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Antibacterial Activity of Kitolod (*Isotoma Longiflora* L.) Herb Extract and Fractions Against the Growth of Dental Carises Causing Bacteria *Streptococcus Mutans*

Oktafianti A. Nurafifah[1], Daniel Lantang[2], Rani Dewi Pratiwi[3], Krisna Dewi[4], Nur Fadilah Bakri[5]

[1, 2,3,4,5,6] Cenderawasih University, Jayapura, Papua Province, Indonesia

ABSTRACT

The Kitolod herb (*Isotoma longiflora* L.) has empirically been used to treat several diseases. Kitolod herb contains some secondary metabolite compounds, such as flavonoids, tannins, and saponins, which are antibacterial. This study aims to determine the inhibition of antibacterial activity and the most effective concentration in inhibiting *Streptococcus mutans* bacteria. The sample used in this study was Kitolod herb obtained from Sentani Regency. Then, the sample was extracted using the maceration method with 96% ethanol solvent. Then, it was fractionated using the solvent of ethanol-water, ethyl-acetate, and n-hexane. The antibacterial activity testing used the disc diffusion method. This study used a completely randomized design with nine treatments that included the concentrations of 50 ppm, 100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, 1000 ppm, amoxicillin (positive control), sterile distilled water (negative control). The results showed that the extract of Kitolod herb has activity as an antibacterial on *Streptococcus mutans* at the highest concentration of 1000 ppm of 10.20 mm (strong category). The testing results on the three fractions showed that the ethyl-acetate fraction has the most significant inhibition activity of 8,16 mm at a concentration of 1000 ppm (medium category). The result showed that the extract and fraction of Kitolod herb (*Isotoma longiflora* L.) have the potential to be an antibacterial to inhibit *Streptococcus mutans* bacteria.

KEYWORDS: *Isotoma longiflora* L., antibacterial, *Streptococcus mutans*, extract, fraction

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I. INTRODUCTION

Dental caries is among the prevalent illnesses globally, presenting as a significant public health concern. According to a World Health Organization report in 2012, dental caries affects 60%-80% of children worldwide, and nearly all adults experience this condition [1]. Dental caries is a dental health problem relatively high in Indonesia, with a prevalence of more than 80%. Dental caries can also cause tooth pain, which will interfere with food absorption [2]. One of the causes of dental caries is the process of carbohydrate fermentation by microorganisms in the oral cavity [3]. It can be caused by inappropriate eating habits and poor oral hygiene, disrupting the symbiosis between the host's immune reaction and microbes in the oral cavity and causing various oral diseases, such as dental caries [4].

Dental caries is a disease in the hard tissue of teeth characterized by the destruction of enamel and dentin caused by microorganism activity in plaque, which causes demineralization due to interactions between microorganism products, saliva, and parts derived from food and enamel [5]. Microorganisms that cause dental caries include *Streptococcus mutans*, Lactobacillus, and Actinomycetes. This dental caries is accompanied by pain and inflammation around the teeth [6;7]. However, the main bacteria that causes and is often found in dental caries is *Streptococcus mutans*. *Streptococcus mutans* can adhere to the tooth surface, produce acid, and survive in acidic conditions [8;9].

Indonesia is rich in biodiversity, namely around 40,000 types of plants, 1,300 of which are plants that can be used as traditional medicine. Medicinal plants are plants that are useful for medicine, cosmetics and health and are

consumed or used from plant parts such as leaves, stems, fruit, tubers (rhizomes) or roots [10;11]. One plant that can be used for treatment is kitolod (*Isotoma longiflora* L.). The Kitolod (*Isotoma longiflora* L.) has been used to treat several diseases empirically. Among them, Kitolod treats various eye diseases in Riau and West Java. In contrast, in the Bogor area, it treats toothache by pounding approximately two leaves [12].

The Kitolod (*Isotoma longiflora* L.) contains several secondary metabolite compounds, such as alkaloids, flavonoids, tannins and saponins, which have antibacterial properties [13], and also as an analgesic [14]. Kitolod (*Isotoma longiflora* L.) can be used as eye drops, dental medicine, asthma, bronchitis, sore throat, wound medicine, cancer medicine and anti-inflammatory [15;16].

Based on this background, research was carried out on antibacterial tests of Kitolod herb extracts and fractions on the growth of *Streptococcus mutans* bacteria.

II. METHODES

A. Materials and Equipments

The research was conducted at the pharmacy laboratory and microbiology laboratory, Cenderawasih University, Jayapura, Indonesia.

The tools used are glassware, analytical scales, incubators, callipers, micropipettes, tube needles, rotary evaporators, water baths, and laminar airflow. The materials used were Kitolod herb (*Isotoma longiflora* L.), 96% ethanol, sterile aquadest, paper discs, 0.9% NaCl solution, ethyl acetate, n-hexane, amoxicillin, NA (Nutrien agar), *Streptococcus mutans* bacteria.

B. Extraction

Kitolod (*Isotoma longiflora* L.) herb samples were collected from Sentani Regency. Kitolod herb were washed clean and drained, dried in the air, and crushed to become simplicia powder. Kitolod herb simplicia was extracted using the maceration method using 96% Ethanol as a solvent. Simplicia powder (400 g) was soaked in 96% Ethanol solvent (1200 ml) at room temperature for 1x24 hours with two remacerations. The filtrate was filtered using a rotary evaporator and water bath until a thick extract was obtained. The percent yield of extract was calculated.

C. Fractination

The extract was fractionated successively using ethanol-water, ethyl acetate and n-hexane (1:1) solvents and repeated six times. Fractioning was done by mixing 5 grams of extract dissolved in warm water with ethanol (25 ml) and water (25 ml) and then placing it in a separating funnel. Then partitioned using n-hexane solvent (50 ml), the mixture was shaken and left until separation occurred. The ethanol water at the bottom was removed first from the separating funnel and followed by the n-hexane solvent in a different container. Next, the previously separated ethanol-water fraction was put back into the separating funnel, partitioned using ethyl acetate (50 ml), shaken, and allowed to be separated. The ethanol-water fraction at the bottom was removed from the separating funnel, followed by the ethyl acetate solvent in a different container. The resulting liquid n-hexane fraction, ethyl acetate fraction, and ethanol-water fraction were evaporated until a thick fraction was obtained.

D. Phytochemicals Screening

Alkaloid

A total of 3 ml of Kitolod herb extract was added with 2 ml of chloroform and 2 ml of ammonia and then filtered. After that, 3-5 drops of concentrated H_2SO_4 were added to the filtrate and shaken until two layers formed. Next, each sample was tested using alkaloid reagents, namely Mayer and Dragendroft. A positive sample contains alkaloids, if a white colour forms in the solution to which the Mayer reagent is added, a brown precipitate occurs in the Dragendroff reagent.

Flavonoid

A total of 3 ml of Kitolod herb extract was added to 10 ml of hot water, boiled for 5 minutes, and then strained. After that, 5 ml of filtrate was added with 0.05 Mg-stearic and concentrated HCL, then shaken. This test is positive if the solution forms a layer of amyl alcohol with a red, yellow or orange colour.

Tannin

A total of 3 ml of Kitolod herb extract was added with a 10% $FeCl_3$ reaction. The sample is positive for containing tannins with a blackish-green colour.

Saponins

A total of 3 ml of Kitolod herb extract was added to 10 ml of hot water while shaking for 1 minute, and then two drops of HCl 1N were added. If foam appears and lasts up to 7 minutes, the sample is declared to contain saponin.

Steroids and Terpenoids

A total of 3 ml of Kitolod herb extract was added with ten drops of glacial CH_3COOH and two drops of H_2SO_4, after which the solution was shaken gently and left for several minutes. A steroid test is said to be positive if the resulting solution initially turns red and then changes to blue or green at the end of the test. Meanwhile, the triterpenoid test is positive if it produces a violet ring.

E. Antibacterial Activity Testing

The disc diffusion method was tested for the antibacterial activity of extracts and fractions of Kitolod herb (*Isotoma longiflora* L.) against *Streptococcus mutans* bacteria. The first step was to put the liquid agar medium in a sterile petri dish until it solidifies. The suspension that had been made was spread evenly on the surface of the agar medium using an L rod. Paper discs that had been soaked in 5 drops of test solution at concentrations (50 ppm, 100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, 1000 ppm), were

then placed on the surface of the agar using sterile tweezers. The positive control was amoxicillin, and the negative was sterile aquadest. After that, it was incubated at 37ºC for 24 hours. After 24 hours, the precise area was observed and measured using a calliper.

F. Data Analysis

The research data was analyzed using the Analysis of Variance (ANOVA) method with a confidence level of 95%. If there is a significant difference, proceed with the Tukey test.

III. RESULT AND DISCUSSION

A. Result of Extraction and Fractination

The yield of Kitolod (*Isotoma longiflora* L.) herb extract obtained was 42 g from 400 g of simplicia, resulting in a yield of 10.5%.

Table 1. Results of Kitolod (*Isotoma longiflora* L.) *herb* extract

Sample	Simplicia	Solute	Extract	Yield
Isotoma longiflora L.	400 g	1200 ml	42 g	10.5 %

Table 1. shows that % yield of Kitolod (*Isotoma longiflora* L.) herb extract was 42 g. Another study showed that the yield of Kitolod herb extract was 12.32% with a 70% ethanol solvent [17]. In another study, the yield value was 6.84% with a 96% ethanol solvent [18]. Several factors, such as the ratio of materials to solvents, extraction methods, and extraction time, can cause differences in yield values [19].

Table 2. Results of phytochemical screening of Kitolod herb extract (*Isotoma longiflora* L.)

Constituents	Result
Alkaloid	+
Flavonoid	+
Tannin	+
Saponin	+
Steroid	+
Triterpenoid	+

Table 2. shows that extract of Kitolod (*Isotoma longiflora* L.) herb contain alkaloid, flavonoid, tannin, saponin, steroid, and triterpenoid. Another research showed that Kitolod herb contains bioactive substances such as flavonoids, tannin, coumarin, terpenoid, alkaloids, and saponins [20]. The previous research also showed that Kitolod herb (*Isotoma longiflora* L.) positively contained flavonoids, steroids, saponins, tannins, and alkaloids [21].

Table 3. Results of fractionation of Kitolod (*Isotoma longiflora* L.) herb extract

Sample	Fraction	Extract Weight	Fraction Weight	Yield
Kitolod *herb* (*Isotoma longiflora* L.)	Etanol-air	30 g	12.26 g	40.86 %
	Etil asetat	30 g	5.60 g	18.66 %
	n-heksana	30 g	3.20 g	10.60 %

Table 3 shows the results of fractionation using three solvents with different polarities. The ethanol-water fraction weighs 12.26 grams; the ethyl acetate fraction is 5.60 grams, and the n-hexane fraction is 3.20 grams. The percent yield of the ethanol-water fraction was 40%; the ethyl acetate fraction was 18.66%, and the n-hexane fraction was 10.60%. Fractionation of Kitolod herb showed more significant results in the ethyl-acetate and water-ethanol fractions because the compounds contained tended to have semi-polar and polar properties. At the same time, there were relatively few non-polar compounds.

The yield results from a sample are essential to determining the amount of extract obtained during the extraction process [22]. The yield data is also related to the active compounds in a sample, so if the yield number increases, the number of active compounds in the sample will also increase. The high level of active compounds in a sample is indicated by the high yield produced [23].

Antibacterial Activity Test

The antibacterial activity test was carried out using the disc diffusion method. Disc diffusion is one of the test methods to see antibacterial activity by looking at the presence of a clear area (inhibition zone) around the disc paper for bacterial growth on solid media [24]. The formation of an inhibition zone around the bacterial colony indicates inhibition of the growth of the test bacteria. The disc diffusion method was chosen. It has the advantage of being fast, easy, and cheap because it does not use special equipment [25].

Table 4. Results of antibacterial activity test of extracts and fractions of Kitolod (*Isotoma longiflora* L.) *herb*

No.	Group (ppm)	Inhibition Zone (mm)			
		Extract	Fraction Etanol-air	Fraction Etil asetat	Fraction n-heksana
1	50	6.21^{b}	6.11^{b}	6.19^{b}	6.08^{b}
2	100	6.41^{c}	6.17^{b}	6.32^{b}	6.12^{b}
3	200	7.80^{d}	6.22^{b}	6.46^{b}	6.18^{b}
4	400	8.21^{e}	6.40^{c}	6.88^{b}	6.31^{c}
5	600	8.79^{f}	7.18^{c}	7.20^{c}	6.79^{d}
6	800	9.42^{g}	7.42^{d}	7.97^{d}	7.02^{e}
7	1000	10.20^{h}	7.90^{e}	8.16^{e}	7.17^{f}

Corresponding Author: Nur Fadilah Bakri

8	Control (+)	13.09^{i}	13.09^{f}	13.09^{f}	13.09^{g}
9	Control (-)	0^{a}	0^{a}	0^{a}	0^{a}

The result of the research showed inhibition of the growth of *Streptococcus mutans* bacteria. The criteria for antibacterial inhibitory strength are that an inhibition zone diameter of 5 mm or less is categorized as weak, an inhibition zone of 5-10 mm is categorized as moderate, an inhibition zone of 10-20 mm is categorized as vital, and an inhibition zone of 20 mm is categorized as very strong [26].

Table 4 shows that the antibacterial activity of *Streptococcus mutans* in ethanol extract with a concentration of 50 ppm - 800 ppm is in the medium category, while at a concentration of 1000 ppm, it is in the strong category. The antibacterial activity of *Streptococcus mutans* in samples of the ethanol-water, ethyl acetate, and n-hexane fractions at concentrations of 50 ppm - 1000 ppm was included in the medium category. Amoxicillin, as a positive control, has an average inhibition zone diameter of 13.09, which is in the strong category. Amoxicillin was chosen because it has a broad spectrum that effectively works to inhibit gram-positive and gram-negative bacteria. The mechanism of action of this antibiotic is to interfere with the synthesis of the bacterial cell wall, causing the cell wall to lyse [27].

The most effective concentration of extracts and fractions in inhibiting the growth of *Streptococcus mutans* bacteria is a concentration of 1000 ppm because it has an inhibition zone of 10.20, which is classified as a strong inhibition zone. The fraction that shows the largest inhibition zone is the ethyl acetate fraction, namely 8.16 mm at a concentration of 1000 ppm, which is classified as a medium inhibition zone. The antibacterial activity of the extract is stronger than the fraction because the secondary metabolite compounds contained in the extract are still complex or multicomponent compounds.

The higher the concentration of the extract, the more active antibacterial ingredients it contains. It is thought that increasing the concentration of antibacterial compounds can increase the penetration of antibacterial compounds into the interior of microbial cells, which will damage the internal cellular metabolic system and result in cell damage. Differences in solvents can cause differences in the inhibitory power against bacteria caused by compounds, the number of compounds, and the strength of the compounds extracted [28].

The results of statistical analysis of data using One Way Anova showed significant results (0.000 < 0.01) for both extracts and fractions with F count > F table α 0.01. In the Tukey test, the negative control is significantly different from the positive control, as well as the seven extract and fraction concentrations. Kitolod herb extract at concentrations of 50 ppm – 1000 ppm shows that the seven concentrations have significantly different effects in inhibiting *Streptococcus mutans* bacteria.

The ethanol-water fraction of Kitolod herb at a concentration of 50 ppm was not significantly different from concentrations of 100 ppm and 200 ppm. The concentration of 400 ppm is not significantly different from 600 ppm. It shows that this concentration has the same effect in inhibiting *Streptococcus mutans* bacteria. Meanwhile, concentrations of 800 ppm and 1000 ppm have significantly different effects in inhibiting *Streptococcus mutans* bacteria.

The ethyl acetate fraction of Kitolod herb at a concentration of 50 ppm was not significantly different from concentrations of 100 ppm, 200 ppm, and 400. It shows that these four concentrations have the same effect in inhibiting *Streptococcus mutans* bacteria. Meanwhile, concentrations of 600 ppm, 800 ppm, and 1000 ppm have significantly different effects in inhibiting *Streptococcus mutans* bacteria.

The n-hexane fraction of Kitolod herb at a concentration of 50 ppm was not significantly different from concentrations of 100 ppm and 200 ppm. It shows that the three concentrations have the same effect in inhibiting *Streptococcus mutans* bacteria. Meanwhile, concentrations of 400 ppm, 600 ppm, 800 ppm, and 1000 ppm have significantly different effects in inhibiting *Streptococcus mutans* bacteria.

Several active metabolite compounds, namely alkaloids, flavonoids, tannins, saponins, and steroids, are thought to have antibacterial activity. Alkaloid compounds have several mechanisms for inhibiting bacteria, namely inhibiting nucleic acid and protein synthesis, modifying the permeability of bacterial cell membranes, damaging cell walls and membranes, inhibiting bacterial metabolism, and inhibiting efflux pumps [29;30]. Flavonoid compounds are essential compounds from the phenolic class. Flavonoids also have several antibacterial activities through several important mechanisms, namely suppressing nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism. Flavonoids are also reported to reduce adhesion and biofilm formation, porins in cell membranes, membrane permeability, and have pathogenicity properties, all of which are supporting factors for bacterial growth [31]. The mechanism of tannin compounds in inhibiting bacterial growth is through several mechanisms, namely forming chelates with iron compounds [32], inhibiting cell wall synthesis [33], and disrupting cell membranes [34]. Saponin inhibits bacterial growth through a mechanism: changing the morphology of the bacterial cell surface by disrupting the integrity of the membrane and cell wall [35;36] and disrupting membrane permeability [37].

CONCLUSIONS

Kitolod (*Isotoma longiflora* L.) herb extract has a strong inhibitory effect on the growth of *Streptococcus mutans*, namely at a concentration of 1000 ppm. The

fraction (*Isotoma longiflora* L.) has moderate inhibitory power against the growth of *Streptococcus mutans*, namely at a concentration of 1000 ppm.

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