International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X
Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 3; Issue 6; November 2018; Page No. 16-18

Growth inhibition test of *Streptococcus mutans* against methanol extract/fractions of Kitolod (*Isotoma longiflora* (L.) C.Presl.)

Arfiandi[1], Zulfisa[2], Indrie Ramdhani[3], Utri Makhfirra IA[4], Mhd. Riza Marjoni[5*]
[1-5] Department of Microbiology, Dwi Farma Academy of Pharmaceutical, Bukittinggi 26121, West Sumatera, Indonesia

Abstract
Kitolod flower (*Isotoma longiflora* (L.) C.Presl.) is one of the native plants of Indonesia which is widespread in various regions in Indonesia. Traditionally, Kitolod flower has been used to treat dental diseases. Kitolod flower contains flavonoids, terpenoids, tannins and alkaloids which can be antibacterial so that it is thought to be able to inhibit the growth of bacteria that cause tooth disease. This study aims to determine the inhibitory test of methanol extract and its fraction on *Streptococcus mutans* bacteria *in vitro*. with diffusion method. The results showed that at a concentration of 1%, hexane and ethyl acetate fractions have a moderate inhibition zone response with a clear zone diameter of 17.98 mm and 17.03 mm. Methanol fraction has a weak inhibition zone response with clear zone diameter of 11.66 mm against the growth of *Streptococcus mutans*. From this study concluded that methanol extract and its fraction have antibacterial activity against *Streptococcus mutans* bacteria.

Keywords: inhibitory test, *Isotoma longiflora*, *Streptococcus mutans*

Introduction
Indonesia is one of the countries that has the largest diversity of flora in the world, around 40,000 species of plants [1]. Plants contain compounds that function to defend themselves from disease. These compounds are secondary metabolites namely phenols and phenolics, terpenoids, flavonoids, saponins, alkaloids, tannins, poliasetylene, polyamines, isothiocyanates, thiosulfinates, and glucosides [2]. In microbiology, these secondary metabolites can be used to inhibit growth or kill bacteria [3]. To obtain secondary metabolites from plant parts various types of organic solvents are used, such as methanol, hexane, and ethyl acetate. Methanol can dissolve polar compounds such as flavonoids and saponins, while hexane is non-polar and ethyl acetate is semi-polar can dissolve terpenoids and alkaloids [4].

One plant that has the potential and has not been widely studied is the kitolod plant (*Isotoma longiflora* (L) C.Presl.). Research by Rosidah *et al*., (2014) stated that the extract of leaves, flowers and stems from the kitolod plant (*Isotoma longiflora* (L) C.Presl) contains phenolic compounds, flavonoids, alkaloids and terpenoids [5]. The activity of alkaloids and flavonoids has been reported to have many pharmacological effects including: anti-inflammatory, antioxidant, anticancer, antidiabetic, antibacterial, antimalarial, antitumor, antimicrobial, antifungal, anti-insecticide, and antiseptic [6, 7]. Alkaloid and flavonoid compounds have high activity against bacteria that cause dental caries [8]. The presence of polar and non-polar compounds in kitolod plants (*Isotoma longiflora* (L) C.Presl) became the reason for the need to conduct research on the activity of fractionation of kitolod flower extract (*Isotoma longiflora* (L) C.Presl.) by using three types of solvents: methanol, hexane and ethyl acetate against bacteria that cause dental caries *Streptococcus mutans*.

Materials and Methods
This research is an experimental laboratory conducted *in vitro* using agar diffusion method. The part of the plant used is Kitolod fresh flowers obtained from the Bukittinggi area, west Sumatra. Fresh ingredients are then made simplicia and extracted by maceration using Methanol solvent. The extract obtained was then fractionated by the Liquid-Liquid Extraction method using n-hexane, and ethyl acetate solvents.

Phytochemical screening
Phytochemical screening is performed on simplicia and fraksinat to identify the content of secondary metabolites found in simplicia, especially on alkaloid compounds, flavonoids, terpenoids and tannins. After the methanol fraction is obtained, n-hexane fraction and ethyl acetate fraction, then the antibacterial activity was determined. Determination of the Minimum Inhibitory Concentration (MIC) against Streptococcus mutans bacteria was carried out on the fraction which showed the best antibacterial activity among the three.

Determination of the antibacterial activity
Determination of Antibacterial activity of each fraction was carried out using the CLSI 2014 Method [9]. *Streptococcus mutans* bacteria are taken from bacterial stock using sterile ose needles, then suspended into 10 ml physiological NaCl in a vial. The turbidity of the bacterial suspension is adjusted to Mc. Farland standard solution 0.5 using a spectrophotometer at a wavelength of 600 - 625 nm with absorbance 0.08 - 0.1 [10]. Each fraction was made a concentration of 1000 µg / disc for inhibitory test, the solvents of each fraction as negative controls and Clindamycin antibiotics with a dose of 2 µg / disc as a positive control. Add 300 µL of the bacterial suspension to each petri dish and add 15 ml of media. The mixture is stirred until homogeneous and leave it to half solid. Plant a

paper disc containing 100 µL of extract / fraction, negative control and positive control. All petri dishes were incubated in an incubator in reverse for 18 - 24 hours at 37 ° C. Measure the diameter of the inhibition indicated by the presence of a clear zone around the paper disc

Results and Discussion

The ability of extracts and fraction of kitolod flowers to inhibit the growth of some bacteria is caused by the presence of an active ingredient which acts as an antibacterial such as phenolic, flavonoids, alkaloids and terpenoids. The results of phytochemical screening showed that methanol extract and fraction of kitolod flowers (*Isotoma longiflora* (L) C.Presl) contains phenolic, flavonoids, alkaloids, terpenoids and tannins. Complete results of phytochemical screening from the extract and fraction of kitolod flower presented in table 1 below

Table 1: Phytochemical screening of extracts and fractions of kitolod

No.	Secondary Metabolites	Reagan	Identification		
			MeOH fraction	n Hexane Fraction	EtoAc Fraction
1.	Alkaloids	Mayern & Dragendorff	++	++	++
2.	Flavonoids	HCl & Mg	+	++	-
3.	Terpenoid	Acetate Anhydrous, H2SO4 p	-	++	-
4.	Tanin	FeCl3	++	++	-

Flavonoids are the largest group of phenol compounds and are the best reducing compounds in inhibiting oxidation reactions. Flavonoids in their function as antibacterial work by forming complex compounds against extracellular proteins that interfere with the integrity of bacterial cell membranes. The mechanism works by denaturing bacterial cell proteins and damaging cell membranes irreparably [11]. Flavonoids in high concentrations totally damage bacterial cell membranes and precipitate cell proteins, whereas in low concentrations it causes bacterial cell leakage, thus causing important metabolites to emerge from bacterial cells. Flavonoids can also inhibit bacterial gyrase DNA enzymes which plays a role in opening the twine of DNA for the DNA replication process. This inhibition of the DNA gyrase enzyme will cause the DNA replication process and transcription is also inhibited, causing damage to bacterial cells and ultimately bacterial cell death [12]. Alkaloid compounds as antibacterial work by inhibiting and disrupting the constituent components of peptidoglycan in bacterial cells. This process causes the bacterial cell wall layer not to form completely and cause cell death [13]. In addition, alkaloid compounds also have a nitrogen base group that can react with amino acid compounds making up bacterial cell walls and bacterial DNA. This reaction will result in changes in the structure and arrangement of amino acids from bacteria. This reaction has an impact on the genetic balance in the DNA chain so that the bacteria are damaged. Damage that occurs, will result in lysis of bacterial cells and cause cell death in bacteria [14]. Tannin compounds in extracts and fractions work as antibacterial by inhibiting the formation of bacterial cell wall polypeptides which cause lysis of bacterial cell walls [15] Tanin has a spasmolytic effect which in addition can reduce intestinal peristalsis, it can also shrink bacterial cell walls, causing disruption of bacterial cell permeability. In addition, tannins can also inhibit the reverse transcriptase and DNA topoisomerase enzymes that play a role in the multiplication process of bacteria so that bacterial cells cannot form and multiply themselves

In this research, *Streptococcus mutans* bacteria are rejuvenated with the aim of getting new cultures so they can reproduce well and can be used according to its function. Then bacterial suspension was carried out by measuring turbidity compared to Mc. Farland standards 0,5. Absorbance value of standard Mc. Farland 0.5 is 0.08 - 0.1 in a spectrophotometer with a wavelength of 625 nm, absorptions obtained during the study were 0.102. Determination of methanol, hexane and ethyl acetate fraction of kitolod flower extract as antibacterial against *Streptococcus mutans* bacteria using a concentration of 1% because the *paper disc* used was absorbed 1000 µg / disc [9].

Based on the measurement of the diameter of the inhibitory zone, the extract and fraction of the kitolod flower against the *Streptococcus mutans* bacteria showed that the biggest obstacle was found in the hexane fraction of 17, 98 mm.

Table 2: Response of Inhibitory Zones of Methanol, Hexane and Ethyl Acetate Zone of Kitolod Flower Extract.

Sample	Diameter of inhibition area	Inhibitory Response of Bacteria
Methanol Fraction	11, 66 mm	Weak
Hexan fraction	17, 98 mm	middle
Ethyl Acetate fraction	17, 03 mm	middle
Antibiotics	42, 6 mm	Strong
Negative Control	0 mm	-

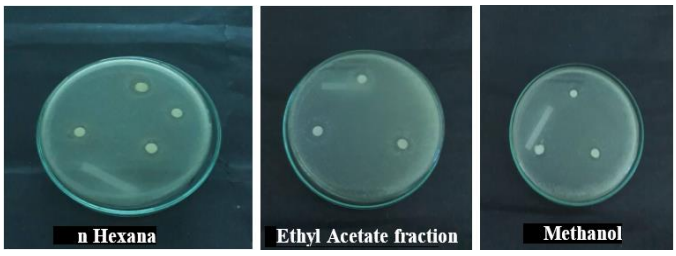

Fig 1: Response Zone of Inhibitory Growth of Streptococcus mutans Bacteria on Kitolod Extract and fraction using n hexane, EtoAc, and MeOH fractions

The ability to extract hexane fractions in inhibiting bacterial growth is caused by the active compounds contained in the extract such as terpenoids. This is in accordance with the book Indonesian Medicinal Plant Inventory Edition I and according to research conducted by Rosidah *et al* (2014) also proves that

70% ethanol extract of kitolod leaves has the same chemical content, namely terpenoids. The mechanism of terpenoids as antibacterial is reacting with porin (transmembrane protein). Porin is the entrance and exit of compounds that will reduce the permeability of bacterial cell walls. Porin damage, will cause bacterial cells to lack nutrients so that bacterial growth is blocked or dead [16].

The testing of antibacterial activity in Streptococcus mutans with ethyl acetate fraction showed resistance to bacterial growth of 17, 03 mm. The ability to inhibit bacterial growth is caused by the active substances contained in the extract, such as alkaloids. Alkaloids also have the same antibacterial effect as terpenoids. Alkaloids as antibacterial, work by disrupting the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed intact and causes cell death.

Antibacterial activity testing in Streptococcus mutans bacteria with methanol fraction showed the lowest bacterial growth inhibition of 11.66 mm. This is caused by the dissolution of most active substances in the fractions of n hexane and ethyl acetate, so that the active substance dissolved in the methanol fraction is less.

Antibacterial activity testing in *Streptococcus mutans* bacteria was also carried out on positive and negative controls using Clindamycin antibiotics and solvents from each fraction. On Clindamycin antibiotics showed bacterial growth inhibition of 42.6 mm while for solvents from each fraction did not show any inhibition of bacterial growth. This is caused by the absence of active substances contained in the solvent.

Based on the data obtained, the active substance contained in the kitolod flower also has antibacterial properties against the *Streptococcus mutans* bacteria as well as the leaf parts that have been studied previously [17]. The results of the study also show that, hexane fraction and ethyl acetate fraction of kitolod flower extract have a stronger effect than the methanol fraction.

Conclusion

From this study it was concluded that the methanol extract and its fraction had antibacterial activity in the *Streptococcus mutans* bacteria

Reference

1. Sholikhah EN. Indonesian medicinal plants as sources of secondary metabolites for pharmaceutical industry. J Med. Sci., 2016.
2. Mazid M, Khan TA, Mohammad F. Role of secondary metabolites in defense mechanisms of plants," *Biology and Medicine*. 2011.
3. Ruiz B, *et al.* Production of microbial secondary metabolites: Regulation by the carbon source, Critical Reviews in Microbiology, 2010.
4. Koşar M, Göger F, Hüsnü Can Baer K. In vitro antioxidant properties and phenolic composition of Salvia halophila Hedge from Turkey, *Food Chem.*, 2011.
5. Putri DD, Hazar S, Fitrianingsih SP. Antibacterial Activity Test of Kitolod Herba Extract (*Isotoma longiflora* (L.) C. Presl) against *Bacillus cereus*, *Pros. Farm.*, 2016.
6. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. The Scientific World Journal, 2013.
7. Pietta PG. Flavonoids as antioxidants. Journal of Natural Products, 2000.
8. Da Silva IF, De Oliveira RG, Mendes Soares I, Da Costa Alvim T, Donizeti Ascêncio S, De Oliveira Martins DT. Evaluation of acute toxicity, antibacterial activity, and mode of action of the hydroethanolic extract of Piper umbellatum L. J Ethnopharmacol., 2014.
9. CLSI, C, LS. Institute, *M100-S23* Performance Standards for Antimicrobial Testing. 2014.
10. Church DL, Baxter H, Lloyd T, Larios O, Gregson DB. Evaluation of StrepBSelect chromogenic medium and the fast-track diagnostics group B streptococcus (GBS) real-time PCR assay compared to routine culture for detection of GBS during antepartum screening. J Clin. Microbiol., 2017.
11. King A, Young G. Characteristics and occurrence of phenolic phytochemicals. Journal of the American Dietetic Association, 1999.
12. Yin LM, Edwards MA, Li J, Yip CM, Deber CM. Roles of hydrophobicity and charge distribution of cationic antimicrobial peptides in peptide-membrane interactions. J Biol. Chem., 2012.
13. Hamoen LW, Wenzel M. Editorial: Antimicrobial Peptides - Interaction with Membrane Lipids and Proteins. *Front. Cell Dev. Biol.*, 2017.
14. Reardon JT, Sancar A. Nucleotide Excision Repair, Progress in Nucleic Acid Research and Molecular Biology., 2005.
15. Kamonwannasit S, Nantapong N, Kumkrai P, Luecha P, Kupittayanant S, Chudapongse N. Antibacterial activity of Aquilaria crassna leaf extract against Staphylococcus epidermidis by disruption of cell wall, *Ann. Clin. Microbiol. Antimicrob.*, 2013.
16. Cowan MM. Plant products as antimicrobial agents, Clinical Microbiology Reviews. 1999.
17. Rosidah A. DAntibacterial Cataract Leaf Extract (*Isotoma longiflora* (L.) C.Presl.) against the growth of *Streptococcus mutans*, Univ. Jember, 2014.