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Article

Antibacterial Test of *Ziziphus spina-christi* (L.) Desf. Leaves Extract Againstgram-Positive Bacteria

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Abstract. *Ziziphus spina-christi* (L.) Desf. is a member of the Rhamnaceae family, which has efficacy values as herbal plants used in traditional medicines. The spread of infectiousdiseases in humans has increased in recent years, raising public awareness of the importance of using traditional medicine as the first line of prevention against infectious diseases. This research aimed to determine the antibacterial activity of fresh extract, infusion, and ethanol extract and to determine the greatest inhibition zone of *Z. spina-christi* leaves against Gram-positive bacteria (*B. cereus, B. subtilis, S. aureus*,and *S. epidermidis*). This research used the survey method with a purposive samplingtechnique. The results showed that fresh extract, infusion, and ethanol extract of the *Z. spina-christi* leaves had antibacterial activity against Gram-positive bacteria (*B. cereus, B. subtilis, S. aureus*, and *S. epidermidis*) and the greatest inhibition zone was obtained from the ethanol extract with a strong inhibition category.

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1. Introduction

Indonesia provides a wide range of biodiversity that can be used as raw materials for modern and traditional medicines. Traditional medicine has long been known and usedby Indonesians to cure a variety of diseases. The rising cost of modern medicine on the market is one reason to reconsider the use of traditional medicine. Many different medicinal plants have been used as raw materials in Indonesia, and some of them have even been experimentally tested for phytochemical content, efficiency, and safety of usage [1].

Traditional medicine uses Christ's Thorn Jujube plant (*Ziziphus spina-christi* (L.) Desf.) as one of the herbs. It herb contains phenolic compounds that have a widerange of biological effects, including antioxidants, anti-inflammatory, antibacterial, antifungal, and tumor prevention [2]. Alkaloids, phenols, flavonoids, terpenoids, polyphenols, saponins, tannins, sitosterols, phytosterols, triterpenoids, andglycosides are some of the chemical components that function as treatments in the *Z. spina-christi* plant [3]. In Indonesia, *Z. spina-christi*is also known as "Tumbuhan Bidara Arab". *Z. spina-christi* is used by people all around the world to treat diarrhea, diabetes, fever, and malaria, and also beauty issueslike acne, wrinkles, and dark circles under the eyes [4]. *Z. spina- christi* leaves are used to cure diarrhea, vomiting, laxatives, skin infections, ulcers, liver ailments, rheumatism, asthma, and fever by the people of NTT in Indonesia [5].

Infectious diseases are one of the health-care issues that have been growing inrecent years. Bacteria, viruses, fungus, and protozoa are among the microorganisms that cause infections [6]. Many people choose antibiotics and other anti- infective medicines as their first treatment. Due to the establishment of resistance, oneclass of antibiotics is no longer utilized in therapy. Nevertheless, because antibiotics are expensive, the use of diverse plants in the treatment of infectious diseases may bean option for the Indonesian people [7].

Some Gram-positive bacteria are capable of causing disease in humans. *Staphylococcus aureus* is a normal flora in humans, especially on the skin; it can be found on the nose lining, skin, hair follicles, boils, and wounds [8]. *Bacillus subtilis* is a pathogenic organism that causes ulcers and food poisoning. *Bacillus cereus* is an aerobic Gram-positive rod-shaped bacterium that canform endospores and cause poisoning if a person consumes the bacteria or its spore form, reproduces and produces toxins in the intestine, or consumes food that already contains the toxin. Toxins that cause diarrhea and toxins that cause vomiting (emesis)are both produced by *Bacillus cereus* [9]. Skin infections, itching, and acne can all be caused by *Staphylococcus epidermidis* [10]. The results of Mardhiyani and Afriani's [11], indicated that a 70% ethanolic concentrationextract of *Z. spina-christi* leaves can inhibit the development of *S. aureus* in the presence of an inhibition zone with a diameter of 12.25 mm.

According to preliminary research, infusion of *Z. spina-christi* leave extract can inhibit the growth of *S. aureus* bacteria by forming an inhibition zone. Based on this and given the lack of information on the potential of *Z. spina-christi* leaves extract as an antibacterial against Gram-positive bacteria, an antibacterial test of Christ's Thorn Jujube (*Z. spina-christi*) leaves extract against Gram-positive bacteria was conducted.

2. Experimental Section

2.1. Field Sampling

Z. spina-christi leaves samples are collected in the Bukittinggi and Agam regions. Theleaves selected are not too old, yellow, or fungus-infested. Leaves samples put in plastic bags and moved to the laboratory.

2.2. Sample Extraction

2.2.1. Fresh Extraction

100 g of *Z. spina-christi* leaves were washed and wiped with tissue paper. The *Z. spina-christi* leaves then cut into smaller pieces. According to [12], the smaller pieces of leaves were crushed in a sterile mortar and stamped before being filtered using filter paper.

2.2.2. Infuse Extraction

100 g of *Z. spina-christi* leaves were washed and wiped with tissue paper. The *Z. spina-christi* leaves are then cut into smaller pieces. Then add 100 ml of distilled water as asolvent with temperature up to the boiling point for a set time period (at least 30 minutes) [13].

2.2.3. Ethanol Extraction

Fresh *Z. spina-christi* leaves were wiped with tissue paper. The dried leaves then grounded with a grinder. A 50 mesh sieve was used to filter the mashed sample. 800 gdried *Z. spina-christi* leaves powder were weighed and placed in a maceration container with a 96 % ethanol solution. The maceration container was placed at roomtemperature for 3x24 hours, shaking often to ensure that the active substance was completely extracted and then filtered. The extract was concentrated using vacuum distillation and a rotary evaporator to separate the solvent from the active compound, producing a condensed extract. The condensed extract was weighed [14].

2.2.4. Creating Bacterial Suspension for Testing

The test bacteria were collected from a slanted agar (1x24 hours), 1 ose needle was taken, and the bacteria were suspended in a sterile 0.9 % physiological NaCl solutionuntil a turbidity comparable to Mc Farland's 0.5 was reached [15-16].

2.2.5. Inhibitory Activity Testing using the Disc Diffusion Method

25 mL of NA (Nutrient Agar) medium in Petridish was poured and allowed until solid.Then, disperse the bacterial suspension used a cotton swab onto solid NA medium. Paper discs with a diameter of 5 mm are dipped in each leaves extract of *Z. spina- christi*. Paper discs are placed on the surface of the media. Inhibition zone was produced after 24 hours of incubation at 37°C. A caliper was used to measure the inhibition zone diameters, and then the average was taken [17].

2.3 Phytochemical Test

2.3.1. Alkaloid Test

The extract were heated for 5 minutes in 1.5 mL of HCl 2N and then filtered. Add 5 drops of Dragendorff's reagent. A positive test is indicated by the presence of an orangeprecipitate [18].

2.3.2. Flavonoid Test

The extract were added with a pinch of magnesium (Mg) powder 0,1 g and 1 mL of HCl 2N. Red, yellow, or orange color indicated a positive test of flavonoid [19].

2.3.3. Saponin Test

The extract were added with 1 ml distilled water then shake it for a moment. A positivetest is indicated by the formation of permanent foam within 15 minutes [20].

2.3.4. Steroid/Triterpenoid Test

The extract were added with 5 drops of acetic anhydride and 2 drops of H_2SO_4 2N were added. The presence of triterpenoid compounds were shown by the developmentof orange and purple colored solutions, which probably turn blue and green that indicated a positive reaction containing steroid compounds [21].

2.3.5. Tannin Test

The extract were added 2 drops of 1% ferric chloride ($FeCl_3$), a greenish brown or black-blue color develops, that indicated the presence of tannins [22].

3. Results and Discussion

3.1. Antibacterial Test of *Z. spina-christi* (L.) Desf. Leaves Extract

The results of an inhibition test of *Z. spina-christi* leaves extract against Gram-positivebacteria (*B. cereus, B. subtilis, S. aureus, and S. epidermidis*) using the diffusion method are shown in Table 1 and Figure 2.

Table 1. Inhibition zone average of *Z. spina-christi* leaves extract against *B. cereus, B. subtilis, S. aureus,* and *S. epidermidis*

No	Variety of Extract	Inhibition Zone Average Diameter (mm)			
		B. cereus	*B. subtilis*	S. *aureus*	S. *epidermidis*
1.	Fresh Extract	1.805*	1.585*	1.460*	1.570*
2.	Infusion Extract	2.670*	2.820*	2.100*	2.560*
3.	Ethanol Extract	12.070**	11.180**	11.855**	12.090**
4.	Control (-) distilled sterile water	0	0	0	0
5.	Control (+) chloramphenicol	18.020**	18.210**	19.905**	19.065**

Description: (*) : low category

(**) : strong category

Table 1 shows that the fresh extract, infusion, and ethanol extract of *Z. spina- christi* leaves can inhibit the growth of *B. cereus, B. subtilis, S. aureus,* and *S. epidermidis*. The antibacterial activity of *Z. spina-christi* leaf extract against the test bacteria *B. cereus, B. subtilis, S. aureus,* and *S. epidermidis* can be seen from the clearzone around the paper disc [23]. The ethanol extract of *Z. spina-christi* leaves produced a greater inhibition zone for all test bacteria, compared to the fresh extract and infusion.

Z. spina-christi leaves infusion and fresh extracts produced inhibition zones with that almost the same diameter. Most secondary metabolites foundin plants cannot be dissolved by polar solvent like water. Due to the general prevalenceof nonionic bonds in their organic structures, most polyphenols have a low solubility in water [24]. The diameter of the inhibition zone produced by the fresh extract, infusion, and ethanol was lower than the diameter of the inhibition zone of chloramphenicol as a positive control.

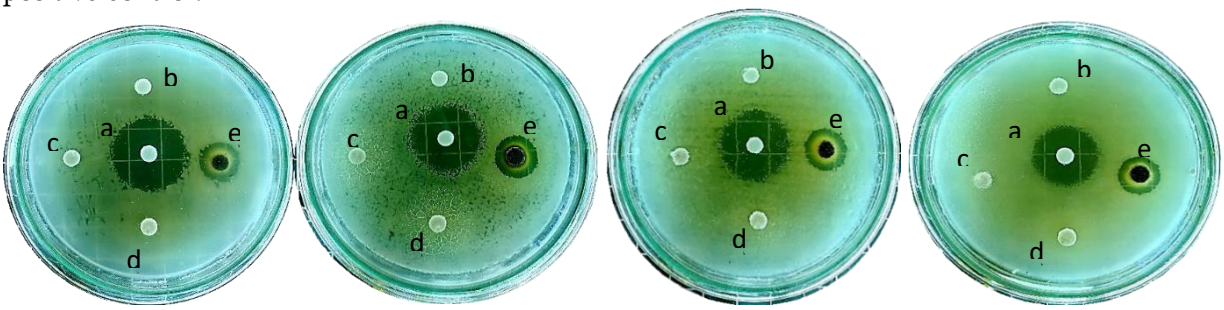

Figure 2. Antibacterial Test of *Z. spina-christi* Leaves Extract Description: (1) *B. cereus,* (2) *B. subtilis,* (3)*, S. aureus* and (4) *S.epidermidis*; a. control (+) chloramphenicol; b. control (-) distilled sterile water; c. freshextract; d. infusion extract e. ethanol extract.

The antibacterial activity comparison of each extract based on its solventproperties, the extraction process was carried out in three ways: fresh extract, infusion,and maceration. Ethanol is known to be more dissolving of secondary metabolitecompounds, has good absorption power, and does not react with extracted components [25], it is probable that it contains saponins, flavonoids, steroids,tannins, and alkaloids [26]. Secondary metabolites that werenot detected in the infusion extract could be affected by these compounds' low polarity [27]. The distilled water used in the extract is a polar solventbinding polar compounds. Meanwhile, fresh extract is used to preserve secondary metabolites in samples that would be damaged if dried by heating [28].

Each extract has a different inhibitory ability. This is because the number of available compounds in each extract varies depending on the polarity of the solventused [2 9] . Different polarity of solvents affect yield becausedifferent solvents dissolve different compounds depending on their polarity andavailability in the extracted material [30]. Ethanol is used as a solventbecause ethanol can attract non-polar, semi-polar, and polar compounds [31]. Ethanol extracts have high bioactivity due to the presence of activecompounds from the group of alkaloids, flavonoids, triterpenoids, tannins, and hydroquinones.

According to [32], the greater the diameter of the inhibitionzone formed, the less bacteria will grow. This indicates that the extract, whichproduces a great diameter of the inhibition zone, has inhibitory activity by damaging the bacterial cell wall and membrane, denaturing or inhibiting protein synthesis and nucleic acid synthesis, and changing membrane permeability. According to [33], an extract is categorized as having good antibacterial activity if the material has an inhibition zone formed that is greater than the diameter of the disc paper; otherwise, the extract is classified as having poor antibacterial activity if the diameterof the inhibition zone formed is smaller than the diameter of the disc paper or no inhibition zone is formed. These are classified as having no antibacterial activity.

The antibacterial activity produced by the ethanolic extract of *Z. spina-christi* leaves was strong category, while the antibacterial activity produced by the infusion and fresh extracts was low category. According to [34], antibacterial power is classified into four types: inhibition area greater than 20 mm (more strong category), inhibition area 10-20 mm (strong category), inhibition area 5-10 mm (medium category), and inhibition area less than 5 mm (low category).

Chloramphenicol inhibited the four test bacteria effectively: *B. cereus, B. subtilis, S. aureus, and S. epidermidis*. Because chloramphenicol inhibits bacterial growth by inhibiting protein synthesis, preventing protein chain elongation, andinhibiting the activity of the peptidyl transferase enzyme on bacterial ribosomes, this is used to treat infections [35]. Sterile aquadest was used as a negative control function to indicate whether or not the media used was contaminated, with thenegative control indicating that there was no inhibition zone in the antibacterial test against the test bacteria, proving that the inhibition formed was not influenced by the solvent but also by the activity of the active compounds contained in the extract [36].

3.2. Phytochemical Test of *Z. spina-christi* (L.) Desf. Leaves Extract

The results of the phytochemical test of the *Z. spina-christi* leaves extract are shownin Table 2 below. Table 2 shows that the ethanol extract contains more active compounds than the infusion and fresh extract, results in a greater inhibition zone produced by the ethanol extract than the infusion and fresh extract. Ethanol is a solvent that dissolvesa broader spectrum of plant secondary metabolites than water [37]. Getting fresh extracts without using a solvent, while the infusion extracts are created by filtering water-soluble bioactive substances as polar solvents, resulting in extracts that are unstable and easily contaminated by microorganisms. As a result, the extractedmaterial should not be kept for more than 24 hours [38].

Table 2. Phytochemical Test of *Z. spina-christi* Leaves Extract

No	Compounds	Variety of Extract		
		Fresh Extract	Infusion Extract	Ethanol Extract
1.	Alkaloid	+	+	+
2.	Flavonoid	+	+	+
3.	Steroid	-	-	+
4.	Triterpenoid	-	-	-
5.	Saponin	+	+	+
6.	Tannin	+	+	+

Description: (+) : It contains a group of compounds

(-) : It does not contain a group of compounds

The content of secondary metabolites that act as antibacterial compounds strongly influences the antibacterial ability of fresh extract, infusion, and ethanol extract of *Z. spina-christi* Lleaves in inhibiting the growth of *B. cereus, B. subtilis, S. aureus*, and *S. epidermidis*.of steroids as antibacterials in inhibiting bacterial growth, according to [39], is related to lipid membranes and sensitivity to steroid components The phytochemical test results revealed that steroid compounds were found only in ethanolextracts, while triterpenoids were not found in fresh extracts, infusions, or ethanol extracts. The action mechanism that cause leakage in bacterial liposomes. Steroids can interact with cell phospholipid membranes, which are permeable to lipophilic compounds, resulting in reduced membrane integrity and changes in cell membrane morphology, due to cell fragile and lysis [40]. Damage to the bacterialcell membrane ruptures the plasma membrane, the cell loses its cytoplasm, substancetransport is disrupted, and metabolism is inhibited, resulting in growth inhibition and even death due to bacterial cell lysis [41].

Ziziphus extracts (leaves, seeds or pulps) were rich on fatty acids (linolenic, palmitic, oleic, linoleic acids), alkaloids, tannin, steroid, sterols (sitosterol, stigmasterol, etc.) and flavonoids (rutin and apigenin). *Z. spina- christi* and *Z. lotus* had been used in folk medicine as a demulcent, depurative, anodyne, emollient, stomachic [42-43]. Secondary metabolites found in *Z. spina-christi* include flavonoids, triterpernoids, steroids, saponins, tannins, and alkaloids [44]. Alkaloids, flavonoids, saponins, tannins, and phenols are a group of compounds indicated in *Z. spina-christi* leaves extract which are thought to have antibacterial activity. Alkaloids have the ability to act as an antibacterial and inhibitory mechanism in bacterial cells by interfering with the peptidoglycan constituent components, allowing the cell wall layer to not fully form and causing cell death [45]. Alkaloids are simple compounds (in the presence of N atoms). They generally contain N or more atoms thatare linked together as part of a cyclic or heterocyclic system. Alkaloids have prominent physiological activities, so they are widely used in medicine. Alkaloids are generally colorless, often optically active, crystalline, and, to a smaller extent, liquid (e.g. nicotinic) at room temperature [46].

Flavonoids have antibacterial properties, with the mechanism of action being the formation of complex compounds with extracellular and dissolved proteins to damage bacterial cell membranes, followed by the release of intracellular compounds [47]. Flavonoids are antimicrobial because they disrupt metabolicfunctions by destroying cell walls and denaturing microbial proteins. Flavones, flavonoids, and flavanols are phenolic compounds that plants produce in response to microbial infection. The ability to form complexes with extracellular proteins and dissolve with microbial cell walls is the mechanism of action as an antibacterial [48].

The presence of saponins in the extract is indicated by the height of the foam. The more saponins are present in the extract, the higher the foam formed after shaking [49]. Saponins have antimicrobial activity by interfering with cell wall surface tension. When the surface tension is disturbed, antimicrobial substances can easily enter the cell and disrupt metabolism, eventually leading to bacterial cell death [50]. Saponins' mechanism of action as antibacterials is that they can cause protein and enzyme leakage from the cell. Saponinshave antibacterial properties due to active ingredients that reduce the surface tension of the bacterial cell wall and damage membrane permeability [51]. Saponins diffuse through the outer membrane and vulnerable cell walls before binding to the cytoplasmic membrane, disrupting and reducing the cell membrane's stability. This causes the cell's cytoplasm to leak out, resulting in cell death. Antimicrobial agents disrupt bactericidal agents that disrupt the cytoplasmic membrane [52].

Triterpenoids as antibacterials, can form a very strong polymeric bond with porin (transmembrane protein) on the outer membrane, causing porin damage. As a result, the cell wall's permeability is reduced, and the mobility of nutrients or essentialcell compounds is disrupted [53]. Tannins as antibacterials, can disrupt bacterial metabolism and permeability [54]. Tannins can also

react with cell membranes, inactivate enzymes, and destroy genetic material functionby forming protein complexes via hydrogen and hydrophobic bonds. Reduced surfacetension of bacterial cell walls can also result in cell leakage, allowing intracellular compounds to exit. Bacterial cell growth is decreased as a result of this. Tannins haveantibacterial activity because they can shrink cell walls or cell membranes, interferingwith cell permeability and causing cells to be unable to carry out activities, possibly cause growth to be inhibited or even death [55].

4. Conclusion

Based on research that has been done, it can be concluded fresh extract, infusion, and ethanol extract of Christ's Thorn Jujube leaves (*Ziziphus spina-christi* (L.) Desf.) can inhibit the growth of *B. cereus, B. subtilis, S. aureus,* and *S. epidermidis.* The ethanol extract of Christ's Thorn Jujube leaves (*Ziziphus spina-christi* (L.)Desf.) produces the greatest inhibition zone and was classified as strong category

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