of antibiotics increases the infection pneumonia, TB [tuberculosis], gonorrhea, salmonellosis; candidiasis and infectious diseases are becoming increasingly difficult to eradicate; hospital stay time is longer, the cost of treatment is higher, and mortality is increasing [3]. On the other hand, the damage to crops and processed by fungi and molds are also a problem in the availability of food, feed, and traditional medicines. Research for the discovery of new compounds that can suppress or kill microbes is expected to be able to reduce medical costs and losses due to crop damage, especially in the increasingly expensive cost of using antibiotics. Besides, "new" antibiotics can also reduce rest periods in the hospital and increase productivity, and can ensure the availability of plant and animal foodstuffs [4].

As a country located in the tropics, Indonesia's biodiversity is very high and has been used by the ancestors of this nation in various daily lives [ethnobotany], including to treat human, animal, and plant diseases caused by bacterial and fungal infections. Various types of native Indonesian plants have become known sources of medicine to foreign countries. This potential has not been fully utilized, even many plants are threatened with extinction because they are cut down and burned to change forest land use [2].

Calabash [*Crescentia cujete* L.] is a plant that has the potential to be used as a medicine that has not been widely explored by the Indonesian people, especially in the Special Region of Yogyakarta, even this plant is feared and considered dangerous or poisonous so it is not used except for biopesticides][5]. Whereas in the Philippines this plant is known as a miracle medicinal plant [6], while in Central to South America it is widely used for household furniture [7].

The existence of flavonoids, saponins, and terpenoids from the research of Proximate, phytochemical screening and mineral analysis of *Crescentia cujete* L. by Oliniyi, et al. [8] inspired to conduct research related to the use of calabash as an antibiotic.

The content of bioactive compounds produced by Calabash in both the fruit and leaves is a source of materials that can be controlled for harvesting and are not harmful to plants. Parvin et al [9] have reported the potential for ethanol extract of calabash leaves and stems which can be used for antibacterial *Staphylococcus aureus* and *Escherichia coli*, although the results were inconsistent in several tests. This time the explosive antibiotic ability was examined from the leaves and fruit tested for pathogenic bacteria such as *Staphylococcus epidermidis* and *Pseudomonas aeroginosa*, and two food and feed destroying fungi, namely *Saccharomyces cerevisiae* and *Aspergillus niger*.

RESEARCH METHODS

A. Time and Place of Research

This research will be carried out in September 2019-March 2020 and will be carried out at the Technobio-Industrial Laboratory of the Faculty of Biotechnology, Atma Jaya University Yogyakarta, and the Laboratorium Penelitian dan Pelayanan Terpadu [LPPT] UGM.

B. Experiment Design

This experiment was designed using a completely randomized design to compare 2 plant organs of leaf and fruit extract sources with indicators of 2 types of pathogenic bacteria and types of fungi. This research was conducted using 3 replications. The best experimental results compared to positive controls.

Table 1. Design for measuring the minimum inhibitory concentration of ethanol extract of calabash leaves and fruit.

Concentration of extract %	*Stapylococcus aereus* [Sa]	*Pseudomonas aeroginosa* [Pa]	*Saccharomyces cerevisiae* [Sc]	*Aspergillus niger* [An]
100	Sa100	Pa100	Sc100	An100
75	Sa75	Pa75	Sc75	An75
50	Sa50	Pa50	Sc50	An50
25	Sa25	Pa25	Sc25	An25
0 [akuades]	Sa0	Pa0	Sc0	An0

C. Materials and Tools

The material used is the calabash plant [*Crescentia cujete* L] verified by the Indonesian Institute of Sciences. This plant grows a lot in the courtyard of the Church of Saint Alfonsus Gemawang Sinduadi Mlati Sleman, Yogyakarta Special Region. Extracting chemicals and measuring the chemical content of extracts such as methanol, alcohol, sulfuric acid, hydrochloric acid, acetic acid, and magnesium, as well as growth media to qualify for analysis [p.a]. The tools used include petridish, shaker, vortex, shimadzu spectrophotometer, GC-MS shimadzu.

D. Sample Preparation

Calabash plant ingredients are chosen which look healthy and fresh and bear a lot of fruit. 5 kg of fresh leaves measuring 10-15 cm long are taken and then dried naturally for 5 days. Selenuk fruit which is ripe is marked with dark green and yellowish-green hard skin weighing 1.5-2.0 kg, taken 10 pieces. The broken fruit is taken pulp and then dried using an oven then ground into a powder and stored in a closed dark jar.

For microbial materials were obtained from the microbiology laboratory, Gadjah Mada University, Yogyakarta. Furthermore, it is cultured and reproduced for the stock of experimental test materials.

E. Extraction process

Leaf and fruit samples were extracted using 70% ethanol solvent with a ratio of 1:10 by maceration, then separated and dried using a rotary evaporator. The extract is stored in dark bottles for stock samples [10]

F. Analysis of Secondary Metabolite Content [Bioactive compounds]

The sample obtained was diluted 10 times as needed, then measured the content of alkaloids, flavonoids, triterpenoids, saponins qualitatively carried out at the Techno Bioindustri Laboratory of Atma Jaya University Yogyakarta and continued with the analysis of the chemical composition using GC MS at LPPT Gadjah Mada University.

G. Measurement of Antibiotic Activity

The ethanol extract obtained was used for the antibiotic test using the agar diffusion method by inserting 10 μL into the well on an agar plate, then inoculating the preparations of each test bacterium on a petri dish and leveling it. Subsequently incubated for 24-48 hours at 37o C, then seen and measured the clear zone or inhibition zone formed [11][12].

H. Measurement of Minimum Inhibitory Concentration

The advanced antibiotic test applied in this study is the Minimum Inhibitory Concentration [MIC] with the liquid dilution method with stratified dilutions. A serial dilution of fruit and leaf ethanol extracts was obtained in the order of 100, 75, 50, 25, and 0% and tested for 2 types of bacteria and two types of microscopic fungi [11] [12].

RESULT AND DISCUSSION

A. Content of secondary metabolites Calabash fruit and leaves

Plants produce a variety of organic compounds, most of which do not play a direct role in plant growth and development. Metabolites are classified into two, namely primary metabolites and secondary metabolites. Primary metabolites which are formed in limited quantities are important factors for the growth and life of living things. Secondary metabolites are not used by plants for growth and are produced more when the plants are under

stress[13]. The function of secondary metabolites is to defend themselves from unfavorable environmental conditions, for example to cope with drastically changing environments such as temperature and salinity, pests and diseases, attract pollinators, and as signaling molecules. In other words, secondary metabolites are used by plants to interact with their environment.

Based on the origin of biosynthesis, natural plant metabolite products can be divided into three main groups, namely terpenoids, alkaloids, and phenylpropanoids as well as groups of phenolic compounds [14]. The Terpenoid group is synthesized through the mevalonic acid metabolic pathway. For example, monoterpene, sesquiterpene, terpene, triterpene, and terpene polymers. Phenolic Group compounds are made from simple sugars and have benzene, hydrogen, and oxygen ring in their chemical structure. Examples include phenolic acids, coumarins, lignins, flavonoids, and tannins. The alkaloid group is a compound containing nitrogen. These compounds are produced in a limited manner in certain taxonomic groups [14]. The result of extraction and qualitative test showed in figure 1 and table 2.

Fig 1. Ethanol Extract of Fruit [F] and Leaf [L] *Crescentia cujete*

Table 2. The results of the qualitative phytochemical analysis of calabash fruit and leaf extracts

Test	Ethanol extract of the fruit	Leaf ethanol extract
Flavonoid	+++ Intense yellow	+++ Intense yellow
Alkaloid 1.Dragendorff reagent 2.Mayer reagent 3.Wagner reagent	 + [yellow oil sediment] + [brick red sediment] -	 - + [brick red sediment] -
Triterpenoid/Steroid	+ Triterpenoid	+ Triterpenoid
Tanin	+	++
Saponin	+++	++

Based on the results of the qualitative analysis listed in table 2, it is known that calabash both fruit and leaves contain secondary metabolites of flavonoids, alkaloids, and terpenoids. This means that this calabash plant contains complete secondary metabolite compounds. Like other plants, calabash produce secondary metabolites and use these compounds to defend themselves and compete with other living things around them. Calabash produces secondary metabolites [such as flavonoids, terpenoids, and alkaloids.] Which can be used as pesticides, insecticides, and bactericides, or antibiotics. Secondary metabolite production is triggered by stress in plants [15]. Increased radiation and low air temperature affect secondary metabolites [17], besides that, the existence of biotic stress also plays a role in the secondary metabolic activities of plants.

The results of secondary metabolite analysis on calabash fruit and leaves can be seen in table 2. It can be seen that the most prominent ones are flavonoids and saponins. As is well known, flavonoids are a class of secondary metabolites produced by plants [19]. These compounds can be toxic to other organisms, working by disrupting the function of cell proteins. Several metabolites interact with molecules that have fundamental cellular functions, such as DNA [deoxyribonucleic acid] and proteins involved in cell division [18]. Nofiani [23] states that the formation of secondary metabolites is regulated by nutrition, decreased growth rate, feedback control, enzyme inactivation, and enzyme induction [13].

Apart from flavonoids, other secondary metabolites contain saponins. Saponin is a glycoside that has an aglycone in the form of sapogenin. Saponins can reduce the surface tension of the water so that it will result in the formation of foam on the surface of the water after being shaken. This property has in common with surfactants. Drop-in surface tension caused by soap compounds can damage the hydrogen bonds in water. This soap compound has two parts that are not the same polarity. The chemical structure of saponins is a glycoside composed of glycons and aglycones. The glycon part consists of sugar groups such as glucose, fructose, and other types of sugar. The aglycone part is sapogenin. This amphiphilic nature can make natural materials containing saponins function as surfactants. Surfactants are a common ingredient in soap preparations. A surfactant is a molecule that also has a hydrophilic group and a lipophilic group so that it can unite a mixture consisting of water and oil. Surfactant molecules have a polar part that likes water [hydrophilic] and a non-polar part that likes oil/fat [lipophilic]. The polar part of the surfactant molecule can have a positive, negative, or neutral charge. Saponins will bind to sterols and damage the integrity of the membrane which causes microbial death [21].

B. Anti-microbial Activity

Antibiotics are a class of drugs used to treat the infection or prevent infection. Antibiotics work in different ways in killing or inhibiting the growth of microorganisms. The antibiotic activity of a substance can be measured based on the area or diameter of the inhibition zone, which means that the substance's ability to inhibit microbial growth.

Table 3. Minimum inhibitory concentration of calabash fruit ethanol extract in the form of growth inhibition diameter [mm]

Extract concentration%	*Stapylococcus aereus* [Sa]	*Pseudomonas aeroginosa* [Pa]	*Saccharomyces cerevisiae* [Sc]	*Aspergillus niger* [An]
100	12.33	15.33	10	0
75	10.6	11.66	8	0
50	10.33	8.5	0	0
25	0	0	0	0
0 [aquadest]	0	0	0	0

Information on positive control of chloramphenicol with an inhibition zone diameter of 20 mm

Table 4. Minimum inhibitory concentration of ethanol extract of leaves in the form of growth inhibition diameter [mm].

Extract concentration%	*Staphylococcus aereus* [Sa]	*Pseudomonas aeroginosa* [Pa]	*Saccharomyces cerevisiae* [Sc]	*Aspergillus niger* [An]
100	10.6	10.33	7	0
75	9.36	8	7	0
50	6. 27	6	6	0
25	0	0	0	0
0 [aquadest]	0	0	0	0

The content of secondary metabolites of plants can play a role in inhibiting the growth of other organisms to preserve and protect plants from attacks by other organisms in a healthy or injured condition. The content of flavonoids, alkaloids, and terpenoids extracts of calabash fruit and leaves seems to be able to inhibit the growth of both gram-positive and negative bacteria, as well as unicellular fungi, but it is unable to inhibit the growth of multicellular fungi. This can be seen in Tables 3 and 4. The minimum inhibitory concentration was obtained at a concentration of 50% for both leaves and fruit for bacterial resistance and 75% for unicellular fungi. The ability to inhibit the growth of the ethanol extract of calabash fruit and leaves is similar to the research of Parvin et al. [9] regarding the antibacterial and anti-inflammatory properties of the bark and leaves of calabash which occurred in the range of 45% extracts. Similar results were obtained in the research of Ardianti and Kusnaedi [23]. The results of this study were better than the research on the antibiotic activity of the leaves and bark of calabash conducted by [24] which was conducted on E. *coli* and S. *aureus*.

Apart from antibacterial, the ethanol extract of calabash leaves and fruit has antifungal properties. In this study, its fungal activity only applies to the unicellular fungus Saccharomyces cerevisiae with an inhibition zone of 9 mm at the minimum inhibitory concentration using both fruit and leaf extras 75%. Its antifungal activity is moderate because it is less than 10 mm. These results are equivalent to the research of Dewi [25] which used the *Candida albicans* test fungus and obtained an inhibition zone with a diameter of less than 10 but at an extract concentration of 25%. The small anti-fungal activity of calabash is thought to be due to the low saponin content, so it does not cause damage to the fungal cell membrane [26].

The antibiotic activity of the calabash fruit and leaf extracts was classified as low-moderate. Greenwood [27] classifies the antibiotic activity based on the inhibition zone diameter which is less than 10 low, less than 15 moderate, less than 20 strong, and very strong above 20 mm [24]. The ability of this antibiotic activity is supported by the presence of flavonoids, alkaloids, terpenoids, and also the presence of various kinds of alcohol compounds in small amounts in calabash fruit. Various alcohols such as propanediol and butanol can be seen from the results of gas chromatography analysis carried out at LPPT UGM. As is known, alcohol is an antiseptic compound that can kill microbes by dissolving cell wall lipid.

CONCLUSION AND SUGGESTIONS

Based on the results of the above research, it can be concluded that the ethanol extract of calabash leaves and fruit contains flavonoids, alkaloids, saponins, and terpenoids. as well as the existence of various kinds of alcohol compounds that support the ability of anti-microbial activity against tested bacteria and fungi with a minimum inhibitory concentration of 50%, except for Aspergillus thread fungus. Suggestion: it is necessary to continue research to obtain a definite inhibitory concentration value and the main compound in its role as an antibiotic.

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PENULIS

Patricius Kianto Atmodjo, prodi Biologi, Fakultas Biologi, Universitas Atma Jaya Yogyakarta.

Boy Rahardja Sidharta, prodi Biologi, Fakultas Teknobiologi, Universitas Atma Jaya Yogyakarta.