TABLE OF CONTENT

COVER ..... **ii**
TITLE PAGE ..... **iii**
APPROVAL PAGE ..... Error! Bookmark not defined.
STATEMENT OF THESIS AUTHENTICITY AND COPYRIGHT TRANSFER ..... **v**
APPRECIATION REMARKS ..... **vi**
ABSTRACK ..... **vii**
TABLE OF CONTENT ..... **viii**
FIGURE LIST ..... **x**
TABLE LIST ..... **xi**
APPENDIX LIST ..... **xii**
CHAPTER I **1**
1.1. Background **1**
1.2. Problem Formulation **2**
1.3. Research Objective **2**
1.4. Research Benefit **2**
1.4.1. Scientific field 2
1.4.2. Clinical Field of Dentistry 2
1.4.3. Benefits for the Community 2
CHAPTER II RESEARCH METHOD **3**
2.1. Type of Research **3**
2.2.1. Research Location 3
2.2.2. Research Time 3
2.3. Research Variables and Operational Definitions **3**
2.3.1. Research Variables 3
2.3.2. Operational Definition 3
2.4. Research Techniques and Sample Size **4**
2.4.1. Sampling Techniques 4
2.4.2. Research Sample 5
2.4.3. Preparation of Tools and Materials 5
2.4.4. Extract Processing 6
2.4.5. Flavonoid Test 6
2.4.6. Dilution preparation 6
2.4.7. Antibacterial Test 7
2.4.8. Observation 8
2.4.9. Research Flow 9

CHAPTER III RESEARCH RESULTS AND DISCUSSION 10
3.1. Research result.....10
3.1.1. Determination of Flavonoid Level.....10
3.1.2. Bacterial Inhibitory Activity Test Results 11
3.1.3. Data analysis.....12
3.2. Discussion.....14
CHAPTER IV CONCLUSION AND SUGGESTIONS.....16
4.1. Conclusion 16
4.2. Suggestion 16
REFERENCES.....17
APPENDIX.....18

FIGURE LIST

Figure 1. Quercetin Standard Curve ……..……...10
Figure 2. Inhibitory Activity Test..……..……..11

TABLE LIST

Table 1. Results of Determination of Level.....11
Table 2. Inhibition Zone Diameter Result.....11
Table 3. Statistical Test Result.....12
Table 4. Mann-Whitney Test Results.....13

APPENDIX LIST

Appendix 1. Ethics Recommendation Letter…….....18
Appendix 2. Research Permit Letter ……..……..……19
Appendix 3. Activity Documentation (UMI Pharmacognosy Lab)…..……20
Appendix 4. Results of Determination of Levels ……..……21
Appendix 5. Activity Documentation (Microbiology Lab, Faculty of Medicine)..……..22
Appendix 6. Normality Test…...……..……...23
Appendix 7. Homogeneity Test ……..……23
Appendix 8. Tukey's Test …….....24
Appendix 9. Certificate of Research and UMI Pharmacognosy Lab Fees …...……..25
Appendix 10. Research Ethics Letter ……...…...27
Appendix 11. Thesis Control Card ……..……....29
Appendix 12. Attendance of Supervisors and Examiners.....30

CHAPTER I
INTRODUCTION

1.1. Background

A member of the Rhamance Family, red jujube fruit (Ziziphus Jujuba Mills), ‘kurma merah’ in Indonesia, ‘annab’ in Iran, ‘ber’ in India, ‘pomme sourette’ in France, ‘dazao’ in China is a fruit that has been consumed in various countries as food and herbal medicine because of its effects on human health (Rodriguez Villanueva, J. and Rodriguez Villanueva, 2017), Red jujube fruit originates from China and has been cultivated for more than 4000 years, mostly cultivated in subtropical and tropical regions. Red jujube fruit is traditionally used to treat and improve stomach health, strengthen the spleen, improve blood health, and is known to strengthen the body as a whole (Liu, S.J., Lv, Y.P., Tang, et al, 2021). It has been proven that jujube fruit is rich in flavonoids which can inhibit biofilm formation and improve the pH environment of bacteria (Wang, Z., Yang, Q., Zhang, H, et al, 2023)

Lactobacillus acidophilus bacteria, part of the firmicutes division, are gram-positive bacteria known for their ability to produce lactic acid, the acidic nature of lactic acid lowers the pH level in the oral cavity, causing demineralization on the tooth surface.

Flavonoids are polyphenol compounds that help through various mechanisms of action and provide antibacterial activity, flavonoids can also suppress acid synthesis, and can reduce adhesion in biofilm formation. Flavonoids can be a potential method for inhibiting the potential for dental caries in the mouth. (Shamsudin, N.F., Ahmed, Q.U. et al, 2022)

Since ancient times, humans have been very dependent on the environment to meet their needs. For example, for food, shelter, clothing, and medicine. The abundant and diverse natural wealth is very useful, but has not been fully explored, utilized, or even developed. (Harefa, D., 2020)

Traditional medicine is also called herbal medicine because the ingredients used are natural ingredients. Traditional medicine not only helps physical healing, but also has social, cultural, and spiritual dimensions. Many communities maintain the heritage of traditional medicine as part of their cultural identity. The use of traditional medicine is often associated with local beliefs and certain rituals that are at the heart of local traditions. (Adiyasa, M.R. and Meiyanti, M., 2021).

Efforts towards the development of herbal medicine in Indonesia have been carried out in accordance with the Decree of the Minister of Health of the Republic of Indonesia Number HK.01.07/MENKES/1163/2022 Concerning Phytopharmaca Formulary, it is stated that phytopharmaca are the result of natural materials that have been developed, have gone through product standardization, and have been scientifically proven for their safety and efficacy in order to improve the health of the Indonesian people. The purpose of this study was to determine the inhibitory power and flavonoid test of dried red jujube fruit extract against Lactobacillus acidophilus bacteria, as well as to contribute to the development of herbal medicines in Indonesia.

1.2. Problem Formulation

Based on the background described above, the formulation of the research problem is as follows:

1.2.1. Can dried red jujube fruit extract (*Ziziphus jujuba mill*) inhibit the growth of *Lactobacillus acidophilus* bacteria?

1.2.2. How much the flavonoid content in dried red jujube fruit extract (*Ziziphus jujuba mill*) quantitatively?

1.2.3. How is the inhibitory activity of dried red jujube fruit extract (*Ziziphus jujuba mill*) against *Lactobacillus acidophilus* bacteria?

1.3. Research Objective

1.3.1. To determine whether dried red jujube fruit extract (Ziziphus jujuba mill) can inhibit the growth of Lactobacillus acidophilus bacteria.

1.3.2. To determine the flavonoid content in dried red jujube fruit extract (Ziziphus Jujuba M.) quantitatively.

1.3.3. To determine the inhibitory activity of dried red jujube fruit extract (*Ziziphus jujuba mill*) against *Lactobacillus acidophilus* bacteria.

1.4. Research Benefit

1.4.1. Scientific field

The researcher hopes that this research can increase the researcher's insight into the content and benefits of black cumin. The researcher also hopes that this research can increase the researcher's experience in conducting research and become a description of the activities carried out in experimental laboratory research. Thus, it can increase the interest of researchers to become future researchers and continue their studies to a higher level.

1.4.2. Clinical Field of Dentistry

The results of the study are expected to be developed in the field of dentistry and can be used as reading material and reference for further research. This study is also expected to provide scientific data that can support the use and development of jujube fruit extract as an antibacterial agent.

1.4.3. Benefits for the Community

Can provide information on the inhibitory power of red jujube fruit extract against Lactobacillus acidophilus bacteria and the content of compounds contained in dried red jujube fruit.

CHAPTER II
RESEARCH METHOD

2.1. Type of Research

This type of research is a *true experimental laboratory* research with *the post test only control group design* to assess the inhibitory activity of antibacterial from red jujube fruit extrack (*Ziziphus jujube mill)* on *Lactobacillus acidophilus* bacteria.

2.2.1. Research Location

1. The preparation of dried red jujube fruit extract was held at the Phytochemistry Laboratory, Faculty of Pharmacy, Muslim University of Indonesia.
2. Inhibitory activity test was held at the Microbiology Laboratory, Faculty of Medicine, Hasanuddin University

2.2.2. Research Time

The research was conducted in July 2024 – November 2024

2.3. Research Variables and Operational Definitions

2.3.1. Research Variables

1. Dependent variable: Lactobacillus acidophilus bacteria
2. Independent variable: Red jujube fruit extract
3. Controlled variables: Incubation time, media type and test bacteria type

2.3.2. Operational Definition

1. Dried red jujube fruit (*Ziziphus jujube mill*), fruit obtained in Chinese herbal medicine stores that have been dried to a water content of 0%.,
2. Lactobacillus acidophilus bacteria, are gram-positive bacteria with bacteria that are available and obtained from the Microbiology Laboratory of the Faculty of Medicine, Hasanuddin University.
3. Flavonoids are phenolic compounds obtained from phytochemical screening tests quantitavely.
4. Medium, Nutrient Agar (NA) made from preparations provided by this laboratory is used as a medium to see the bacterial inhibitory activity.
5. The antibacterial inhibitory activity of red jujube fruit is the ability of red jujube fruit extract to inhibit bacterial growth. Inhibitory activity is presented as the diameter of the inhibition zone in the form of a clear zone around the disc.
6. The inhibition zone is the clear area in the bacterial medium culture after incubation, the diameters were measured by using a calipper (mm), carried out 4 times with different diameters and then averaged. Resistant if the diameter of the bacterial inhibition zone is ≤ 13 mm, Intermediate if the diameter of the bacterial inhibition zone is 13-16 mm and is categorized as sensitive if the diameter of the bacterial inhibition zone is ≥ 17 mm.
7. The sample concentration is the concentration of red jujube fruit extract (*Ziziphus jujuba mill*) which is made by crushing the samples

using a blender and adding 96% ethanol.

8. Positive control using Chlorhexidine 0.2%, is a sample group that uses chlorhexidine as a comparison material to inhibit the growth of *Lactobacillus acidophilus* bacteria. The results of chlorhexidine will provide a positive clear zone
9. Negative control using DMSO 10% (Dymethyl Sulfoxide), is a sample group that uses DMSO 10% on the disc to inhibit the growth of *Lactobacillus acidophilus* bacteria. The results of DMSO will provide a negative clear zone

2.4. Research Techniques and Sample Size

2.4.1. Sampling Techniques

This study used *purposive sampling* technique for dried red jujube fruit and *simple random* for grouping test of bacteria. The sample size of the study was calculated using the Federer's formula to determine the number of repetitions in experimental research in order to obtain valid data. The Federer formula as follows:

Federer's Formula: $(n-1)(t-1) \geq 15$

This study used 5 concentrations of 15%, 25%, 50%, 75%, 100% and an additional 1 from the positive control and negative control

$(n-1)(t-1) \geq 15$

$(n-1)(7-1) \geq 15$

$(n-1)(6) \geq 15$

$(6n-6) \geq 15$

$(6n) \geq 21$ $n \geq 3.5$

Round up to 4

(so there are 4 repetitions)

Description:

n = sample size for each group.

p = number of groups, number of interventions or observations.

Group Division

Group A: Red jujube extract with a concentration of 100%

Group B: Red jujube extract with a concentration of 75%

Group C: Red jujube fruit extract with a concentration of 50%

Group D: Red jujube fruit extract with a concentration of 25%

Group E: Red jujube fruit extract with a concentration of 15%

Group F: Red jujube fruit extract with positive control using Chlorhexidine 0.2%,

Group G: Red jujube fruit extract with negative control using DMSO 10% (Dymethyl Sulfoxide)

The samples in this study were *Lactobacillus acidophilus* bacteria, red jujube fruit extract (*Ziziphus jujuba mill)* in 5 dilutions, each 15%, 25%, 50%, 75%, 100%. In each treatment group, 4 replications were carried out.

2.4.2. Research Sample

1. Inclusion Criteria
   a. Red jujube fruit that has been dried to 0% water content
   b. *Lactobacillus Acidophilus* bacteria obtained from the laboratory
2. Exclusion Criteria
   a. Dried red jujube fruit still contains water in the fruit

2.4.3. Preparation of Tools and Materials

1. Tools
   a. Maceration vessel
   b. Scales
   c. Test tube
   d. Glass funnel
   e. Tissue spatula
   f. Autoclave
   g. Stirring rod
   h. Beaker
   i. Glass
   j. Blue tip
   k. Petri dish
   l. Hot plate
   m. Incubator
   n. Newspaper
   o. Oven
   p. Blender
   q. Disc paper
   r. Test tube
   s. Erlenmeyer
   t. Cotton
   u. Refrigerator
   v. Vernier caliper
   w. Tweezers
2. Materials
   a. Lactobacillus acidophilus bacteria
   b. Jujube fruit
   c. 10% DMSO (Dymethyl Sulfoxide)
   d. Handscoon
   e. Mask
   f. Label paper
   g. Aluminum foil
   h. 70% Ethanol

i. Chorhexidine 0.2%
j. BaCl2 1% (Barium Chloride)
k. Medium nutrient agar (NA)

2.4.4. Extract Processing

The method used in this extraction process is the maceration method. Maceration is a common method used in making extractions. This method is carried out by soaking the sample in a solvent that can attract substances in the sample with different time variations, in this study the sample used was dried red jujube fruit (Ziziphus jujuba M.).

Jujube fruit that has been dried using an oven at a temperature of 50 ° C, then ground using a grinder using a 40 mesh sieve to get the same size. Jujube fruit samples (Ziziphus jujube M.) as much as 1200 grams and macerated in 96% ethanol organic solvent. After 24 hours the sample was filtered to separate the ethanol liquid and the pulp. This process is carried out with 3 separate repetitions using the same amount of material and solvent volume. The liquid extract is then put into an Erlenmeyer flask and evaporated using a vacuum rotary evaporator for 50 hours to obtain a thick extract. After that, the extract yield is calculated using the equation:

$$\boldsymbol{Total\ Yield}(\%) = \frac{\boldsymbol{Jujube\ Fruit\ Extract\ Mass}}{\boldsymbol{Jujube\ Fruit\ Mass}} \boldsymbol{X100}$$

Jujube fruit extract sample (Ziziphus Jujuba M.) was weighed as much as 0.5 grams, then diluted with 96% ethanol as much as 50 mL in a beaker glass. Ethanol was added little by little and stirred until homogeneous.

2.4.5. Flavonoid Test

The process of quantitative flavonoid content testing in dried red jujube fruit extract using a UV-Vis spectrophotometer with a colorimetric method. Determination of total flavonoid levels, weighing 10.0 mg using quercetin standard then dissolved with ethanol in a glass beaker, put into a 10.0 ml measuring flask and dissolved with ethanol p.a to the limit mark, until the concentration is obtained. Weigh 10.0 mg of quercetin then dissolve it with ethanol p.a up to 10.0 mL and obtain the parent standard solution (1000 ppm), then make a concentration series of 4 ppm, 5 ppm, 6 ppm, 7 ppm, 8 ppm, 9 ppm, 10 ppm, then the parent standard solution is pipetted 0.04 ml, 0.05 ml, 0.06 ml, 0.07 ml, 0.08 ml, 0.09 ml, 0.10 ml, put it into a measuring flask then add 3 mL of ethanol p.a, 0.2 AlCl3 10%, 0.2 mL of potassium acetate p.a and add distilled water up to 10 mL after that incubated for 30 minutes, before the measurement is carried out first optimize the wavelength in the wavelength range of 400-500 nm, after that the absorbance is measured by Spectrophotometry UV-Vis at a wavelength of 428 nm. (Hasanah, N. and Novian, D.R., 2020.)

2.4.6. Dilution preparation

The dilution aims to produce several concentrations of jujube fruit extract (Ziziphus Jujuba M.) which will be used for the minimum inhibitory level of jujube fruit extract (Ziziphus Jujuba M.) which can inhibit the growth of Lactobacillus

acidophilus bacteria. In this study, dilutions of 15%, 25%, 50%, 75%, 100% were made.

2.4.7. Antibacterial Test

a. Preparation of Bacterial Suspensions and Cultures

Bacterial suspensions are made according to the McFarland standard. The preparation of the McFarland standard is done by preparing two tubes for the 0.5% McFarland standard and the bacterial suspension. 0.1 ml of 1% BaCl2 is pipetted and then put into the first tube. 9.9 ml of 1% H2SO4 is pipetted and put into a tube containing 0.1 ml of BaCl2, mixed until homogeneous. 5 ml of the mixture is taken, then put into the second tube and 5 ml of distilled water is added and mixed until homogeneous. The 0.5% McFarland standard is ready to be used as a comparison for the bacterial suspension (the turbidity formed is proportional to the cell density of 1.5 million CFU/ml). The preparation of the bacterial suspension to be tested is done by culturing bacteria. 1-2 oses of bacteria are taken, then suspended in 15 mL of MHB or 0.9% saline solution, and incubated for 24 hours. Turbidity is adjusted to the 0.5 McFarland standard or equivalent to the number of bacteria 108 (CFU)/mL. (Sidharta et al., 2021), (Rini, Supriatno and Rahmatan, 2017)

b. Preparation of Agar Medium

Preparation of agar medium is done by dissolving 3.8 grams of Mueller Hinton Agar in 100 ml of distilled water, then heated to boiling with stirring until the powder is completely dissolved, then MHA is sterilized in an autoclave at 121°C for 15 minutes, and poured into a petri dish to a depth of 4 mm. After solidifying, the agar is dried at 30°C - 37°C in an incubator.

c. Disc Diffusion Steps

1. Using aseptic technique, take each bacteria from broth culture 108 CFU/ml and swab on agar medium using sterile swab.
2. Let dry for 5 minutes
3. Prepare and cut paper discs into 6 mm size
4. Soak each disc with 10 µl of jujube fruit extract with concentrations of 15%, 25%, 50%, 75%, 100% mg/mL, positive control, and negative control separately and let dry.
5. Use discs with 10 µl of 0.2% chorhexidin as positive control and DMSO% (Dymethyl Sulfoxide) discs as negative control
6. The dried discs are then placed and pressed gently on agar with prepared Lactobacillus acidophilus bacteria
7. Turn all plates over and incubate at 37°C for 24 hours
8. Measure the inhibition zone and the results of the measurements are then analyzed. The inhibition zone of the antimicrobial compound of jujube fruit extract was measured separately based on the diameter (mm). Inhibition is a clear area around the disc. Diameter measurement (mm) was carried out using a caliper

(accuracy 0.05 mm) on seven inhibition zone diameters and then averaged.

9. The criteria for assessing inhibition power were measured from the average inhibition zone diameter according to CLSI (Clinical Laboratory Standards Institute), namely sensitive if the diameter of the bacterial inhibition zone is ≥ 17 mm, intermediate category if the diameter of the bacterial inhibition zone is 13-16 mm, and resistant category if the diameter of the bacterial inhibition zone is ≤ 13 mm.

2.4.8. Observation

Observations were made twice by repeating the experiment, then the data results obtained were analyzed using ANOVA. The normality test was carried out using the Shapiro-Wilk test which is useful for determining whether the data is normally distributed or not. The homogeneity test was carried out using the Levene's test. Statistical tests were carried out using the one-way ANOVA test to see the differences in the inhibitory power of each sample group. After the One-Way ANOVA test was carried out, Post Hoc Multiple Comparison Equal Variance by Tukey was carried out to determine significant differences in each group.

2.4.9. Research Flow