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Antibacterial Activity Test Against *Cutibacterium acne* and Contaminant Testing of Spirulina (*Arthrospira platensis*) Anti-Acne Serum

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ABSTRACT

Acne is a skin problem caused by the *Cutibacterium acne* bacteria, which has increasing bacterial resistance. Therefore, this study aimed to evaluate the antibacterial activity of spirulina extract serum against *C. acne*, as well as to test for microbial contamination, heavy metals, and microplastics in the serum. Antibacterial activity tests were conducted using the well diffusion method with varying concentrations of spirulina extract (0.25%, 0.5%, and 1%) compared to the positive control clindamycin. Microbial and heavy metal contamination tests were conducted based on standard laboratory methods. Also, in order to verify a microplastic-free status the "Beat the Microbead" application was used. The test results show that spirulina extract does not produce a significant inhibition zone. However, after being formulated into a serum, there is an increase in antibacterial activity classified as strong, with an average largest inhibition zone diameter of 16.27 ± 0.25 mm. Contamination testing shows that the serum meets Indonesian Food and Drug Authority cosmetic safety requirements, and is free from the Total Plate Count, Mold and Yeast Count, pathogenic microbes, and heavy metals within permissible limits. The microplastic test also showed that the serum does not contain microplastics, with the scanning results appearing green. In conclusion, spirulina extract serum has antibacterial activity and potential as a safe and environmentally friendly anti-acne product.

INTRODUCTION

Acne is a common skin problem experienced by almost all teenagers and adults. This condition should not be underestimated, because acne is not a minor issue; it can leave scars on the skin and cause long-term emotional impacts, such as depression and loss of self-confidence. The loss of self-confidence is often linked to facial appearance, where acne lesions, including papules, pustules, and nodules (inflammatory lesions), and comedones (non-inflammatory lesions), can be very disruptive to one's appearance (Kirsten *et al*., 2021). To date, the exact cause of acne remains unclear; however, the colonization of *Cutibacterium acne* (*C. acne*) is believed to be the most common cause of skin infections and plays a key role in acne pathophysiology (Hastuti *et al*., 2019; McLaughlin *et al*., 2019). *C. acne* contributes to acne by breaking down triglycerides in sebum into free fatty acids, facilitating its colonization, and triggering an inflammatory response (Sifatullah and Zulkarnain, 2021).

Topical treatments commonly used for acne include the application of antibiotics, with clindamycin being one of the most effective options. However, continuous use can lead to side effects such as skin irritation and bacterial resistance. Given the growing concern over bacterial resistance (Sinha *et al*., 2014), there is a need to develop natural antibacterial topical treatments for acne-causing bacteria. One natural ingredient with the potential for acne treatment is spirulina. Spirulina, a marine

resource with abundant availability in Indonesia, a country with the second-longest coastline in the world (Permadi *et al*., 2022), offers various health benefits, particularly as an active cosmetic ingredient. However, more research needs to be conducted on spirulina's benefits for skin health. Spirulina has been widely used in the pharmaceutical and functional food industries and as an active ingredient in natural cosmetic products (Ragusa *et al*., 2021; Suryaningtyas, 2019). The increasing availability of spirulina extract as an active cosmetic ingredient, especially in luxury market segments, indicates a growing interest in its use within the cosmetic industry.

Several cosmetic products using spirulina on the market are mostly formulated in the form of face creams, clay masks, and powder masks (Ragusa *et al*., 2021). One of the most popular cosmetic forms gaining popularity recently is facial serum. Serums are low-viscosity formulations classified as emulsions that spread easily on the skin, providing a comfortable and light feel upon application. Additionally, serums have the advantage of containing high concentrations of active ingredients and are quickly absorbed into the skin, delivering fast results (Kurniawati, 2018). The spirulina species commonly used in cosmetic formulations is Spirulina platensis (*Arthrospira platensis*) (Józsa *et al*., 2020). Previous studies have reported that spirulina exhibits antibacterial activity against gram-positive and gram-negative bacteria, yeast, and fungi (Hoseini *et al*., 2013). It has also been shown to be effective in treating acne caused by *C. acne* and *Staphylococcus epidermidis* (*S. epidermidis*) due to its active compounds such as phycocyanin (Nihal *et al*., 2018), alkaloids, steroids, saponins, and phenols (Setyaningsih *et al*., 2020). These findings suggest that spirulina extract has the potential to be developed as an anti-acne serum.

Quality assurance and safety are crucial in enhancing the quality and ensuring the efficacy of cosmetic formulations. Therefore, it is essential to ensure that cosmetic products are free from contaminants. In this context, microbial and heavy metal contamination are the main concerns due to the possibility of direct interaction with the skin and their potential to penetrate the skin and enter the circulatory system, posing risks to various organs (Almukainzi *et al*., 2022). Additionally, the issue of microplastic contamination has become a global concern. Microplastics are plastic fragments less than 5 micrometers to nanometers in size, often found in cosmetic and skincare products such as microbeads, ranging from 1 to 1,000 micrometers. Microplastics from cosmetic and personal care products are now recognized as major contributors to marine pollution (Suardy *et al*., 2020) and pose risks to human health because they can be absorbed through the skin (Yee *et al*., 2021).

The Android application "Beat the Microbead" can be used to verify the presence of microbeads, which are microplastics, in cosmetic formulations by scanning the list of ingredients. Accordingly, this study was conducted to test the antibacterial activity and contamination of Spirulina extract anti-acne serum to assess its antibacterial activity against *C. acne* and ensure that the spirulina serum is free from cosmetic contaminants.

METHODS

Materials and Instrumentations

The materials used for antibacterial testing and serum preparation include spirulina extract obtained from PT. Organic Lombok Indonesia and additional ingredients such as pearl extract, seaweed extract (*Ulva lactuca*), propylene glycol, glycerin, distilled water, potassium sorbate, sodium benzoate, citric acid, tetrahydroxyethyl ethylenediamine (EDTA), phenoxyethanol, and hydroxyethylcellulose (HEC). Nutrient Broth (NB) and Nutrient Agar (NA) media were sourced from Merck, and Clindamycin Phosphate gel (MediKlin) served as the positive control. The bacterial strain used was *C. acne* (formerly *P. acne*) (ATCC).

For contamination testing, spirulina serum samples were prepared using selective culture media such as *Modified Letheen Broth* (Himedia), Mannitol Salt Agar (MSA), MacConkey Agar (MCA), and Potato Dextrose Agar (PDA) were sourced from Merck. Other reagents included BPW (0.1%) sterile solution (Merck), standard lead solution 1000 ppm (Merck), aqua regia (HCl: HNO_3), distilled water, and concentrated H_2SO_4 (Merck).

The instruments used for antibacterial testing and serum preparation included an analytical balance (Kern ABJ-NM), spatula, beakers, stirring rods, micropipettes (Dlab), droppers, pH meter (Hanna HI9813-5), hot plate (Ika C-Mag HS4), Petri dishes, test tubes, tube racks, inoculation loop, Bunsen burner, watch glass, autoclave (Tomy Seiko LSX-300), incubator (Labnet 311-D), calipers, serum containers, and personal protective equipment (PPE).

For contamination testing, instruments such as an incubator (Labnet 311-D), autoclave (Tomy Seiko LSX-300), glassware (Pyrex), pH

meter (Hanna HI9813-5), sterilized plastic petri dishes, sterile pipettes, Bunsen burner, water bath, Kjeldahl flasks, atomic absorption spectrophotometer (AAS) for lead testing (Thermo ICE 3000), Mercury Analyzer for mercury testing (Merx-T Epa 1631), and the "Beat the Microbead" Android application were utilized.

Procedures

Preparations of spirulina extract samples with concentrations of 0.25, 0.5, and 1% were done by taking successively 1, 2 , and 4 g of spirulina extract and diluting it with aquadest up to 100 mL. The preparation of nutrient broth (NB) media was done by weighing 3.25 g of NB. Then, it is placed into an Erlenmeyer flask and added with 250 ml of distilled water. The NB and distilled water in the Erlenmeyer flask are heated using a hotplate for approximately 10 min until the NB dissolves. The homogenized media is sterilized in an autoclave for 15 min at 121°C. The sterile NB media is poured into test tubes, each containing 10 mL (Gerung *et al*., 2021; Indarto *et al*., 2019).

The preparation of Nutrient Agar (NA) media was done by weighing 7.25 g of NA. Then, it was placed into an Erlenmeyer flask and added with 250 mL of aquadest (28 g/1000 mL). The Nutrient Agar (NA) and aquadest in the Erlenmeyer flask were heated using a hotplate for approximately 10 min until the NA dissolved. The homogenized media was sterilized in an autoclave for 15 min at 121°C. The sterile NA media was poured into test tubes and allowed to solidify at an angle of approximately 30°C to obtain slanted agar media. Next, the required number of Petri dishes are prepared, and a sterile NA medium is poured into each dish with approximately 20 mL, allowing it to solidify (Gerung *et al*., 2021).

Inoculation of *C. acne* bacteria from old media to new media was performed by taking a bacterial culture from the old media and planting one loop into 10 mL of NB, then incubating for approximately 48 h (Indarto *et al*., 2019). The bacteria that were incubated for approximately 48 h were taken with one loop and streaked onto slanted NA media using a sterile inoculating loop. Slant NA media and test bacteria were incubated at 37°C for approximately 24 h. The *C. acne* bacteria that were inoculated are then suspended by taking one loop of the cultured bacteria from the slanted NA medium and placing it into a test tube containing 10 mL of 0.9% NaCl until the turbidity matches the McFarland turbidity standard for antibacterial activity testing.

Test the antibacterial activity of the extract using the well diffusion method. 100 µL of bacterial suspension is dropped and spread over the NA medium using a spreader; wait until the suspension is absorbed for about ±15 min. Next, make wells in the NA medium using a blue tip until wells are formed (diameter ± 7.6 mm). Add 40 µL of the spirulina extract test sample (concentration 0.25, 0.5, and 1%), negative control (k-), and positive control (k+) into each well aseptically, performing three repetitions. Cover the petri dish and provide further instructions for incubation for approximately 24 h.

The preparation of spirulina extract serum used extract concentrations of 0.25, 0.5, and 1%, as F1, F2, and F3.

Table 1. Formula Design of Facial Serum Formulation

Material Name	F1 (0.25%)	F2 (0.5%)	F3 (1%)	F- (%)	F+ (%)	Function
Aqua	50.2	50.2	50.2	50.2	-	Solvent
EDTA	1	1	1	1	-	Stabilizer
Phenoxyethanol	0.8	0.8	0.8	0.8	-	Preservative
Spirulina extract	1	2	4	-	-	Active ingredient
Seaweed LE	1.5	1.5	1.5	-	-	Botanical solvent
Pearl LE	4	4	4	-	-	Botanical solvent
Aqua ^{for} HEC	37.5	37.5	37.5	37.5	-	Solvent
HEC	0.7	0.7	0.7	0.7	-	Thickener
Citric acid	0.3	0.3	0.3	0.3	-	Buffer
Clindamycin phosphate	-	-	-	-	1	Antibacterial

Clindamycin Phosphate Gel 1% (Medellin) served as the positive control (F+), and the serum without extract (Placebo) as the negative control (F-). The formula design can be seen in Table 1**.** After the serum preparation, the quality evaluation of the serum preparation was conducted, including physical tests (color, aroma, and form), chemical tests (pH test), and the antibacterial activity test of the serum preparation.

The antibacterial activity test of the serum used a method similar to the antibacterial test of the extract. Each well plate has five wells filled with 40 µL of serum sample solution of spirulina extract F1, F2, and F3 using a micropipette tip. Clindamycin Phosphate 1% serves as the positive control, and serum without spirulina extract (placebo) serves as the negative control.

Microbial contamination testing includes tests for the Total Plate Count (TPC), Mold and Yeast Count (MYC), *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* contamination. The testing procedure consists of preparing the initial suspension of the test sample. The test sample is a preparation of the selected serum formula. Then continued with isolation and identification referring to the contamination testing standards (Badan Pengawas Obat dan Makanan Republik Indonesia, 2011) modifications of the contamination testing procedures at the Testing and Calibration Health Laboratory Hall (BLKPK) NTB.

Heavy metal contamination testing includes tests for lead (Pb) and mercury (Hg) contamination. The procedure for testing lead (Pb) and mercury (Hg) contamination begins with sample preparation using the wet destruction method. This involves weighing 6 mL of spirulina extract serum sample into a Kjeldahl flask, adding 6 mL of concentrated H_2SO_4 solution, and using aqua regia to dissolve lead in the serum sample. The sample mixture is heated using a Kjeldahl term apparatus at a temperature of 350ºC for ±2–3 h until a clear solution is formed. After heating, proceed with cooling. Measure the volume of the extract obtained from the destruction, then add 50 mL of aquabidest. Filter using Whatman 41 paper to separate the solution from the insoluble residue. Transfer the filtrate into a 100-mL volumetric flask and dilute with aquabidest up to the mark.

After the sample preparation is complete, proceed with measuring the lead (Pb) and mercury (Hg) content in the test sample. The lead (Pb) content measurement uses an atomic absorption spectrophotometer instrument by first creating a standard regression curve. (variation of pb metal standard concentration, which is 1, 2, 4, 6, and 8 ppm). Measurement of mercury (Hg) levels was done using the Mercury Analyzer instrument.

The verification test for microplastic-free serum samples is conducted by downloading the "Beat the Microbead" application from the Play Store and installing it on a smartphone. Next, scan the label of the spirulina extract serum preparation in the composition section of the preparation formula.

Analysis

There are several data obtained from this research, including data on the antibacterial activity test of spirulina extract and spirulina extract serum preparation, data on microbial contamination tests, data on heavy metal contamination tests, and data on microplastic contamination verification.

The data on the antibacterial activity test of spirulina extract serum is presented in tabular form, including the average inhibition zone (mm) and standard deviation. (SD). The obtained data were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 26 (IBM Corp., Chicago, USA) with the Kruskal-Wallis test method, followed by a post-hoc test using the Mann-Whitney test until a selected formula was obtained as the test sample in the subsequent testing stages.

Data from the observation of microbial contamination were consisting of TPC, MYC, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. The calculation of each colony follows the existing literature for TPC and MYC using the equation N = m/(Vx d) with units of colonies/mL (*Badan Pengawas Obat dan Makanan Republik Indonesia*, 2011). The observation results for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* are presented in tabular form with positive (+) and negative (-) indications.

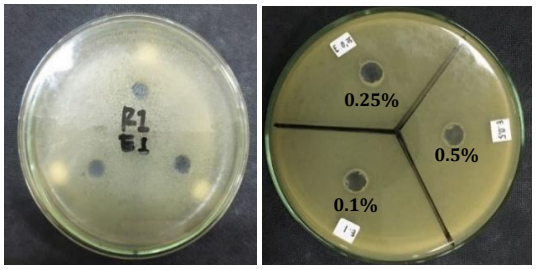
Data from the test measuring lead (Pb) levels using atomic absorption spectrophotometers and mercury (Hg) levels using a mercury analyzer. Subsequently, the data were analyzed using the metal content calculation formula according to the guidelines (*Badan Pengawas Obat dan Makanan Republik Indonesia,* 2011*)* and presented in the form of a test result table. The data from the microplastic-free verification test were analyzed based on the scanning results using the "Beat the Microbead"

application on the serum formulation. The scanning results will yield three (three) possible colors: red (the formula is confirmed to contain microplastics), orange (the formula contains synthetic polymer microplastics), and green (the formula does not contain microplastics), which will then be categorized based on the scanning results.

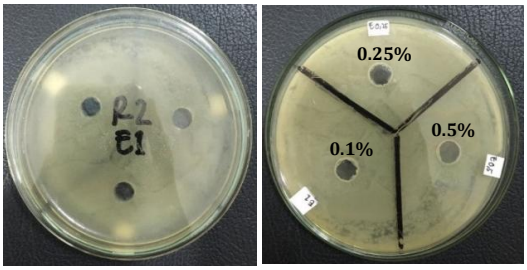
RESULTS AND DISCUSSION

The results of the antibacterial activity test of Spirulina extract show that there is no significant inhibitory effect on bacterial growth at all tested concentrations (0.25, 0.5, and 1%). Based on the observations in Figure 1**.** it is shown that all concentrations of Spirulina extract (0.25, 0.5, and 1%) did not produce inhibition zones, indicated by the absence of clear areas around the wells, demonstrating no antibacterial activity against *C. acne* at these concentrations. The factor suspected to cause the undetected antibacterial activity of Spirulina extract in this study is the relatively low concentration of the extract used, as previous studies have shown that at very high concentrations, such as 100% Spirulina extract, a strong inhibition zone diameter against *C. acne* was produced (Nihal *et al*., 2018). Another factor is the content of less stable active compounds. Some antibacterial compounds in Spirulina, such as phycocyanin and phenol, are known to have low stability against environmental changes, including temperature and light. During storage or processing, this compound can degrade and lose its effectiveness; therefore, this can contribute to the undetected antibacterial activity of spirulina extract (Suryaningtyas, 2019). The interaction of compounds with the medium can also be involved. Some active compounds can bind to proteins or salts in the test medium, making them unavailable to inhibit bacterial growth, which affects the reduction of the antibacterial activity of the active compounds (Indarto *et al*., 2019).

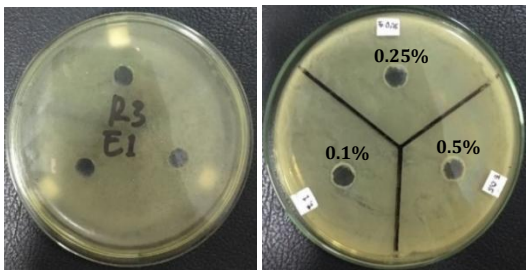
As a comparison, the positive control (K+) with the antibiotic clindamycin phosphate showed an inhibition zone indicated by a clear area around the well, demonstrating that the testing method used is valid and that the *C. acne* bacteria tested are sensitive to the antibiotic clindamycin phosphate.



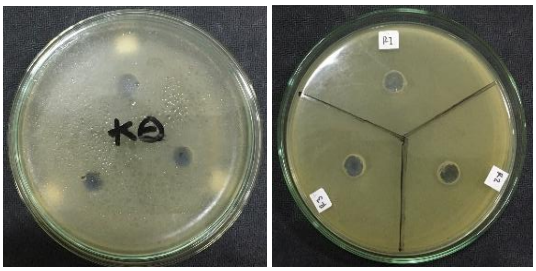
Results of Extract Variation Test Replication 1



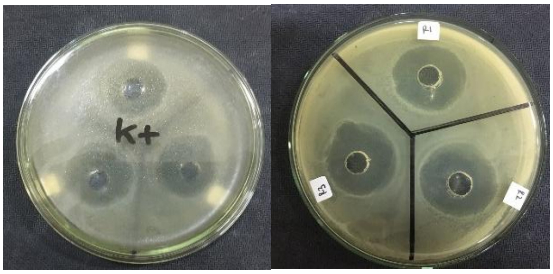
Results of Extract Variation Test Replication 2



Results of Extract Variation Test Replication 3



Results of Negative Control Test



Results of Positive Control Test

Figure 1. Antibacterial Activity Test Results of Spirulina Extract

Table 2. Physical and Chemical Test Results of Spirulina Serum

Formula	Color	Aroma	Texture	Average ± SD pH
F(-)	Clear white	No aroma	Liquid and tends to be slightly viscous	6.20 ± 0.00
F1	Clear white	No aroma	Liquid and tends to be slightly viscous	5.80 ± 0.00
F2	Clear white	No aroma	Liquid and tends to be slightly viscous	5.90 ± 0.00
F3	Clear white	No aroma	Liquid and tends to be slightly viscous	5.90 ± 0.00

*F (-) serum formula without spirulina extract; SD, standard deviation.

The preparation of facial serum using spirulina extract as the active ingredient involves several additional components that function to create an effective and stable facial serum formula. The serum preparation that has been made is then followed by physical tests (color, aroma, and texture) and chemical tests (pH test).

The results of the physical and chemical tests of the spirulina extract serum preparation show several parameters that are important for the evaluation of the serum preparation quality. Based on the data presented in Table 2, the results of the color observation of all formulas (F (-), F1, F2, F3) show a clear white color, indicating consistency in the manufacturing process and no visible contamination. The aroma observation results indicate that no distinctive aroma was detected in all formulas, as there were no additional ingredients that impart aroma, such as natural or synthetic fragrances, in the formulation. The texture observation results show that all formulas have a liquid texture and tend to be somewhat thick according to the number of thickening agents and solvents added, so when applied to the skin, they do not feel sticky and are easily absorbed. This texture indicates that the formula is quite good in terms of viscosity. The results of the pH measurement using a pH meter indicate that the serum preparation as a whole meets the skin's pH requirements, which range from 4.1 to 6.7 (Gite, 2023).

The next test was the antibacterial activity of spirulina extract serum preparation against *C. acne* bacteria. The test results are presented in **Table 3**, which shows the greatest antibacterial activity is in the positive control with an inhibition zone diameter of 19.69 ± 0.03 mm, followed by serum formulas F1, F3, and F2 with inhibition zone diameters of 16.27 ± 0.25 mm, 16.17 ± 0.76 mm, and 15.83 ± 0.29 mm, respectively. Formula F (-) is a serum formula that does not contain extracts showing the diameter of the inhibition zone of 13.11 ± 0.19 mm. The results obtained in the F (-) treatment against the positive control (F (+)) (p<0.05) are marked with the letter b in the table. This result shows a significant difference between F (-) and positive control, which explains that formula F (-), which does not contain spirulina extract, does not have antibacterial activity. The three formulas (F1-F3) gave significant differences (p<0.05) both against F (-) marked with the letter a and against the positive control marked with the letter b in the table. These results explain that formulas F1, F2, and F3 have antibacterial activity, but the resulting activity is lower than the positive control. As for the comparison between concentration variations (F1, F2, and F3), it gives no significant difference (p>0.05), which is indicated by the letter d in the table. These results explain that Formula F1 gives no significant difference (p>0.05) to Formula F2, likewise with Formula F2 against Formula F3. This result shows that the three formulas provide equivalent antibacterial activity. There is no relationship between increasing the concentration of spirulina extract and the antibacterial activity against *C. acne* bacteria produced. Therefore, the F1 formula is recommended for further testing.

Based on the results of testing the antibacterial activity of spirulina extract serum, the average diameter of the inhibition zone produced is classified as strong (Bilal and Sari Lubis, 2022). The dosage formulation in serum form is able to increase antibacterial activity; this is due to the increased penetration of spirulina extract through the skin. This increased penetration is due to the serum dosage formula, which is known to have a light texture, low consistency, and rapid absorption into the skin, which allows the active ingredients to be absorbed better and reach the target more effectively than pure extracts applied directly. Thus, causing an increase in the antibacterial activity of the extract against *C. acne*, as shown by an increase in the size of the inhibition zone diameter (Kurniawati, 2018).

Table 3. Inhibition Zone Results of Spirulina Serum

Formula	Inhibition Zone (mm ± SD)
F(+)	19.69 ± 0.03[a,c]
F(-)	13.11 ± 0.19[b,c]
F1	16.27 ± 0.25[a,b,d]
F2	15.83 ± 0.29 [a,b,d]
F3	16.17 ± 0.76 [a,b,d]

*F (+) positive control: Clindamycin phosphate gel equivalent to 1% Clindamycin (MediKlin)
*F (-) Serum formula without extract

Mann-Whitney test results are expressed as, a : $p<0.05$ vs F (-), b : $p<0.05$ vs Positive control, c : $p<0.05$ vs variation (F1, F2 and F3), d : $p>0.05$ vs variation (F1, F2 and F3); SD, standard deviation.

Table 4 shows the results of the TPC and MYC tests on the tested spirulina extract serum formulas, indicating that there was no microbial growth, characterized by an average colony count of 0 colonies/mL in all dilutions and replicates. These microbial contamination test results indicate that the tested spirulina extract serum meets the microbiological requirements for topical cosmetic products. The absence of microbial growth in TPC and YMC tests indicates that this formula is sterile from microbial contamination that can harm the skin (Almukainzi *et al*., 2022). These results are in accordance with cosmetic safety standards that require cosmetic preparations to be sterile or contain microbial contamination within the specified safety limits, namely for TPC and MYC no more than $5x10^2$ colonies/mL (BPOM, 2019). The use of preservatives such as Phenoxyethanol, which is known to be effective in preventing bacterial and fungal growth in water-based cosmetic products, is important to avoid microbial contamination during product storage and use (Ragusa *et al*., 2021). Good sterilization of tools and packaging materials, as well as the application of appropriate production processes during the manufacture of serum preparations, are important steps that can affect the safety of products from microbial contamination (Almeida *et al*., 2022).

The results of the pathogenic microbial contamination tests consisting of *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Candida albicans* are presented in Table 4. Negative results were shown on all three pathogenic microbes tested in each replication. These results indicate that spirulina extract serum is not contaminated by pathogenic microorganisms that can cause skin infections. Microbes such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* are bacteria that are often found in contaminated cosmetic products and are known as pathogens that have the potential to cause skin infections, especially on skin with wounds and inflammation such as acne (Bilal and Sari Lubis, 2022). The negative results of this pathogenic microbial test indicate that spirulina extract serum is safe for use on sensitive and acne-prone skin, as it does not contain microorganisms that can aggravate skin conditions. This result is in accordance with cosmetic safety standards that require cosmetic preparations not to contain pathogenic microbial contamination (BPOM, 2019) and is supported by international standards regarding the safety of cosmetic products that require freedom from pathogenic microbes (Almukainzi *et al*., 2022).

The results of the metal contamination test in Table 4 show that the serum preparation contained lead metal contamination with a level of 7.32 ± 0.023 ppm. This result still meets the requirements in accordance with the BPOM contamination limit, which is no more than 20 ppm or 20 mg/L (BPOM, 2019). The results of the mercury (Hg) metal contamination test showed that there was mercury (Hg) metal content in serum preparation samples with levels of 0.00023 ± 0.0004 ppm. This result still meets the requirements in accordance with the BPOM contamination limit of no more than one ppm or one mg/L (BPOM, 2019). This relatively low value indicates that the production and processing of spirulina extract serum preparations are in accordance with cosmetic safety standards, especially in ensuring that the levels of heavy metals used do not exceed the permissible tolerance limits, which indicates that spirulina extract serum is safe from heavy metal contamination, especially mercury, which is a major concern in skin care preparations (Almukainzi *et al*., 2022).

The next test is a microplastic-free verification test using the Beat the Microbead application. This application is used to scan the composition of the spirulina extract dosage formula and detect the presence of microplastics in the dosage formula, such as microbeads, which are microplastics with sizes ranging from 1 to 1000 micrometers. Microbeads are commonly

Table 4. Test Results of Selected Serum Contaminants

Type of Contamination	Test Results (Average ± SD)	Limits of Contamination
Microbes		
TPC (colonies/mL)	0.00 ± 0.00	Not more than 5 x 10^2 Colonies/mL
MYC (colonies/mL)	0.00 ± 0.00	Not more than 5 x 10^2 Colonies/mL
Pseudomonas aeruginosa	Negative	Negative
Staphylococcus aureus	Negative	Negative
Candida albicans	Negative	Negative
Heavy metals		
Lead (ppm)	7.32 ± 0.023	Not more than 20 ppm or 20 mg/L
Mercury (ppm)	0.00023 ± 0.0004	Not more than 1 ppm or 1 mg/L

*Limits of contamination according to (BPOM, 2019); Mold and Yeast Count, MYC; SD, standard deviation; Total Plate Count, TPC.

Table 5. Microplastic Content Scan Results

Serum Composition	Scan Result	Description
Aqua, *Arthrospira plantesis* extract (spirulina), Pearl LE, *Ulva lactuca* LE, Tetrahydroxyethyl Ethylenediamine (EDTA), Phenoxyethanol, Hydroxy ethylcellulose, Citric acid.	Green color	Does not contain microplastics

used in skin care and cosmetic products (Suardy *et al*., 2020).

Table 5 shows the verification test results that indicated the spirulina extract serum formula does not contain microplastics, which is indicated by the green color result on application. This color indicates that the spirulina extracts serum preparation is an environmentally friendly skincare preparation because it is free from microplastic microbeads, considering the negative impact of microplastics on the environment and human health through the cycle of microplastics that are released into the environment and enter the food network, thus contributing to human health problems through microplastic-contaminated food (Yee *et al*., 2021) Additionally, the research conducted by Praveena *et* al. (2018) reported that skin care products and cosmetics are considered to be a source of microplastic contamination released into the environment such as the sea, thus disrupting marine ecosystems.

CONCLUSIONS

This study shows that spirulina extract serum has significant antibacterial activity against *C. acne* with a strong inhibition zone. The serum was found to be free from microbial pathogens, heavy metals, and microplastics, thus meeting cosmetic safety standards and being environmentally friendly.

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CONFLICT OF INTEREST

The authors declare no conflict of interest related to the research, writing, and publication of this article.

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