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Optimization of Solvent Type on Antioxidant Activity of *Spirulina platensis* Extract and Its Pharmaceutical Tablet Formulation

Optimasi Jenis Pelarut terhadap Aktivitas Antioksidan Ekstrak *Spirulina platensis* dan Formulasi Tablet Farmasinya

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ABSTRACT

Spirulina platensis contains nutrients such as proteins, essential amino acids, fatty acids, minerals, pigments, carotenoids, and vitamins, which have strong antioxidant properties. This study evaluated the impact of extraction solvents (70% methanol, 70% ethanol, and 70% acetone) on antioxidant activity, with the most effective extract formulated into tablets. Extraction was performed by maceration. Tablet preparations were characterized by moisture content, flow rate, angle of repose, organoleptic properties, weight uniformity, hardness, disintegration time, and friability. The results showed that the 70% methanol extract exhibited the highest antioxidant activity (IC_{50} = 45.57 ± 0.01 ppm), superior to 70% acetone (IC_{50} = 67.15 ± 0.07 ppm) and 70% ethanol (IC_{50} = 85.32 ± 1.21 ppm), although lower than vitamin C (IC_{50} = 3.52 ± 0.01 ppm). Tablets prepared from 70% methanol extract fulfilled all pharmaceutical quality requirements.

ABSTRAK

Spirulina platensis memiliki kandungan protein, asam amino esensial, asam lemak, mineral, pigmen, karotenoid, dan vitamin yang berpotensi sebagai antioksidan. Penelitian ini menguji pengaruh jenis pelarut ekstraksi (metanol 70%, etanol 70%, dan aseton 70%) terhadap aktivitas antioksidan, dengan ekstrak terbaik diformulasikan dalam sediaan tablet. Ekstraksi dilakukan dengan metode maserasi. Karakteristik sediaan tablet meliputi kadar air, laju alir, sudut diam granul, sifat organoleptik, keseragaman bobot, kekerasan, waktu hancur, dan kerapuhan. Hasil menunjukkan ekstrak metanol 70% memiliki aktivitas antioksidan tertinggi (IC_{50} = 45,57 ± 0,01 ppm), lebih baik dari ekstrak aseton 70% (IC_{50} = 67,15 ± 0,07 ppm) dan etanol 70% (IC_{50} = 85,32 ± 1,21 ppm), namun lebih rendah dibanding vitamin C (IC_{50} = 3,52 ± 0,01 ppm). Tablet ekstrak metanol 70% memenuhi semua persyaratan mutu farmasi.

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1. INTRODUCTION

Free radicals are molecular species with unpaired electrons in atomic orbitals, capable of damaging critical macromolecules such as DNA, proteins, carbohydrates, and lipids, causing cellular injury and disrupting homeostasis (Qazi & Molvi, 2018). Damage caused by free radicals contributes to the onset of chronic and degenerative diseases, including cancer, autoimmune disorders, aging, cataracts, rheumatoid arthritis, and cardiovascular and neurodegenerative disorders (Pham-Huy et al., 2008). Antioxidants neutralize free radicals by donating electrons, thus preventing further cellular damage. Natural antioxidants obtained through dietary supplements in tablet form are increasingly favored (Mutmainah et al., 2019). *Spirulina* is one natural ingredient known for its significant antioxidant properties (Asghari et al., 2016).

Spirulina, belonging to the genus *Arthrospira*, is a symbiotic microalga recognized for its spiral filamentous structure. Known as blue-green algae due to its photosynthetic capability and rich pigment content, it is now classified within the Cyanobacteria phylum, specifically in the Spirulinaceae or Pseudanabaenaceae family (Neyrinck et al., 2017; Nuhu, 2013). *Spirulina* naturally thrives in highly saline and alkaline waters found in tropical and subtropical regions. The widely studied edible species include *Spirulina platensis (Arthrospira platensis), S. maxima* (*A. maxima*), and *S. fusiformis* (*A. fusiformis*) (Ma et al., 2025).

S. platensis has become prominent due to its substantial nutritional profile, including high protein content, essential amino acids, fatty acids, minerals, pigments, carotenoids, and vitamins (Kamaludin & Holik, 2022). This study aims to determine the optimal solvent for extraction to maximize antioxidant activity and develop a pharmaceutical tablet formulation from the most potent extract. Tablets are advantageous due to their practicality, precise dosage, ease of administration, and stability during storage, requiring thorough evaluation to confirm quality (Son et al., 2019).

2. METHODS

2.1. Materials

Spirulina platensis was obtained from PT ALGA Bioteknologi Indonesia, Cepoko, Gunung Pati, Semarang. Analytical grade chemicals used were 70% ethanol, 70% methanol, 70% acetone, 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma), and Vitamin C (Sigma). Excipients for tablet formulation included Avicel pH 102, sodium starch glycolate (SSG), talc, and magnesium stearate.

2.2. Extraction

Extraction was performed by maceration method using three solvents: 70% methanol, 70% ethanol, and 70% acetone. *S. platensis* powder (10 g) was immersed in each solvent (40 mL) and replicated five times, protected from light exposure. Maceration was carried out for 3x24 hours (Departemen Kesehatan Republik Indonesia, 2020). The filtrate obtained was concentrated using a rotary evaporator at 30°C and subsequently dried under nitrogen gas to yield a thick extract.

2.3. DPPH Radical Scavenging Assay

Samples were dissolved in methanol at varying concentrations. The concentrations of the sample used were 10, 30, 50, 70, and 90 ppm, while the concentrations of the vitamin C standard used were 2, 2.5, 3, 3.5, and 4 ppm. Antioxidant activity was determined by mixing 0.2 mL of sample solution with 4 mL of 0.1 mM DPPH solution. The mixture was vortexed for 1 minute, incubated for 30 minutes at room temperature, and absorbance measured at 517 nm. Vitamin C served as a reference standard. Antioxidant activity (percentage inhibition) was calculated as follows:

$$\%\,Inhibition = \frac{A_c - A_s}{A_c} \times 100\%$$

where Ac and A_s represent the absorbance of the control and sample, respectively. IC_{50} values were determined from the percentage inhibition data (Margiati et al., 2019)

2.4. Tablet Formulation

The extract with the highest antioxidant activity (70% methanol extract) was used for tablet formulation. The tablet contained methanol extract (0.1%), Avicel pH 102 (80.9%), sodium starch glycolate (4%), talc (10%), and magnesium stearate (5%). Initially, the extract was thoroughly mixed with aerosil. The dry mixture was then blended with Avicel and SSG, followed by the addition of magnesium stearate and talc. The final mixture was homogenized, sieved through a 40 mesh, and compressed into 300 mg tablets.

2.5. Granule Evaluation

Granules were evaluated by determining moisture content using an Ohaus moisture balance. Flow properties were assessed by measuring the time required for 100 g of granules to flow through a funnel. Angle of repose was calculated based on the granule heap formed using the formula $\tan \alpha = h/r$, where h is the height and r is the radius of the granule cone.

2.6. Tablet Evaluation

Tablets underwent several evaluations. Organoleptic properties were assessed visually for appearance, odor, and taste. Weight uniformity was determined by individually weighing 20 tablets, with deviation calculated according to Indonesian Pharmacopoeia III standards. Tablet hardness was measured using a hardness tester. Disintegration time was evaluated using a disintegration tester with distilled water at 37.5 ± 0.5°C until complete disintegration occurred. Friability testing involved rotating tablets at 25 rpm for 4 minutes in a friability tester, and percentage friability was calculated using the formula:

$$\text{Friability (\%)} = \frac{W_i - W_a}{W_i} \times 100\%$$

where W_i is the initial tablet weight and W_a is the weight after testing.

2.7. Data Analysis

All data were expressed as mean ± standard deviation (SD) of triplicate experiments. Statistical analysis was performed using GraphPad Prism (version 9.1.2; GraphPad Inc., San Diego, CA, USA). IC_{50} values were determined with statistical significance set at $p < 0.05$.

3. RESULTS AND DISCUSSION

Solvent extraction is commonly employed to isolate active compounds from plant materials, involving the dissolution of compounds from the plant matrix followed by diffusion into the solvent medium (Sultana et al., 2009). Extraction efficiency depends significantly on factors including solvent polarity, extraction duration, temperature, physical and chemical properties of the material, and the solvent-to-sample ratio (Nawaz et al., 2020). The extraction yields for *S. platensis* were strongly influenced by the solvent type, with 70% methanol resulting in the highest yield (22.82%), followed by 70% acetone (18.30%), and 70% ethanol (13.07%) (**Figure 1**). Statistical analysis indicated significant differences in yield, with methanol showing superior extraction efficacy ($p < 0.0001$) compared to ethanol and acetone ($p < 0.001$). This result aligns with the principle of "like dissolves like," suggesting that methanol's polarity closely matches the compounds in spirulina.

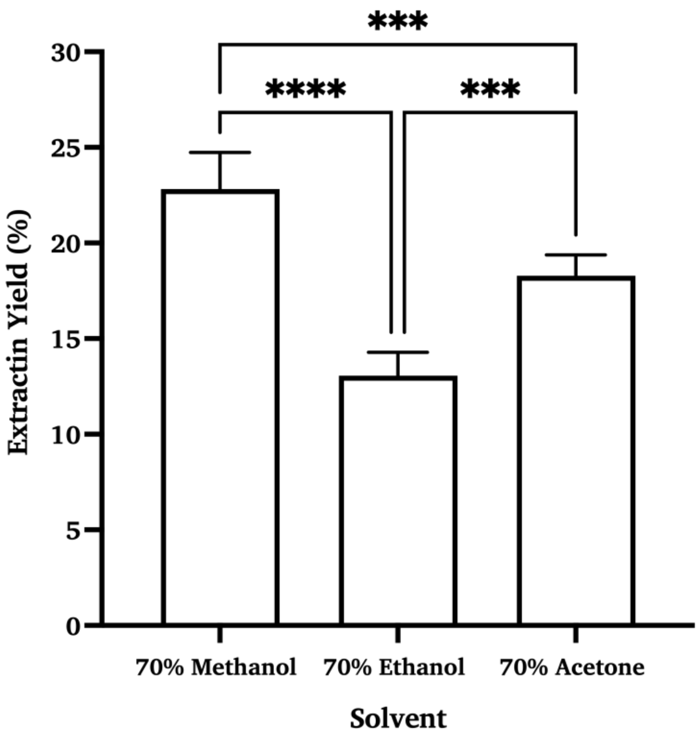

Figure 1. Comparison of Extraction Yield (%) from *Spirulina platensis* Using Methanol, Acetone, and Ethanol Solvents (mean ± SD, n=5). *** = $p < 0.001$, **** = $p < 0.0001$.

Antioxidants neutralize free radicals by electron donation, thus reducing cellular damage (Zhuang et al., 2021). Spirulina extracts contain various potent antioxidants such as phycocyanin, β-carotene, vitamins, and minerals, contributing significantly to their antioxidant capability (Lü et al., 2010). The antioxidant activities measured by the DPPH method showed that the 70% methanol extract exhibited the highest radical-scavenging activity (IC_{50} = 45.57 ± 0.01 ppm), significantly outperforming 70% acetone (IC_{50} = 67.15 ± 0.07 ppm) and 70% ethanol (IC_{50} = 85.32 ± 1.21 ppm) (**Figure 2**). These differences were statistically significant ($p < .0001$), although all extracts were less potent than Vitamin C (IC_{50} = 3.52 ± 0.01 ppm). According to Philip (2004), the antioxidant capacity of the methanol extract falls into the "very strong" category (<50 ppm), whereas acetone and ethanol extracts were classified as "strong" (50-100 ppm). Previous research supports these findings, highlighting solvent choice as critical in determining antioxidant effectiveness (Zaid et al., 2015; Gheda et al., 2021).

Granule evaluation prior to tablet production assessed moisture content (%), flow rate (g/sec), and angle of repose (°), as presented in **Table 1**. Granules demonstrated acceptable moisture content (1.88%), aligning with optimal humidity standards of 1%-5% (Jones, 2016). This parameter ensures sufficient binding without brittleness or capping issues during compression (Mustarichie & Priambodo, 2018). Flow rate (10.16 g/sec) met the recommended standard (>10 g/sec), influenced by granule particle shape, size distribution, and cohesiveness (Buang et al., 2023; Nawangsari, 2019). Additionally, the angle of repose was satisfactory at 21.07°, within the ideal range (20°–30°), reflecting optimal flow characteristics (Frenning & Alderborn, 2018).

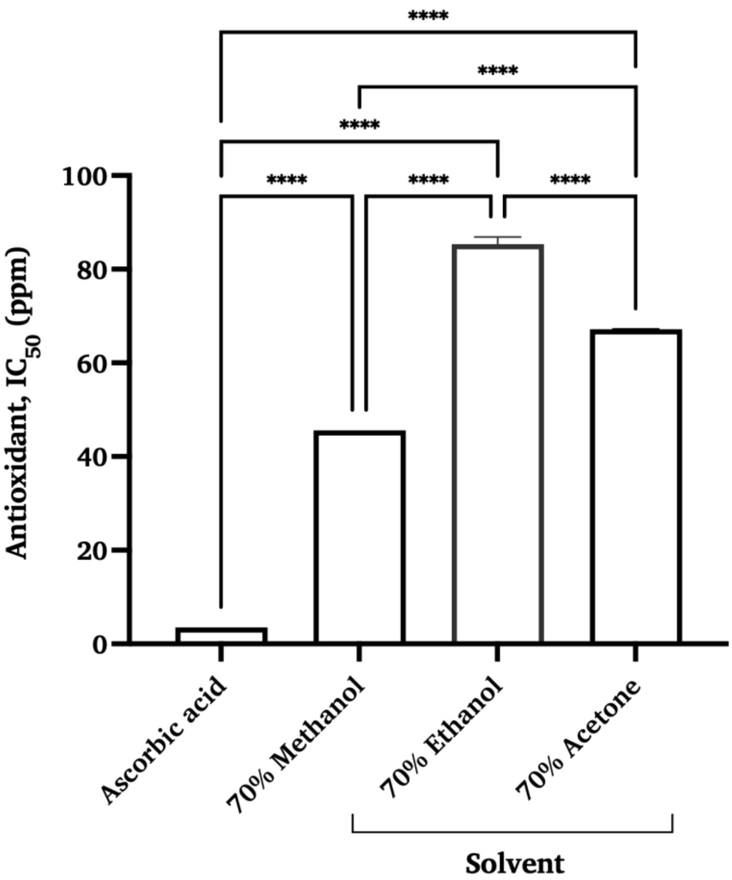

Figure 2. Comparison of Antioxidant Activities (IC_{50}, ppm) of *Spirulina platensis* Extracts and Vitamin C Standard (mean ± SD, n = 3). **** = $p < 0.0001$.

Table 1. Granule Evaluation Parameters of *Spirulina platensis* Methanol Extract (mean ± SD)

Parameter	Result	Literature Reference
Moisture content (%)	1.88 ± 2.48	Good granule moisture is 1%-5% (Jones, 2016)
Flow rate (g/sec)	10.6 ± 1.14	Good flow rate is > 10 g/sec (Frenning & Alderborn, 2018)
Angle of repose (°)	21.07° ± 1.47	Below 40° (Frenning & Alderborn, 2018)

Table 2. Tablet Evaluation Parameters of *Spirulina platensis* Methanol Extract Tablets Compared to Indonesian Pharmacopeial Standards (mean ± SD)

Parameter	Result	Indonesian Pharmacopoeial Standards Reference
Weight uniformity (mg)	302 ± 4.99	No more than 2 tablets deviate by > 5% and none by > 10% (Ministry of Health, 1979)
Hardness (kg)	6.21 ± 0.57	4–8 kg (Buang et al., 2023)
Disintegration time (sec)	34.2 ± 1.30	< 15 sec (Ministry of Health of the Republic of Indonesia, 2020)
Friability (%)	0.49 ± 0.22	< 0.8% (Frenning & Alderborn, 2018)

Tablet evaluations further confirmed product quality (**Table 2** and **Figure 3**). Tablets presented consistent organoleptic characteristics, uniform weight distribution (302 ± 4.99 mg), meeting Indonesian Pharmacopoeia standards (Ministry of Health, 1979). Tablet hardness was optimal (6.21 kg), complying with the 4-8 kg requirement, ensuring mechanical stability during packaging and transport (Buang et al., 2023; Mustarichie & Priambodo, 2018). Disintegration time (34.2 sec) and friability (0.49%) also conformed to pharmacopeial standards (< 15 min disintegration and < 0.8% friability), ensuring rapid tablet breakdown and robust physical integrity during handling (Departemen Kesehatan Republik Indonesia, 2020; Sari & Setiyadi, 2024). Tablet characteristics such as hardness, disintegration time, and friability were influenced by binder concentration, highlighting the importance of formulation optimization (Khabibah & Ernawati, 2023; Sari & Setiyadi, 2024).

Figure 3. Tablets Prepared from Methanol Extract of *Spirulina platensis*

4. CONCLUSION

This study concludes that the 70% methanol extract of *S. platensis* exhibited the highest extraction yield and the most potent antioxidant activity. Furthermore, tablets formulated from the 70% methanol extract successfully met all pharmacopeial quality standards, demonstrating appropriate characteristics including uniform weight, optimal hardness, acceptable friability, and efficient disintegration time. These findings highlight the suitability of 70% methanol as the optimal solvent for extracting antioxidant-rich compounds from *S. platensis* and the subsequent feasibility of formulating these extracts into high-quality pharmaceutical tablets.

AUTHOR CONTRIBUTIONS

Conceptualization, I Kadek Bagiana and Lia Kusmita; methodology, Yuvianti Dwi Franyoto and Mutmainah; software, Yuvianti Dwi Franyoto; validation, I Kadek Bagiana, Lia Kusmita, and Mutmainah; formal analysis, I Kadek Bagiana; investigation, I Kadek Bagiana; resources, Lia Kusmita; data curation, Yuvianti Dwi Franyoto; writing—original draft preparation, I Kadek Bagiana; writing—review and editing, Lia Kusmita; visualization, Yuvianti Dwi Franyoto; supervision, I Kadek Bagiana.; project administration, I Kadek Bagiana; funding acquisition, Lia Kusmita. All authors have read and agreed to the published version of the manuscript.

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Not applicable.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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