Antibacterial Activity of Buffalo Curd against Propionibacterium acnes Bacteria and its Antioxidant Content as a Natural Facial Treatment

Delli Lefiana[1], Dihan Kurnia[2], Devi Kumala Sari[2]

[1]Department of Veterinary Paramedics, Agricultural

State Polytechnic of Payakumbuh West Sumatra 26271, Indonesia

[2]Department of livestock production technology, Agricultural

State Polytechnic of Payakumbuh West Sumatra 26271, Indonesia

*e-mail: dellilefiana@yahoo.co.id

Abstract. Dadih is a traditional West Sumatra dish derived from buffalo milk that is naturally fermented in bamboo vats at room temperature for 24-48 hours. The aim of this study was to determine the antibacterial activity of buffalo salt against the acne-causing bacteria Propionibacterium acnes, and the antioxidant content in buffalo salt can inhibit the formation of melanin pigment in the skin. This study used 12 curd samples including 4 treatment groups and 3 replicates each, in which the first treatment (D0) was 100% buffalo curd, the second treatment (D1) with 75% milk concentration. Eastern buffaloes supplemented with aquatic products, the third treatment. Treatment (D2) with 50% concentration of buffalo curd supplemented with aquadest and treatment 4 (D3) with 25% concentration of curd supplemented with aquadest. Each group was tested for bacterial inhibition by diffusion method and tested for antioxidant content by DPPH method. The obtained data were analyzed by ANOVA and continued with the DMRT test. The results of statistical tests on antibacterial activity of buffalo dung mushrooms showed that there was no significant difference (P 0.05) between groups D0, D1 and D2, but significantly different from D3. While the statistical results on antioxidants in buffalo curd showed no significant difference (P 0.05) compared with the four treatment groups. The highest antibacterial activity was shown in the D1 group (12.11 ± 2.94 mm) and the highest antioxidant content was in the D2 group (32.76 ± 2.67 ppm). Based on this, it is concluded that buffalo curd can be used as an alternative to natural facial skin care.

1. Introduction

Raw milk consumption in Indonesia is increasing every year as milk is one of the livestock products that can supply protein, energy, vitamins and minerals. According to data from the Central Statistical Agency in 2021, the consumption of fresh milk by the Indonesian population in 2020 is 16.27 kg/capita/year, an increase of 0.25% compared to 2019. One of the cattle capable of producing milk is buffalo. Judging from its production in West Sumatra, there was an increase in buffalo milk production in 2014 as much as 1.188.438 liters, in 2015 as many as 1.219.395 liters and in 2016 as many as 1.231.588 liters [1]

Milk is widely consumed not only in fresh form but also in processed form, such as curd. The production of curd involves several types of microorganisms such as Lactic Acid Bacteria (LAB), molds and yeasts [2]. Curd is a traditional dish of West Sumatra derived from buffalo milk that is naturally fermented in bamboo vats at room temperature for 24-48 hours [3]. The bacteria that play a role in this process are dominated by Lactobacillus sp.[4].

Buffalo curd processing business can be developed as an effort to maintain food security for animals and a source of income as well as animal protein factories can be developed widely, especially in West Sumatra, Currently, curd is still rarely used as a non-food item as yogurt and kefir have been widely used as facial treatments, although when viewed from the content, curd has potential similar function as an antioxidant and antibacterial agent[5]. Ingredients with antioxidant content can be used

to reduce oxidative stress that causes acne development and fight free radicals, thus helping to prevent the formation of melanin pigment in the skin [6]. The lactic acid bacteria Lactobacillus plantarum fermented in whey can inhibit the growth of the acne-causing bacteria Propionibacterium acnes [7]. Based on its content, buffalo curd has good potential as an alternative skin care product.

Skin care products are now very important to the public, especially women, as treatment is an attempt to improve, maintain and facial skin health [8]. Beautiful skin is synonymous with bright and acne-free skin, which can be achieved with regular facials using natural ingredients that can minimize side effects. Based on the above, a study is needed to use buffalo curd as a natural ingredient that can lighten facial skin and eliminate acne based on its ability to inhibit the growth of P.acnes and antioxidant. Through this study, it is hoped to increase the use value of buffalo curd and show the potential of non-food bufalo curd as a useful natural skin care ingredient to increase the economic value of buffalo curd as a traditional fermented milk in West Sumatra.

2. Methods

2.1 Research Time and Place

This research was conducted from June to September 2022. The curd samples came from the Dadiah cattle farmer group in Nagari Batu Payung Halaban. Sample testing was carried out at the Quality Testing and Analysis Laboratory of the Payakumbuh State Agricultural Polytechnic, Payakumbuh State Agricultural Polytechnic Laboratory of Animal Diseases and Health and FATETA Central Instrumentation Laboratory, Andalas University.

2.2 Research Implementation

Curd is made with each dilution treatment using aquadest. The treatment group D0 = 100% curd with the ratio of curd: aquadest (1:0), treatment group D1 = 75% curd with the ratio of curd: aquadest (3:1), treatment group D2 = 50% curd with the ratio of curd: aquadest (1:1) and the treatment group D3 = 25% with the ratio of curd: aquadest (1:3). Each treatment group was tested for antioxidants, the inhibitory power of Propionibacterium acnes and PH tests.

2.2.1 Measurement of Antioxidant Activity

Measurement of antioxidant activity using the free radical inhibition method 1,1-diphenyl-2-picrylhydrazyl (DPPH) in ethanol with modified DPPH concentration and the ratio of the sample to DPPH, which is 1:1 with a DPPH concentration of 0.1 mM. Tests were carried out in a 96-well microplate [9].

2.2.2 Propionibacterium acnes Bacterial Growth Inhibition Testing

1. **Test Sample Preparation.** Each treatment was taken 5 ml and then put into a petri dish as a test sample for the antimicrobial activity inhibition contained in the curd.
2. **Preparation of Nutrient Agar (NA) Medium.** NA medium (Nutrient Agar) was made by weighing 2 grams of Nutrient agar then dissolved with distilled water up to 100 ml in an Erlenmeyer flask and heated until dissolved then sterilized in an autoclave at 1210C, 1 atm pressure for 15 minutes. For bacterial inoculation as much as 3 mL of the heated medium [10].
3. **Bacterial Setup.** Test The test bacteria used were Propionibacterium acnes bacteria.
4. **Rejuvenation of Pure Cultures of Propionibacterium acnes**. Pure cultures of Propionibacterium acnes were scratched and inoculated aseptically by streaking on agar slanted from NA medium, then anaerobically incubated at 37°C for 24 hours.
5. **Preparation of Pure Suspension of Propionibacterium acnes**. The rejuvenation test bacteria were suspended with 3 ml of physiological solution (NaCl 0.9%) into the vial. Preparation of Test Bacteria Pure suspension of P. acne was scratched and put into a vial containing NA medium, shaken or homogenized, then poured into a petri dish containing NA medium.
6. **Inhibitory Antimicrobial activity testing.** Curd as an anti-acne against the growth of Propionibacterium acnes bacteria was carried out by agar diffusion method using disc paper. Each curd treatment was dipped in disc paper, allowed to stand for approximately 3 minutes, then removed and allowed to dry slightly for approximately 3 minutes, then placed aseptically

on the solid surface of the test medium [10]. Antimicrobial activity observations were carried out after an incubation period of 24 hours and 48 hours. The diameter of the resistance is measured by KHM [11].

2.2.3 Measurement of Degree of Acidity (pH)

The pH meter was turned on, then the pH meter electrode was put in buffers of 4.31 and 6.86 then a sample of 5 grams was weighed and given 100 ml of distilled water, after that it was mashed. Then the electrode is immersed in the sample solution and left for a while until a stable reading is obtained. The value obtained from the reading on the pH meter until the digital number shows a fixed pH value.[12]

2.3 Research design

This study used a completely randomized design (CRD) pattern with 4 treatments and 3 replications. The treatments included: D0 = 100% curd, D1 = 75% curd, D2 = 50% curd and D3 = 25% curd. The equation model used is as follows [13]:

$$\mathbf{Yij = \mu + \alpha i + \varepsilon ij}$$

Information :

Yij : the observed value of the -i curd dilution treatment, in the -j replication (1, 2, 3)
μ : the average value of the quality of the curd treatment
αi : the effect of the -i curd dilution treatment on the quality of each treatment
εij : the effect of error from the treatment of dilution of curd i in the -j replication on the quality each treatment (1, 2, 3)

The data was processed by analysis of variance (ANOVA) using the Statistical Analysis System's Procedures software (SAS Institute Inc. Cary, NC, USA, 2002). If the treatment has a significant effect on the observed variables, then the multiple comparison test is continued using Duncan's test (Steel dan Torrie 1995). The data from the sensory test results of the curd mask were analyzed by the Kruskal-Wallis . nonparametric test [14].

3. Results and Discussion

3.1 Measurement of Antioxidant Activity

The average antioxidant content of buffalo curd can be seen in table 1.

Table 1. Buffalo Curd Antioxidant Activity

Parameters/ Treatment	D0	D1	D2	D3
Antioxidant	24,21 ± 6,58	29,93 ± 3,51	32,76 ± 2,67	31,89 ± 0,96

Table 1 shows the results of the antioxidant activity tests that have been carried out, there are no significant differences between treatments. The curd in this study ranged from 24.21 to 32.76 ppm. The highest antioxidant content was found in treatment D2 which used a ratio of curd and aquadest 1:1 with a value of 32.76 ± 2.67 ppm and the lowest was found in treatment D0 with a value of 24.21 ± 6.58 ppm.

The antioxidant results in this study were slightly lower than research [5] which stated that curd has antioxidants ranging from 44.86 ppm for fat-free curd. This difference is thought to be due to differences in the quality of the milk used. According to [15] the protein content in curd ranges from 5.7-6.6%, fat content 7.9-8.2%, water content 69-73%. Dadih has a high protein content with essential amino acids, calcium, vitamins B and K which is quite complete which is formed during the fermentation process [16].

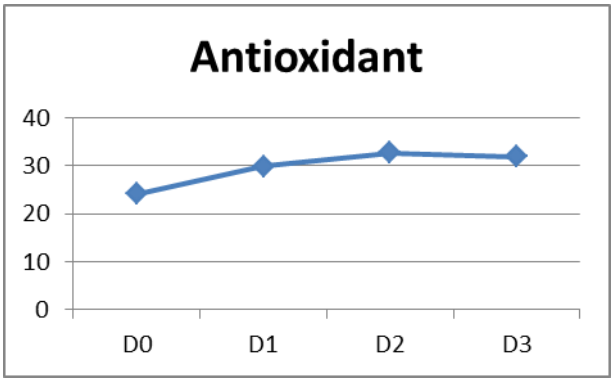

Figure 1. Graph of antioxidant levels in Buffalo curd

Seen from the graph above, it can be seen that there is an increase in antioxidant levels along with an increase in the portion of aquadest as the solvent. This is because the active compounds that act as antioxidants can be free from the fat contained in the curd. According to [17] curd has a thicker texture than cow's milk yogurt because curd uses buffalo milk which has a high fat content. This high fat content must be removed, because the presence of fat in the curd can reduce the ability of the curd to ward off free radicals [18].

The component of curd which is thought to act as an antioxidant is a peptide. Peptides are produced from the breakdown of buffalo milk proteins by microbial enzymes when buffalo milk is being fermented. This protease enzyme breaks down the peptide bonds that make up proteins to produce short peptides that have biological activity, known as bioactive peptides. According to [19] curd contains 16 amino acids 13 essential amino acids and three non-essential amino acids) which are compounds formed as a result of protein breakdown during fermentation.

3.2 Inhibitory test of buffalo curd against Propionibacterium acnes

The average diameter of the inhibition zone of buffalo curd against Propionibacterium acnes can be seen in table 2.

Table 2. The results of the measurement of the diameter of the inhibition zone (mm) of buffalo curd against the bacteria Propionibacterium acnes

Parameters	Resistance (mm)	Antibacterial Strength Criteria
D0	9,14 ± 0,35 ab	Weak Inhibition
D1	12,11 ± 2,94 a	Weak Inhibition
D2	9,99 ± 0,38 a	Weak Inhibition
D3	7,83 ± 0,49 b	Weak Inhibition

Keterangan : weak: 10-15 mm, currently 16-20 mm, strong: >20 mm [20]

Based on table 2, buffalo curd in the treatment group D0 = 100% curd with the ratio of curd: aquadest (1:0); D1 = 75% curd with the ratio of curd: aquadest (3:1); D2 = 50% curd with the ratio of curd: aquadest (1:1) and D3 = 25%, i.e. with the ratio of curd: aquadest (1:3) has the ability to inhibit so that it can be said that buffalo curd is able to inhibit the growth of Propionibacterium acnes. Based on the results of statistical tests on the antibacterial activity of buffalo curd, there was no significant difference (P 0.05) between the treatment groups D0, D1, D2 and D3. However, the highest inhibition zone in the D1 treatment group was 12.11 ± 2.94 mm. The inhibitory ability of buffalo curd against Propionibacterium acnes has a weak inhibition zone ability because it is in the 10-15 mm range, where the medium category is in the 16-20 mm range and strong > 20 mm [20].

Until now there has been no research on the ability of buffalo curd to inhibit the growth of Propionibacterium acnes and other acne-causing bacteria, but buffalo curd is able to inhibit the growth of other pathogenic bacteria such as Staphylococcus aureus which is a gram-positive bacterium that

causes skin diseases such as boils [5]. In the research of Rahman [7] Lactic acid bacteria from various fermented milk products such as yogurt were proven to be able to inhibit the growth of Propionibacterium acnes bacteria by lactic acid bacteria from whey yogurt by 4.35 mm. Another study by Rantono [21] tested the inhibition of Propionibacterium acnes bacteria using goat's milk kefir which has the ability to inhibit the growth of Propionibacterium acnes bacteria in the weak inhibitory category. This shows that lactic acid bacteria derived from fermented milk have the ability to inhibit the growth of acne-causing bacteria even though their activity is lower than antibiotics.

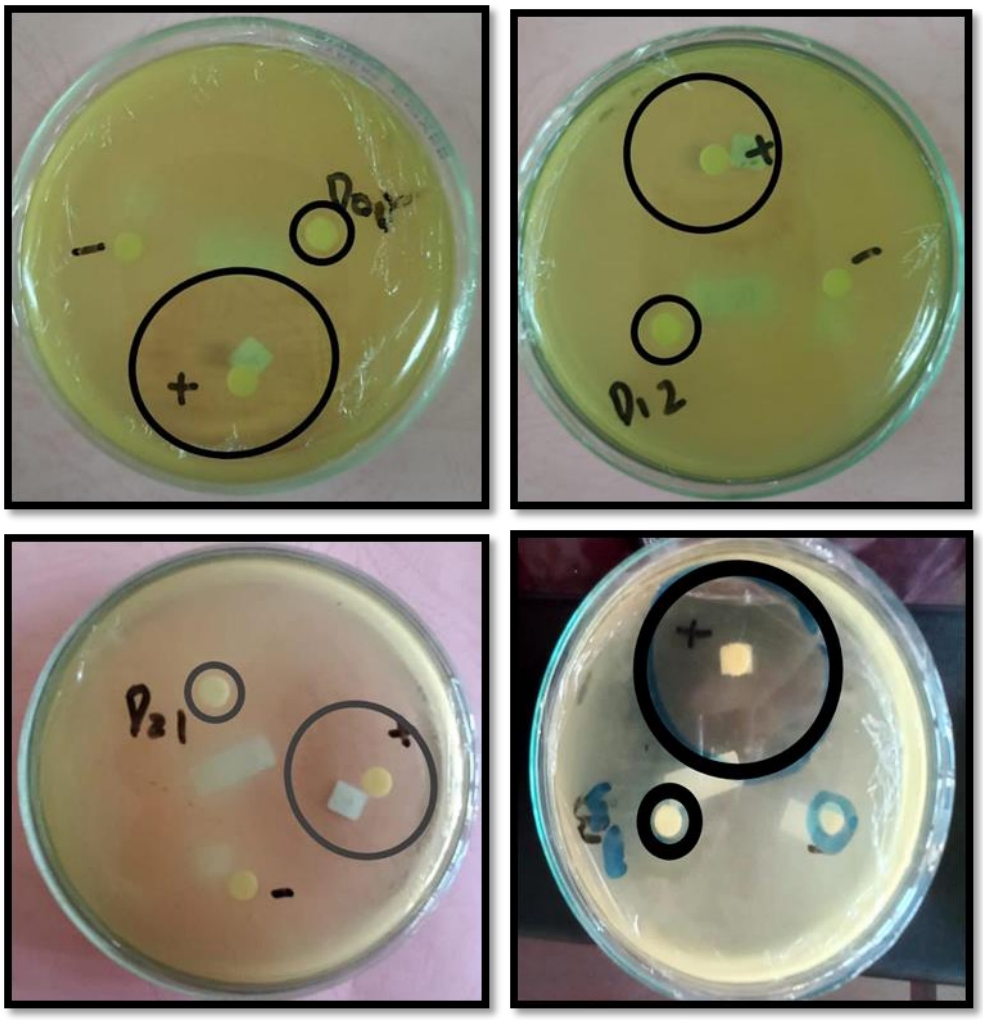

Figure 2. Zone of inhibition of growth of Propionibacterium acnes bacteria D0 (a) D1(b) D2 (c) D3 (d) on agar media

Figure 1 shows the inhibition zones in Curd buffalo curd D0, D1, D2 and D3 where a clear zone is seen on agar media which is an inhibition zone for Propionibacterium acnes growth, the positive control (+) which is an anti-acne gel containing antibiotics has a higher inhibition zone. broad and included in the category of strong inhibition compared to buffalo curd. In the negative control (-) there was no inhibition zone because the negative control was not given any treatment using only blank paper discs. This weak inhibition of buffalo curd may be due to Propionibacterium acnes belongs to the group of Gram-positive bacteria, where Gram-positive bacteria have cell walls and thick peptidoglycan layers. According to Prescott [22], the inhibition of LAB against pathogenic bacteria is influenced by differences in the cell wall and the peptidoglycan layer that composes the cell wall.

3.3 Buffalo curd pH value

The average pH value of buffalo curd can be see

Table 3. Buffalo curd pH value

Parameters/ Treatment	D0	D1	D2	D3
pH	5,42 ± 0,10 [a]	5,15 ± 0,05 [b]	4,95 ± 0,04 [c]	4,80 ± 0,03 [d]

The pH test aims to determine the pH value of buffalo curd which can be used for facial skin care so as to ensure the preparation does not cause irritation to the skin. Table 3 shows that the pH values between the treatment groups D0, D1, D2 and D3 were significantly different, but the four treatment groups could be used as natural ingredients for facial treatments. This is supported by research by Aulton [23] which states that a good topical preparation for facial skin is in the pH range of 4.5 -6.5. This shows that buffalo curd can be used as a natural ingredient for facial skin care.

Changes in the pH value can indicate a reaction or damage to the constituent components in the preparation so that it can decrease or increase the pH value of the preparation. The more alkaline or acidic the material hits the skin, the harder it is for the skin to neutralize it and the skin will become dry, cracked, sensitive and prone to infection. While a pH that is too alkaline can cause scaly skin [24].

4. Conclusions

Based on the results of research and discussion, it can be concluded that buffalo curd can be used as a natural ingredient for facial skin care. The D2 treatment group had the highest antioxidant content while the strongest inhibiting the growth of Propionibacterium acnes was the D1 treatment group, this indicates that the dilution of buffalo curd using distilled water with concentrations of 3:1 and 1:1 is better for skin care products. face. Therefore, it is recommended to conduct further research on the application of buffalo curd as an ingredient for making facial skin care products.

5. References

[1] Badan Pusat Statistik Provinsi S B 2016 Provinsi Sumatera Barat Dalam Angka

[2] Yurliasni And Zakaria Y 2013 Kajian Penambahan Khamir 15 54–9

[3] R S And Marwoto B 2012 Isolasi, Identifkasi, Dan Karakterisasi Bakteri Asam Laktat Dari Dadih Susu Kerbau J. Sains Dan Teknol. Indones. 14(3):228

[4] Jayanti Y D 2014 Uji Organoleptik Dan Kadar Protein Dadih Susu Sapi Dengan Penambahan Ekstrak Jeruk Nipis Dan Lama Pemeraman (Program Studi Pendidikan Biologi Fakultas Keguruan Dan Ilmu Pendidikan Universitas Muhammadiyah Surakarta)

[5] Chalid, Sri Y H F 2013 Potensi Dadih Susu Kerbau Fermentasi Sebagai Antioksidan Dan Antibakteri (Universitas Lampung.: Prosiding Semirata Fmipa) Pp 369–75

[6] Batubara I And Mitsunaga T 2013 Use Of Indonesian Medicinal Plants Products Against Acne Rev. Agric. Sci. 1 11–30

[7] Rahman A, Taufik E, Purwantiningasih S And Purwanto B P 2014 Kajian Potensi Whey Yogurt Sebagai Bahan Alami Pencegah Jerawat (Study Of Fermented Whey As Natural Treatment For Acne Prevention) J. Ilmu Produksi Dan Teknol. Has. Peternak. 02 238–42

[8] Darwati 2013 Cantik Dengan Herbal, Tribun Media: Jakarta

[9] Shori A B And Baba A S 2013 Antioxidant Activity And Inhibition Of Key Enzymes Linked To Type-2 Diabetes And Hypertension By Azadirachta Indica-Yogurt J. Saudi Chem. Soc. 17 295–301

[10] Fifendy M 2017 Mikrobiologi (Depok: Kencana.)

[11] Waluyo L 2019 Mikrobiologi Umum (Malang: Umm Press.)

[12] Oac 1995 Official Methods Of Analysis Association Of Official Analytical Chemist

[13] Steel, G.D. T H Dan T 1995 Prinsip Dan Prosedur Statistik. Suatu Pendekatan Biometric". (P.T Gramedia Pustaka Utama Jakarta.)

[14] Mattjik,A.A. Sumertajaya I M 2006 Perancangan Percobaan Dengan Aplikasi Sas Dan Minitab (Bogor: Ipb Press.)

[15] Alzahra H, Melia S And Susmiati 2021 Nutrient Analysis Of Dadih From Lintau Regency, West Sumatra, Indonesia Iop Conf. Ser. Earth Environ. Sci. 888

[16] Venema K And Surono I S 2019 Microbiota Composition Of Dadih – A Traditional Fermented Buffalo Milk Of West Sumatra Lett. Appl. Microbiol. 68 234–40

[17] Arnold M, Rajagukguk Y V And Gramza-Michałowska A 2021 Characterization Of Dadih: Traditional Fermented Buffalo Milk Of Minangkabau Beverages 7

[18] Maharani A I, Riskierdi F, Febriani I, Kurnia K A, Rahman N A, Ilahi N F And Farma S A 2021 Peran Antioksidan Alami Berbahan Dasar Pangan Lokal Dalam Mencegah Efek Radikal Bebas Pros. Semin. Nas. Bio 1 390–9

[19] Usmiati S And Risfaheri 2013 Pengembangan Dadih Sebagai Pangan Fungsional Probiotik Asli Sumatera Barat J. Litbang Pert. 32 20–9

[20] Rohan H ., Hartini N, Sriwahyuni E, Rokhmad K And Ambarini T . 2017 Mikrobiologi Dasar (Yogyakarta: Deepublish)

[21] Rantono S And Jusnita N 2020 Uji Aktivitas Antibakteri Kefir Susu Kambing Terhadap Bakteri Propionibacterium Acnes Test Of Goat Milk Kefir Antibacterial On Bacteri Propionibacterium Abstrak Masyarakat Berkumpul , Sebanding Juga Dengan Banyaknya Jumlah Kendaraan , Sehingga Tingkat Di 5 20–30

[22] Prescott, L. M. And Horley, J. P., And Klein D A 2002 Microbiology 5th Ed. (Boston: Mc Graw-Hill.)

[23] Aulton And Michael E 2005 Pharmaceutics The Science Of Dosage From Design. Elsevier, United Kingdom

[24] Tranggono, Iswari R, Latifah And Fatmah 2007 Buku Pegangan Ilmu Pengetahuan Kosmetik (Pt. Gramedia Pustaka Utama.Jakarta)