Antibacterial Activity of Extract Ethanol Bidara Leaves (*Ziziphus spina-Christi L)* on *Enteropathogenic coli*

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Abstract

Prokaryotic bacteria are microorganisms can beneficial as normal flora, but also can have health due to disease pathogens and is for the host. The purpose of this study is to find compounds in extract ethanol bidara leaves those compounds and activities against bacteria *Enteropathogenic coli*. This study shows the flavonoid compounds and tannin in extract ethanol bidara leaves (*Ziziphus spina-Christi L)* to minimum inhibitory zone at concentrations 50% and minimum bactericidal at concentrations 75%. The higher concentration extract ethanol Bidara leaves the larger the drag zone produced. The research is experimental study looking the extract ethanol antibacterial activity Bidara leaves with the methods in vitro test diffusion. This study found a chemical compound which is found in extract ethanol Bidara leaves (*Ziziphus spina Christi L*) is flavonoid and tannin have antibacterial activity10^6 CFU/ml against bacteria *Enteropathogenic coli* to minimum inhibitory zone at concentrations 50% and minimum bactericidal at concentrations 75%.

Keywords: *antibacterial, bidara leaves (Ziziphus spina-Christi L), EPEC, MIC, MBC*

Introduction

Prokaryotic bacteria are microorganisms that can be beneficial to health as normal flora, but it could also impact because it is detrimental to the host pathogens and cause disease[1].

Antibacterial is a substance that can interfere with growth or kill bacteria by the mechanism of disturbing the metabolism of bacteria. An antibacterial ideal having been selective toxicity, antibacterial substance that was only harmful to bacteria causing infections but are not dangerous for host Hospes or body[2].

Antibacterial substance is compounded capable of inhibiting the growth of microbes and can be used in the treatment of in humans, animals and plants. Based on the nature of selective toxicity, antibacterial divided into two, bakteriostatic is working in a way inhibits the multiplication of bacteria and which is a bactericidal which kills bacteria. Bakteriostatik can act as a bactericide in high concentration[3]. An antibiotic among bactericide among them are penicillin, cephalosporin, aminoglikosida, kotrimoksazol, and isoniazid rifampisin. While among those targets are a sulfonamide bakteriostatik, tetracycline, kloramfenikol, erytromisin, trimetropin, linkomisin, klindamisin peraminosilat and acid[2].

Treatment of diseases caused by bacteria could be done with the purpose of hinder/kill pathogenic bacteria infecting humans in synthetic antibiotic. But research finds treatment with antibiotics this risk having synthetic resistant so as to cause treatment failure and patients being infected for a long time[4]. When viewed from risk of antibiotic resistance to bacteria, synthetic pathogenic it takes to find alternative solution medicine safe, cheap, easily obtained a new drug and better as a substitute for

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synthetic antibiotic. Of compound substances can be obtained antibacterial of microbes, herbs and animals. The opportunity to obtain antibacterial natural material in Indonesia very large, considering that Indonesia is a rich in biodiversity[5]. The use of traditional medicines in Indonesia is substantially is part of the Indonesian nation, culture this will require the existence of the development and research new drugs are derived from plants. The advantage of the use of traditional medicines is easy to get around us and empirically traditional medicine's ability to heal various kinds of diseases, but efficacy and their ability not proven clinically[6,7].

Antibacterial activity can be measured by in vitro methods to determine the antibacterial a substance in solution and sensitivity to concentration presented by a bacteria. Bacterial sensitivity to an antibacterial can be tested by diffusion and dilution methods. The diffusion method is an antibacterial activity test that is often used because it's relatively easy, affordable, a stage of work does not require special skills and the result are obtained faster[8].

Bidara (*Ziziphus spina-Christi L)* has been proven could heal some diseases as indigestion, weakness, complaints, hearts obesity, bladder problems, diabetes, infections of the skin, lost appetite, diarrhea, fever, insomnia, as a tranquilizer and cancer[9,10]. In Saudi Arabia, this plant has been used for the treatment of various diseases such as indigestion, obesity, their complaints, infections of the skin, fever, diarrhea, bronchitis, diabetes, anemia and insomnia[11].

The bidder is an evergreen tree that grows wild throughout the islands of Java, Bali, Madura and Sumbawa (Nusa Tengara Barat) at an altitude below 400 meters above sea level. All parts of bidara are used in traditional medicine (leaves, fruit, seeds, roots, and stems) now supported by several research found plant bidara contain the alkaloid, glycoside, tannin, flavonoid, quinone, saponin and steroid/triterpenoid[12,13,14]. Other research also found that stems, leaves and seeds bidara having antibacterial activity, anti-inflammatory, anti-fungal, anti-cancer by in vitro and in in vivo[15,16,17,18].

Table 1. The compound in Bidara

Filtering of phytochemistry	Leave	Fruit	Seed
Alkaloid	+	+	-
Flavonoid	+	+	+
Saponin	+	+	-
Tannin	+	+	+
Kuinon	+	+	+
Steroid/Triterpenoid	+	+	-

To the best of our knowledge, there have been no reports on the effects of the species on the antibacterial activities of the *Ziziphus spina-christi L.* Thus, the aim of the present study was to i) estimate the levels of total tannin compounds and total flavonoids of leaves, ii) study the antibacterial activities of *Ziziphus spina-Christi L* leaves to *Enteropathogenic coli* 10^6 CFU/ml *in vitro.*

Materials and Methods

Site of the study: The study was carried out at the biochemistry laboratory, Faculty of Medicine, University of Airlangga, Surabaya.

The design of this study was an experimental study by looking at the antibacterial activity test result of bidara leaf ethanol extract carried out by the diffusion test method in vitro. Diffusion test using *disk diffusion* as a medium for ethanol extract of bidara leaves using

EPEC 10^6 CFU/ml as a test bacterium. The required data is the diameter of the inhibitory zone formed on the MHA media when conducting the sensitivity test of the disk diffusion method.

Sample collection and Preparation

Bidara leaf extract is made from 3 kg of fresh bidara leaves harvested from Madura Island-East Java, cleaned and dried leaves at the temperature of an oven 70° c to dry. Simplicia dried bidara leaves are blended and sieved using 40 mesh sieve to form 560 g of powder, powder obtained used for making extract ethanol bidara leaves[19]. Next the process maceration simplicia Bidara leaves by soaking 560g of Bidara leaves with 2.300 ml ethanol 96 % and left be closed for three days and placed in a sheltered from direct sunlight. During the soaking, marinade stirred several times with the purpose of improving the effectiveness of the process diffusion compound dissolved in a search liquid. Simplicia and the search fluid are filtered and squeezed to get the first macerate liquid. The pulp is soaked again with 750 ml ethanol for three days to get the second macerate. The second macerate then combined with the first macerate. Macerate obtained settled for 1x24 hours and deposited. Macerate concentrated using an evaporator rotary at a temperature 50°C order to obtain extract viscous bidara leaves free from a solvent. Next included to Erlenmeyer plus a solvent ethanol 96 % and whipped 2-3 hours. Next undergone a filtering Phytochemistry the flavonoid and tannin.

Making Suspense Bacteria Test

1 ml of *E. coli* stock cultured on nutrient agar (NA) media at temperatures 37°C for 24 hours. Then will be the manufacture of suspension bacteria culture test by taking *E. coli* and dissolved in solution copy (0,9% NaCl) in aseptic in the tube different, each 5 ml solution NaCl 0,9%. A suspension that is formed equalized with a standard McFarland No. 0,5 that is 1,5 x 108 CFU/ml. For the manufacture of the media MHA *(Muller Hinton Agar)* to do with how to weigh 9,5 g Muller Hinton Agar/MHA (38 g/L) with a composition medium (Beef infusion 300 g, Casamino acid 17,5 g, Starch 1,5 g) dissolved in 250 ml aquades then heated to boiling then sterilized in an autoclave for 20 minutes with the air pressure 1 ATM temperature 121°C[20].

In Vitro Antibacterial Study

The antibacterial activity undertaken using pathogenic bacteria *Escherichia coli (EPEC)* in aseptic with the methods *disc diffusion*. Suspension bacteria test spread evenly in a media MHA by using a swab, sterile cotton, then settled for a few minutes until suspension bacteria percolate in a media. A next paper strain that serves as the accommodates extract Bidara leaves lay on plate agar and incubated at a temperature 37°C among 24 hours. The result of the observation obtained of the whereabouts of zone clear formed all over paper discs which indicates the presence of zone obstruent in the growth of bacteria. The greater cleared zone, the large the ability obstruent extract ethanol Bidara leaves against bacteria. Zone category obstruent as on a table 2.

Table 2. The Zone Obstruent Antibacterial Activity Extract Ethanol Bidara leaves

No	Diameter (mm)	Category
1	Diameter > 12mm	Strong (+++)
2	Diameter 9<Ø≤12mm	Moderate (++)
3	Diameter 7<Ø ≤9mm	Weak (+)
4	Diameter = 6mm	No obstacles (-)

Sumber : Pan *et al*., (2009)

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The determination of minimum levels of the barriers do with the antibacterial activity leaves extract ethanol Bidara diffusion tests with a method of in vitro culture with bacteria to the media and be counted in a paper disc with bacteria test *Enteropathogeniccoli* 10^6 CFU/ml. The determination of minimum levels of kill characterized by the whereabouts bacterial growth in a media culture (nutrient agar) by looking at the number of colonies of every series extract. Concentration The colony incubating expressed by growing or do not grow[21].

Data Analysis

Data analysis was conducted in descriptive having acquired an obstruent minimum data concentration and kill minimum concentration extract ethanol leaves Bidara against bacteria *EPEC* 10^6 CFU/ml. The results of the study and compared with parameters and identified as an obstruent kill zone and the ability to see minimum antibacterial activity Bidara leaves extract ethanol as an antibacterial against *Eschericia coli.*

Results

The Results Of The Phytochemistry Extract Ethanol Bidara Leaves (*Ziziphus spina-Christi L).*

The Phytochemistry conducted in this research is the flavonoid tannin and. Tannin, on the 0.1 g extract ethanol leaves bidara added 5 ml aquades then simmer for a few minutes. Filtrat strained and added $FeCl_3$ 1%. Change colors become a deep blue color or black, greenish formed show positive results in the compound tannin extract leaves bidara tested. In the flavonoid, as many as 5 mg extract leaves bidara dissolved in 5ml hot water, simmer for 5 minutes, and strained. Filtrate obtained them added MG powder, 1ml of concentrated sulfuric acid plus 2ml ethanol. Beaten and let separate strong. Formed red, yellow or to the ethanol, orange showed flavonoid compounds

Table 3. Test Phytochemistry Extract Ethanol Bidara Leaves (*Ziziphus spina-Christi L)*

Parameters	Result
Flavonoid (mg/100g)	65,83
Tannins (µg/g)	179,67

Table on shows that an extract ethanol Bidara leaves (*Ziziphus spina-christi L*) containing flavonoid as much as 65,83 mg/100g and taninns179,67 µg/g.

Result Test of Obstruent Zone

Extract ethanol Bidara leaves based on the measurement result after passing the incubation period of 24 hours and formed meaningful obstruent zone that bacteria unable to grow or develop due to the influence of antibacterial substance given, shown in table 4.

Table 4. Measurement Zone Obstruent Extract Ethanol Bidara Leaves (*Ziziphus spina-Christi L*) against *Enteropathogenic coli* 10^6 CFU/ml on Various Concentration

Concentration Extract Ethanol Bidara Eaves	Obstruent Zone	Category
50%	7 mm	Weak
75%	11 mm	Moderate
100%	11,1 mm	Moderate

Extract ethanol Bidara leaves could impede the bacterium Escherichia coli, with a diameter of 11,1 mmat 100%concentration, 75% concentration with a diameter of 11 mm and 25% concentration with a diameter of7 mm. From the research is found in the bacterium*Escherichia coli*, each concentration extract ethanol leaves bidara have differences in Obstruent Zone. At concentrations extract higher having a zone obstruent larger compared by an astronaut with zones around paper disk with low extract lower. This proved that the higher concentration extract ethanol leaves bidara the more high zone obstruent produced.

Image 1. Antibacterial Activity Extract Ethanol Bidara Leaves a Method of Paper Discs to Escherichia *coli*.

The picture above shows the diameter of obstruent sub extract ethanol Bidara leaves (*Ziziphus spina-Christi L*) against *Enteropathogenic coli* 10^6 CFU/mail on various concentrations.

Table 5. The Minimum Bactericidal Concentration The Extract Ethanol Bidara Leaves (*Ziziphus spina Christi L*) against *Enteropathogenic coli* 10^6 CFU/ml on Various Concentration

Extract Ethanol Bidara LeavesConcentration	MBC
100%	-
75%	-
50%	+
25%	+
12,5%	+
6,25%	+

Note: + = growth; - = not growth

Table 5 showed Minimum Bactericidal Concentration had been incubated for 18-24 hours at temperature 37ºC. Based on the results of the antibacterial activity extract ethanol leaves bidara against *Enteropathogenic coli*the results concentration of 75 % and 100 % there is no bacterial growth, on the concentration 6,25 % to 50 % is the growth of bacteria.

Discussion

The extract ethanol leaves bidara have diameter obstruent of different suits distinction concentration. The greater the concentration of the larger the diameter obstruent that the establishment, in order to know the concentration and diameter obstruent is directly proportional each other.In table 4 it is evident that concentration 50 % extract ethanol leaves bidara obtained diameter obstruent zone of 7 mm, 75 % concentration of 11 mm and concentration of 100 % 11,1 mm, it can be said that the higher concentration extract used the more diameter obstruent formed zone. These facts in accordance with statements from Pelczar and Chan[22] that the higher concentration antibiotic substances the more a fast growth microorganisms killed and impeded.

In the levels of kill minimum found that bacteria*Eschericia coli*can not grow in a mediaNA given extract ethanol leaves bidara concentration 75 % dan 100 %. This indicates that extracts ethanol leaves bidara have the ability kill bacteria*Eschericia coli*at concentrations 75% dan 100% but has no power killed at concentrations 50%, 25%, 12,5% dan 6,25%. The results of the study is based on research conducted Edy *et al*[15] who discovered the methanol extract bidara could hinder growth some pathogenic bacteria.

From the observation of MIC and MBC show positive results, this is caused by the existence of a metabolite secondary in extract ethanol leaves bidara so as to give the effect on the growth of bacteria test. Working mechanism substance flavonoid as an antibacterial is form a compound complex with a protein extracellular and dissolved order to be able to destructive the cell membrane bacteria that followed release of intracellular compound[23,24,25]. According to Cushnie and Lamb[26], apart from its role in inhibitory in DNA – RNAsynthesis by intercalation or hydrogen bonds with the accumulation of a nucleic acid, flavonoid would play a role in resisting energy metabolism. Compound with protein through flavonoid locks down into hydrogen bonds resulting in protein structure being broken, instability and the cytoplasm of a cell wall destroyed. Causing integrity destroyed cytoplasm makes macromolecules of ions and the lost its shape and become lysis[27].

Tannin have antibacterial activity associated with its ability to activate adhesion the microbes also activation enzyme, and interfere with the transport protein in bacterial cells[23]. According to Sari[28], the tannin will have targets in polypeptide the wall of the cells so that the formation of the cell walls into less than perfect that causes bacterial cells be lysis because of the pressures of osmotic and physical and the bacteria would die. Microorganisms growing under conditions aerobic need iron for various function, including reduction from a precursor ribonukleotida DNA. This is caused by capacity a fastener solid steel by tannin.Working mechanism tannin as an antibacterial is by means hinder an enzyme reverse reverse transcriptase and DNA topoisomerase and the bacteria from being can be formed[24]. According to Nahak[29], compound mechanism tannin show antibacterial proline bonded with activity in which are rich in protein leakage and damage occurs bacterium cell wall and lead to the death of the bacteria. At low concentration phenol work with destructive membrane cytoplasm and can cause to leak the cells. While in large concentration the substance coagulated with protein cellular, activation is very effective when bacteria in the division, where the phospholipid around cells are on the very thin and phenol penetration and can easily destroy cells[30].

Conclusion

The research is from a chemical compound which is found in extract ethanol left bidara (*Ziziphus spina Christi L*) is flavonoid and tannin have antibacterial activity as against *Enteropathogenic coli*10^6 CFU/ml with zones inhibitory was at concentrations 75 % and 100% and minimum bactericidal at concentration 75%. The higher concentration extract ethanol leaves bidara, the greater the obstruent and the kill against bacteria *Enteropathogenic Coli*

Suggestion

Necessary other experiment by changing concentration treatment to find concentrations of MIC and MBC more effective and antibiotic as comparing and control.

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