Biomedical Journal of Indonesia
Journal Homepage: https://www.jurnalkedokteranunsri.id/index.php/BJI/index

Effectivity of *Jatropha multifida* L. Leaves Extract as Antibacterial on *Streptococcus mutans* using In Vitro Testing Methods

Listyaning Kartiko Kinasih[1*], Idamawati Nababan[1], Suci Erawati[1], Rouli Natasia M Simanjuntak[1]

[1]Dentistry Education, Faculty of Dentistry, Prima Indonesia University, Medan

ARTICLE INFO

Keywords:
Antibacterial
Streptococcus mutans
Jatropha multifida L.

***Corresponding author:**
Listyaning Kartiko Kinasih

E-mail address:
lkkinasih@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.32539/BJI.v7i2.384

ABSTRACT

Intoduction : Health is the condition of the entire body and body parts of an individual who is free from disease both physically, mentally and socially, thus enabling the individual to be able to carry out daily activities more productively. However, many people neglect their own health, especially in the field of dental and oral health. Caries is one of the most common oral and dental problems. The prevalence of dental caries in Indonesia is high, namely 88.8%. Dental caries is caused by Streptococcus mutans bacteria which plays a role in the conversion of sucrose to lactic acid. Infection due to Streptococcus mutans bacteria can be treated by taking antibiotics. Apart from drugs, antibiotics can also be obtained from natural ingredients, one of which is the tintir castor plant (Jatropha multifida Linn) or commonly known as the betadine. **Objective:** To see the effect of the antibacterial content of tintir leaves against Streptococcus mutans bacteria and to determine the differences in the antibacterial effect of betadine leaf extract (Jatropha multifida L.) from a concentration of 25%, 50%, 75%, and 100% against Streptococcus mutans. **Methods:** The type of research used in this study was a laboratory experimental study with a post-test-only control group design as the research design and to test the sensitivity of bacteria using the paper disc diffusion test. Data analysis using One-Way ANOVA. **Result:** The results of this study indicate the effect of tintir castor leaf extract (Jatropha multifida L.) in inhibiting the growth of Streptococcus mutans bacteria.

1. Introduction

Health is one of the important things that must be considered by society today. The meaning of the word healthy itself, when viewed from the Big Indonesian Dictionary, means the state of the whole body and its parts free from illness[1]. Meanwhile, according to the Health Law no. 23 1992, health is defined as a state of well-being in body, soul, and social conditions that enable everyone to live productively socially and economically[1]. This is in line with the definition of health according to the World Health Organization (WHO), which states that being healthy is a state of being physically, mentally and socially well[3]. So it can be concluded that health is the condition of the entire body and body parts of an individual who is free from disease both physically, mentally and socially, thus enabling the individual to be able to carry out daily activities more productively socially and economically. However, not a few individuals do not really pay attention to their own health, especially from the field of dental and oral health. Caries is one of the most common dental and oral health diseases. According to data obtained from the Basic Health Research by the Indonesian Ministry of Health in 2019, Indonesia has a fairly severe caries prevalence rate of 88.8%. From these data, it is also obtained information that when viewed by age group, dental caries can affect all age groups ranging from the age group 3-4 years to 65 years and over and the incidence of caries is relatively high in each age group with the 55-64 age group. years as the highest prevalence rate, namely 96.8%. Meanwhile, in terms of gender, women have a higher incidence of caries than men, with a percentage of

89.9% [4].

Caries is a disease caused by microbial activity that changes the glucose content of food scraps to acids which results in demineralization, cavity and destruction of tooth hard tissue[5]. Caries is a progressive process characterized by demineralization of the hard tissue surfaces of teeth such as enamel and dentin, both in the crown of teeth and in the root of the teeth, which is associated with a dietary diet that supports dental caries.[6] The process of caries takes a relatively long time. There are 4 important factors that play a role in the formation process of dental caries, namely the host factor, namely the teeth itself, food factors, microorganisms and time[7]. As previously known, the onset of caries is caused by the invasion of a microorganism in the form of a bacterium called Streptococcus mutans. Streptococcus mutans is a normal flora in the oral cavity. These bacteria are included in the gram-positive group of bacteria and during their growth period they will pair up to form a chain. Streptococcus mutans has a very important role in the process of metabolizing sucrose to lactic acid, which can cause demineralization of tooth enamel. The main bacteria that causes dental caries is Streptococcus mutans[8].

Infection due to Streptococcus mutans bacteria can be treated by taking antibiotics. Antibiotics can also be obtained from natural ingredients. In Indonesia, there are many medicinal plants that have functions as natural antibiotics, one of which is the tintir castor tree (Jatropha multifida Linn) or commonly known as the betadine plant. Tintir castor plant (Jatropha multifida L.) is a traditional medicinal plant which is known to have various benefits. In Nigeria, the tintir castor plant is used as a remedy for various infections. The use of sap and leaves from this plant can be used to treat wound infections on the baby's tongue and infections in wounds on the surface of the skin. And for oil, fruit, and seeds obtained from plants which is also known as iodine, it can be used as a prevention and treatment of dental caries, wound medicine, and also as a laxative.[9]

From the results of the phytochemical tests that have been studied by Syarfati et al. In his research on the antibacterial activity test of tintir jatropha which was tested on several types of microorganisms such as bacteria and spores that cause genital disease, it was found that the tintir distance (Jatropha multifida Linn) contains saponins, steroids, glycosides, and tannins in it with different levels in each part and the substances contained are what make the tintir function as antibacterial. A study that discusses the ability of tintir castor sap to inhibit bacterial growth was also carried out by Darmawi et al. and Arianingsih et al. Darmawi et al. observed the inhibitory power of tintir leaf sap extract with several different concentrations in Staphylococcus aureus which was carried out in vitro, the results obtained from this study showed a strong zone of inhibition against Staphylococcus aureus bacteria at a concentration of 25%, 50%, 75%, 100% with a concentration of 100% as the most effective concentration of the other concentrations[10]. Meanwhile, Arianingsih et al. observed the role of extracts from the leaves of tintir (Jatropha multifida L.) against Staphylococcus aureus in vitro using negative control concentrations (0%), 25%, 50%, 75%, and 100%. It was found that the results of this study showed the effect of tintir castor leaf extract (Jatropha multifida L.) on the growth of Staphylococcus aureus bacteria, and a concentration of 50% was the best concentration of tintir leaf extract in inhibiting the growth of Staphylococcus aureus bacteria[11].

Based on the description above, the researcher is interested in conducting a study entitled The effect of tintir castor leaf extract (Jatropha multifida Linn) as an antibacterial Streptococcus mutans in vitro.

2. Method

This research is included in the laboratory experimental category with a post-test-only research design with a control group design. To test the antibacterial activity in this study, the paper disc diffusion test was used. This research was conducted at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, University of North Sumatra. This research took place from February 2021 to March 2021. This study used the tintir castor leaf which has

the Latin name Jatropha multifida Linn as the research sample. There are 2 variables used in this study, namely the dependent variable in the form of Streptococcus mutans bacteria, the independent variable in the form of tintir castor leaf extract with a concentration of 25%, 50%, 75%, 100%, aquadest as a negative control and clindamycin as a positive control.

The initial stage in starting the research is the preparation of the tools and materials to be used. The equipment needed for the implementation of this research is micropipette, petri dish, tweezers, scissors, beaker, ose, spatula, analytical balance, erlenmeyer, incubator, ruler, blender, sieve with a size of 0.625 mm, oven, filter paper, autoclave, vial bottles, baskets, large jars, tissue, aluminum foil, measuring cups, blue tips, and paper discs with a diameter of 5 mm.

As for the materials used, namely the leaves of the tintir castor plant (Jatropha multifida L.), Nutrient Agar (NA) media, Mueller Hinton, aquadest, ethanol as a solvent, Streptococcus mutans bacteria, clindamycin 300 mg as a positive control.

First, clean the tool that will be used using running water and then dry it. Wrap the beaker, measuring cup, petri dish, erlenmeyer, spatula, and tweezers using paper and aluminum foil which are then sterilized using an oven at 150^{0}C for ± 2 hours. Equipment made of plastic and tools that are a medium for bacterial growth are sterilized using an autoclave at a temperature of 121^{0}C for up to 15 minutes using a pressure of 1 atm.

Then, choose fresh leaves from the castor plant and wash them thoroughly. After being washed clean, the tintir castor leaves are dried using an oven at a temperature of 40^{0}C-50^{0}C so that it can be ascertained that the moisture content in the tintir leaves is gone, then the dried tintir leaves are sliced thinly. Then, blend the tintir leaves, which have been thinly sliced beforehand to form a fine powder, then sieve using a 0.625 mm sieve. For ingredients with a size larger than 0.625 mm, blender again until smooth so that it can be used as an extraction material. Make sure that the material to be used is smooth. If it is smooth, do the ethanol extraction process of tintir leaves by maceration. Maceration includes an extraction method where the process is carried out by immersing the material in a solvent (solvent) for a while at room temperature and being protected from light[12]. At first, 200 grams of castor leaf powder with 800 ml of ethanol for 3x24 hours and stirred once a day. Then after 3 days, the extraction material in the form of tintir leaf powder which has been macerated with the solvent is filtered with filter paper. Steam the material with a Rotary Evaporator until you get an extract with a thick consistency called tintir castor leaf extract.

The extract from the leaves of the tintir castor plant that has been obtained, measured and taken as much as 12.5 ml which is then divided into four parts, namely 1.25 ml of tintir leaf extract mixed with 3.75 ml of aquadest is a suspension with a concentration of 25%. Then 2.5 ml of castor leaf extract mixed with 2.5 ml of aquadest is a suspension with a concentration of 50%. Furthermore, 3.75 ml of tintir leaf extract mixed with 1.25 ml of aquadest is a suspension with a concentration of 75%, then 5 ml of tintir leaf extract for suspension with a concentration of 100%.

The next step is to test the antibacterial effectiveness of tintir castor leaf extract against Streptococcus mutans bacteria. In this study, the antibacterial effectiveness test was carried out using the disc paper diffusion method. The first step is to make the media as a bacteria culture place by mixing 2.1 grams of Mueller Hinton with 100 ml of water homogeneously. After that, 18 ml of the stirred media is taken and used as a liquid medium for the bacterial inoculum. The remaining media will be mixed in 1.5 grams of gel which will later be used as a solid medium for coating bacteria.

Soak disc paper (5 mm in diameter) in each extract concentration for a duration of 25 minutes. After that, place the soaked disc paper on the surface of the bacterial medium using tweezers and do a little pressing. The media that had been applied to the research samples were then put into an incubator with a temperature of 25^{0}C, until the optimum growth of Streptococcus mutans bacteria was found. Make the first observation after 24 hours. The diameter of the

inhibition zone was measured using a ruler to determine the effectiveness of the antibacterial content of the tintir castor leaf extract[13]. The zone of inhibition is measured by measuring the diameter of the clear area. The diameter of the inhibition zone is the result of reducing the diameter of the area that is not covered by bacteria around the disc paper by the diameter of the disc paper.

Furthermore, the data from this study were analyzed using the SPSS program. The data was tested first using the ShapiroWilk test to see the normality of the data. If the data is normally distributed ($p > 0.05$), then the data can then be tested using the One Way ANOVA test to see if there are significant differences between each treatment group. But if the data is not normally distributed, the data can be continued using the Kruskal-Wallis non-parametric test.

3. Results

The results of the phytochemical screening test carried out qualitatively on tintir castor leaf extract (Jatropha multifida L.) are shown in table 1 as follows.

From testing the antibacterial potential of jatropha leaf extract or Jatropha multifida Linn with a concentration of 25%, 50%, 75%, and 100% along with positive control (clindamycin) and negative control (aquadest) on the growth of Streptococcus mutans bacteria, the results are in table 2 this.

From the data shown in the diameter above, it was carried out for the Shapiro-Wilk normality test with the results of the normality test showing significant $p > 0.05$ and then re-tested using the parametric One Way ANOVA statistical test. Based on the One Way ANOVA statistical test data, it was found that there was a significant value of $p = 0.000$ ($p < 0.05$) which means that there was an increase in the antibacterial potential of each treatment group. Furthermore, the Tukey test was carried out to see the average similarity of the tintir leaf extract with a concentration of 25%, 50%, 75% and 100%, positive control and negative control of inhibitory diameter in inhibiting the growth of Streptococcus mutans bacteria. Tukey's test results show that there are 3 different subsets. Subset 1 used a negative control sample with a value of 0.000, which means that there is no value on the mean of the sample for the growth of Streptococcus mutans bacteria. Extracts with a concentration of 25%, 50%, 75%, and 100% are in subset 2 with values obtained sequentially, namely 8.933, 9.500, 10.700, and 10.967, which means that there is a difference but not significant in the four experimental groups or in words. the other four experimental groups had the same average value in inhibiting the growth of Streptococcus mutans bacteria. For subset 3 using a positive control (clindamycin) as the sample, a value of 34.867 was obtained, which means that there is a significant difference in the experimental group using the sample to the inhibition zone diameter in inhibiting the growth of Streptococcus mutans bacteria.

Table 1. Phytochemical test results on leaf extract of tintir jatropha (*Jatropha multifida* Linn).

Secondary Metabolites	Reactor	Ethanol Extract
Tannins	FeCl3	Positive (+)
Saponins	Hot water Shaken	Positive (+)
Flavonoids	Mg + HCl + Amyl Alcohol powder	Positive (+)

Table 2.Antibacterial effectiveness test results on leaf extract of jatropha multifida linn against streptococcus mutans bacteria

Bacteria	Sample	Clear Zone Diameter (mm)			Mean ± SD
		U1	U2	U3	
Streptococcus mutans	Clindamycin (K +)	34.1	36.9	33.6	34.87 ± 4.811
	Aquadest (K-)	-	-	-	-
	25% castor leaf extract	9.5	9.1	8.2	8.93 ± 0.488
	25% castor leaf extract	10.2	9.2	9.1	9.50 ± 0.583
	25% castor leaf extract	11.2	11.0	9.9	10.70 ± 0.783
	25% castor leaf extract	11.7	11.2	10.0	10.97 ± 0.828

4. Discussion

This study used a sample of tintir distance leaves with the Latin name Jatropha multifida Linn. Initially, the extraction procedure was carried out on tintir castor leaves. Dry the leaves using the heat of the sun until the color turns slightly brown. Furthermore, the dried jatropha leaves are thinly sliced and then blended until smooth and in the form of small powders. The fine powder of the tintir leaves is then soaked (macerated) for 3x24 hours using ethanol so that more tannins (extracts) are produced. This is consistent with the results of research conducted by Sari et al. In their research, Sari et al. said that if the immersion is carried out longer, the more extract will be obtained, this is because the contact time between the solute and the solvent is getting longer[14]. After 3 days, the Jatropha leaf extract solution is put into a Rotary Evaporator which will then produce a tinted leaf extract which has a thick consistency. The extract was then divided into 4 concentration groups, namely concentrations of 25%, 50%, 75%, and 100% and the rest of the extract was taken for phytochemical screening. From the phytochemical screening that has been carried out, it was found that the content of bioactive compounds including tannins, flavonoids, and saponins in the extract of the tintir leaves has the potential as a compound that can inhibit the growth of Streptococcus mutans bacteria. The results of phytochemical screening in this study are in line with research conducted by Sansetyawati et al. which states that the compounds contained in the tintir castor plant have good benefits to be used as antibacterials[15].

Naim et al. In his research, he formulated that as an antibacterial, tannins are considered to have a mechanism of action as a compound capable of inhibiting the action of adhesins on bacterial cells (molecules that bind to the surface of the host cell)[16]. This is because tannins are phenolic compounds that work by targeting polypeptides in the cell wall and will damage the cell wall membrane. If there is damage to the cell membrane, phenol compounds and their derivatives (flavonoids) will release H + ions and will attack the phosphate compounds (polar groups) which result in the breakdown of phospholipids and convert them into carboxylic acids, phosphoric acids and glycerol. In the end, the shape of the cell membrane cannot be maintained by phospholipids which ends with a leak in the cell wall, this can inhibit bacterial growth and can even cause death in bacterial cells[14].

The results of the antibacterial test in this study found that the four groups given tintir leaf extract with different concentrations could have the potential to be antibacterial against Streptococcus mutans bacteria with an inhibition zone diameter of 8.93 mm for the tintir leaf extract with a concentration of 25%, 9.50 mm for tintir leaf extract with a concentration of 50%, 10.70 mm for a leaf extract of tintir with a concentration of 75%, and 10.97 mm for a leaf extract of tintir with a concentration of 100%. It can be seen that there are differences between the four treatment groups which can be concluded that the lower the concentration level of the tintir distance leaves used, the smaller the diameter of the inhibition zone is formed. The treatment group that was given tintir leaf extract with a

concentration of 75% and 100% had a strong inhibitory power against Streptococcus mutans bacteria in accordance with the antibacterial strength provisions stated by David Stout in Sari et al. that the substance is included in the very strong category in inhibiting bacterial growth if the resistance area formed is 20 mm or more, the strong category if the resistance area formed is 10-20 mm, the moderate category if the resistance area formed is 5-10 mm, and weak category if the resistance area formed is less than 5 mm[14].

Data analysis using One Way ANOVA showed a difference in each experimental group with p <0.05, then continued by using the Tukey's advanced test and the results of Tukey's test showed that the effect of tintir leaf extract (Jatropha multifida Linn) with a concentration of 25% , 50%, 75%, and 100% had insignificant differences in inhibiting the growth of Streptococcus mutans bacteria. Clindamycin has a stronger inhibitory ability compared to tintir leaf extract with a concentration of 25%, 50%, 75%, and 100% in inhibiting the growth of Streptococcus mutans bacteria.

5. Conclusion

From the results of antibacterial tests that have been carried out, it was found that the tintir leaves with the Latin name Jatropha multifida Linn can be used to inhibit the growth of Streptococcus mutans bacteria at concentrations of 25%, 50%, 75%, and 100% although they are not as effective as Clindamycin. The tintir leaf extract that was the strongest in inhibiting bacterial growth was the 100% concentration of tintir castor leaf extract with an inhibition zone diameter of 10.97 mm.

6. Referensi

1. Sehat. (2016). At KBBI Online. Retrieved 13 Sep 2020, from https://kbbi.kemdikbud.go.id/entri/sehat
2. Indonesia, P. R. (1992). Law of the Republic of Indonesia Number 23 Year 1992 concerning Health. Minister / State Secretary of the Republic of Indonesia, 3 (1), 1–53. https://peraturan.bpk.go.id/Home/Details/46620/uu-no-23-tahun-1992
3. Organisasi Kesehatan Sedunia (World Health Organization) (1992). Health Education Guidelines for Basic Health Services. ITB-Udayana University: Bandung-Denpasar
4. Kementrian Kesehatan RI. (2019). Dental and Oral Health Risk Factors. Center for Data and Information, Ministry of Health, Republic of Indonesia, 1–10. https://www.kemkes.go.id/resources/download/pusdatin/infodatin/infodatin Gigi.pdf
5. Ningsih SU, Restuastuti T, Endriani R. Description of Knowledge and Attitudes of Brushing Teeth on Students in Preventing Caries at SDN 005 Bukit Kapur Dumai. FK Student Online Journal 2016; 3 (2): 1-11.
6. Sambuaga DAA, Gunawan PN, Mantik MFJ. Description of Knowledge Level and Dental Caries Status in Christian Middle School 67 Manado Students. E-GIGI, 2015; 3 (2). https://doi.org/10.35790/eg.3.2.2015.10346
7. Warganegara E, Restina D. Castor sap (Jatropha curcas L.) as a growth inhibitor of Streptococcus mutans bacteria in dental caries. Majority, 2016; 5 (3), 1–6.
8. Gartika M, Satari MH. Some natural ingredients as alternative caries prevention materials. Available at: pustaka.unpad.ac.id/wp_content/uplads/2013/08/pustaka_unpad_b-Several_bahan_alam_pdf.
9. Syarfati, Eriani K, Damhoeri A. The Potential Of Jarak Cina (Jatropha multifida L.) Secretion In Healing New-Wounded Mice. Jurnal Natural, 2011; 1(1), 16–19.
10. Darmawi, Manaf ZH, Putranda F. Inhibition of Chinese Castor Sap (Jatropha multifida L.) Against Staphylococcus aureus In Vitro. Journal of Veterinary Medicine, 2013; 7 (2), 113–115. https://doi.org/10.21157/j.med.vet..v7i2.2946

11. Arianingsih EP, Uno WD, Kumaji SS. The Effect of Iodine (Jatropha Multifida L.) Leaf Extract on the Growth of Staphylococcus Aureus Bacteria. Gorontalo State University, Faculty of Mathematics and Natural Sciences, Department of Biology. 2015; 3-3.
12. Damayanti A, Fitriana A. Collection of Rose Essential Oil Using the Maceration Method. Renewable Natural Material Journal, 2013; 1 (2), 1–1. https://doi.org/10.15294/jbat.v1i2.2543
13. Volk WA, Wheeler MF. Basic Microbiology. Ed 1. Translation Markham. Jakarta: PT. Gelora Aksara Pratama, 1993.
14. Sari FP, Sari SM. Extraction of Antimicrobial Active Substances from Iodine Plants (Jatropha multifida Linn) as Alternative Raw Materials for Natural Antibiotics. Journal of Chemical Engineering, Diponegoro University. 2011; 1 (1): 7.
15. Sansetyawati MS. Antibacterial Activity Test of Iodine Plant Ethanol Extract (Jatropha multifida L.) Against Staphylococcus aureus ATCC 6538 and Escherichia coli ATCC 11229 by In Vitro. Muhammadiyah University of Surakarta, 2015; 151, 10–17.
16. Naim, R. (2004). Antimicrobial Compounds From Plants. Faculty of Veterinary Medicine and Graduate School, Bogor Agricultural University