In vitro evaluation of face mask containing extract and biomass of *Spirulina platensis* and its antibacterial activity

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Abstract. *Spirulina* is a potential source of bioactive compounds and recently used in skincare products. The aim of the research was to determine characteristics and antibacterial activity of face mask containing extract and biomass of *Spirulina platensis*. *S. platensis* was cultivated indoor at 28°C, using modified media, and lighting intensity of 2500 lux. The *S. platensis* biomass was extracted using ethanol. The crude extracts and biomass of the *Spirulina* were used in the face mask formulation. The characteristic and antibacterial activity of the face mask was observed. The face mask containing extract and biomass of *Spirulina* had the viscosity of 7306.7±9.2 cP, pH of 6, spreadability of 1.1 mm, homogenous, green color, and semisolid. The face mask was able to inhibit *Propionibacterium acnes* with a diameter of inhibition zone was 10±0.4 mm. The antibacterial activity was due to the presence of alkaloids, steroids, saponins, and phenol in *S. platensis* extract. This research promotes the developing of marine natural products as cosmeceutical ingredient.

Keywords: antibacterial, extract, mask, *Spirulina platensis*

1. Introduction

*Spirulina* is a cyanobacterium that rich in carotenoid, chlorophyll, phycocyanin, amino acid, minerals and many other bioactive components (Singh *et al* 2005). This microalga is easy to cultivate and grow in high and low salinity. *Spirulina* contains of 60-70% protein of, 13.5% carbohydrate, 4-7% lipid, and fatty acid (linolenic acid \(\gamma\)-linolenic acid), essential amino acid (leucine, isoleucine, valine), natural pigment (chlorophyll, phycocyanin and carotenoid), and vitamins such as vitamin A and B12 (Koru 2012). According to Andrade *et al* (2019) *Spirulina* is one of the cyanobacterial which can be used in human and animal health supplements because of its high nutritional value. *Spirulina* cultured in Zarrouk medium re-used has composition of fatty acids contained polyunsaturated fatty acids between 37.58-47.49%.
Cyanobacteria are organisms with high added value because they contain proteins, lipids, carbohydrates (Bach and Chen 2017, Andrade et al 2019). *Spirulina* extract contains bioactive compound include phycocyanin, phenolic, terpenoids, alkaloids, flavonoids (Masojídek and Pra’šil 2010, Singh and Dhar 2011, Ibunez and Cifuentes 2013, Morais et al 2015). According to Priyadarshani and Rath (2012), the phycocyanin and biomass of *Spirulina* can be applied to health food and cosmetics

Usharani et al (2015) reported that algae and cyanobacteria have antioxidant characteristics but they are less well documented. The phycobiliproteins extracted from *Spirulina platensis* has antibacterial activity. Some researches showed that *S. platensis* has antibacterial and antioxidant activity. Amri et al (2017) reported methanol extract of *S. platensis* was active against *P. acnes* with an inhibition zone of 8.3±0.5 mm. Antibacterial activity of *S. platensis* was due to its bioactive compounds. Fithriani et al (2015) stated that *S. platensis* contain bioactive compounds including alkaloids, flavonoids, steroids, saponins, and tannins. Mechanism of actions of the bioactive compounds are such as damage bacterial cell wall, denature protein and DNA, and therefore these cause cell death. The antibacterial activity of the *Spirulina* makes this cyanobacterium potential for cosmetic ingredient including in face mask.

A face mask may be used to overcome skin problem caused by bacterial and exposure to free radicals. The benefit of the mask in facial care includes nourishing and softening the skin, cleansing the skin pores, brightening the skin color, relaxing the facial muscles and curing acne and acne scars (Kartikasari 2015). Bacterial skin flora such as *P. acnes* has high affinity to adhere to implanted devices by the production of biofilm (Abdulmassih et al 2016). The aim of the research was to determine the characteristics and antibacterial activity of the face mask containing extract and biomass of *Spirulina*.

2. Materials and methods

2.1. Materials

The inoculum of *S. platensis* obtained from Brackishwater Aquaculture Development Centre, Jepara, Indonesia, was refreshed and cultivated at Laboratory of Aquatic Product Biotechnology, Faculty of Fisheries and Marine Sciences, IPB University (Bogor Agricultural University). The modified media for *Spirulina* culturing was bought from the local shop Bogor, Indonesia. Cultivation of *S. platensis* using marine water 20 ppt. Ingredients of the face mask (polyvinyl alcohol /PVA), hydroxypropyl methyl cellulose/HPMC), methylparaben/MP, glycerin) were bought from the local shop at Bogor. *P. acnes* was obtained from University of Indonesia.

2.2. Methods

2.2.1. Cultivation and drying of *Spirulina*. *Spirulina* was cultivated in the flask containing modified media (GA organic fertilizer for the plant, urea, and plant catalyst) of 15 L, completed with continuous aeration, tube lamp with the intensity of 2500 lux. Cultivation was held in an indoor laboratory for 12 days. The optical density of the *Spirulina* culture was measured every day for checking the growth. After 12 days cultivation, the biomass was separated from the culture using nylon mesh 20 µm, dried using oven at 40°C for 24 hours and crushed for obtaining powder biomass.

2.2.2. Preparation of microalgae extract. The dried biomass of *S. platensis* was extracted with ethanol. Briefly, the dried microalgae were extracted three times for 24 hours, then evaporated at 40°C until the paste was obtained. The ethanol extract obtained was counted of yield and detected for the active compounds. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals. The crude extract was applied in the peel-off face mask as one of the ingredients.
2.2.3. **Formulation of face mask.** Formulation of the face mask *Spirulina* referred to Sukmawati *et al* (2013) and Syarifah *et al* (2015). Polyvinyl alcohol (PVA) was dissolved in aquadest and stirred until homogenous (Mass 1). Hydroxypropyl methylcellulose (HPMC) dissolved in aquadest and stirred until homogenous (Mass 2). Methylparaben was dissolved in aquadest (Mass 3). Mass 2 was added glycerine and stirred, then mixed with Mass 3 and stirred. Mass 1 was added to Mass 3 and stirred. The formula of peel-off face mask is presented in table 1.

| Ingredient            | Quantity (g) |
|-----------------------|--------------|
| Methylparaben         | 0.01         |
| HPMC                  | 0.2          |
| Extract of *S. platensis* | 0.25         |
| Glycerine             | 0.25         |
| PVA                   | 0.6          |
| Biomass of *S. platensis* | 0.75         |
| Aquadest              | 100          |

2.2.4. **Evaluation of Spirulina face mask.** The *Spirulina* face mask was analyzed for consistency, color, pH value, homogeneity, viscosity, and spreadability referred to Chandira *et al* (2010) and Suhery and Anggraini (2016). The consistency and colour were checked visually. The pH value was checked using pH meter. Homogeneity was checked visually. Spreadability was determined with the preparation of a small amount of the face mask on paper charts coated with transparent glass, then left to stand for 60 seconds. The area appeared by this preparation was calculated. The viscosity of the formulated gel bases was determined. The viscosity was determined using Brook-field viscometer with spindle number S-06 and the determinations were carried out in triplicate and the average of three reading was recorded.

2.2.5. **Antibacterial assay.** The antibacterial activity was observed referred to Marselia *et al* (2015) and Amri *et al* (2017) with modification. In this research used test organism *P. acnes* obtained from Microbiology Laboratory, Faculty of Medicine, Indonesia University. The test medium consists of Blood Agar for *P. acnes*. The blood agar media was poured to petri dish, left to harden. The bacterial suspension (OD>0.5) was inoculated to the blood agar media, then leveled and made the well. Each well was filled sample of 50 µL, incubated at 37°C for 48 h. The zones of inhibition formed around the well demonstrated the ability of the samples to inhibit the bacteria. The clindamycin was used as standard drug (positive control).

2.3. **Data analysis**

The data was obtained from three replications and processed using Microsoft Excel 2010 to get the average value and standard deviation. The data was presented in tables and figures, and they were analyzed descriptively.

3. **Results and discussion**

3.1. **The Spirulina culture**

*S. platensis* is able to grow in modified media. This is indicated by the increase of optical density (OD) during cultivation (figure 1). The culture and cell of *S. platensis* are presented in figure 2. Asthary *et al* (2013) reported that *S. platensis* cells increased until day 4 and then were in the exponential phase.
The growth curve of *S. platensis* during cultivation.

![Figure 1](image1.png)

**Figure 1.** The growth curve of *S. platensis* during cultivation.

The diameter of *S. platensis* cell cultivated in modified media containing GA organic fertilizer (fertilizer for a plant), urea and plant catalyst was 5.65-5.87 μm with the length of 7.79 μm. According to Masojídek and Torzillo (2014) *S. platensis* is a planktonic filamentous cyanobacterium with diameter about 8 mm. The media containing GA fertilizer was able to influence diameter and length of microalgae.

*S. platensis* was cultivated for 12 days in the modified media produced a yield of biomass 17.84±0.28 g.L⁻¹. The biomass of *S. platensis* was green, which is the dominant color of chlorophyll. According to Rajalakshmi and Banu (2016), chlorophyll has a potency of antimicrobial activity. *Mimosa pudica* chlorophyllin possesses potential antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Candida albicans*. Dimara and Yenusi (2011) reported that chlorophyll extract of *Caulerpa racemosa* (Forsskal) was able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* at the concentration of 100% with the average of inhibition zone diameter was 2.17 cm and 2.10 cm, respectively. The dried biomass in the study contains water of 5.6%. The water content in this *Spirulina* was low. According to Kumesan *et al.* (2017), the water content in the product can affect the end product quality such as appearance, texture, chemical composition or microorganism activity.

### 3.2. The active compounds of Spirulina crude extracts

In the present study, the crude extract of *S. platensis* cultivated in modified media contain alkaloids, flavonoids, saponins, phenol compound and steroids (table 2). Amri *et al.* (2017) reported that *S. platensis* contain phenolics, flavonoids, steroids, terpenes. The content of active compounds in samples sometimes different, because they used different method for cultivation or extraction. Manoi (2006) reported that drying process affects the quality of sambiloto. Combination drying of sun and blower gave the better result to alcohol extractive matter content than other methods. George *et al.* (2017) stated alkaloids have the ability as an antibacterial. Alkaloids have been well investigated for many pharmacological properties including antiprotozoal, cytotoxic, anti-inflammatory properties but
there are only a few reports about their antimicrobial properties. In the pharmacological field, the alkaloids seem to be very popular.

| Active compound          | Extract of S. platensis cultivated |
|--------------------------|-------------------------------------|
| Alkaloid                 |                                     |
| - Meyer                  | +                                   |
| - Wagner                 | +                                   |
| - Dragendorff            | +                                   |
| Flavonoid                | +                                   |
| Saponin                  | +                                   |
| Tanin                    | -                                   |
| Phenolic                 | +                                   |
| Steroid/Triterpenoid     | +                                   |

Note: +: detected, -: not detected

3.3. Characteristics of Spirulina face mask

Characteristics of the Spirulina face mask is presented in table 3. The Spirulina face mask was green in accordance with the color of Spirulina, semisolid in consistency, but still smells typical of Spirulina. The ideal face mask characteristics according to Sulastri and Chaerunisa (2016) are no coarse, nontoxic, not irritating, skin cleansing, homogeneous particle, capable of giving moist effects on the skin.

| Parameter          | Observation        |
|--------------------|--------------------|
| Organoleptic       | colour: green      |
|                    | consistency: semisolid |
| Viscosity          | 7306.7±9.2 cP      |
| pH                 | 6                  |
| Spreadability      | 1.1 cm             |
| Homogeneity        | homogenous         |
| Odor               | Specific Spirulina |
| Antibacterial activity | Positive inhibit P. acnes |

The Spirulina face mask had pH 6. Rahmawanty et al (2015) stated the pH is in accordance with the pH range of skin. If the pH is lower than 4.5 (too acidic) will cause irritation to the skin, but if higher than 6.5 (too alkaline) will make skin scaly. The viscosity of the Spirulina face mask was 7306.7±9.2 cP. According to Sukmawati et al (2013) viscosity can affect spreadability, releasing active compounds from the gel of product and consistency of the gel. The viscosity of the gel preparation should be in the range of 7100-83144.

3.4. Antibacterial activity of Spirulina face mask

The antibacterial compounds which inhibit the growth of bacteria but not kills them it's called bacteriostatic, while the bactericidal when the compounds kill the bacteria (Madigan et al 2012). Elsheekh et al (2014) stated that S. platensis has antibacterial activity against Gram-positive and Gram-negative bacteria, as well as fungus such as Candida albicans. In this study, S. platensis cultivated in modified media contain active compounds include alkaloids, flavonoids, saponins, phenol compound, and steroids. It may be useful for the development cosmeceutical include face mask. In this case, the Spirulina face mask was formulated with adding biomass and extract of S. platensis. The result obtained in the present study revealed that Spirulina face mask potential
antibacterial activity against \(P.\) \(acnes\). This was indicated by the appearance of clear zone around the well (table 4 and figure 3).

| Treatment                                   | Zone of inhibition diameter (mm) |
|---------------------------------------------|---------------------------------|
| Face mask without \(Spirulina\) extract     | 0                               |
| Face mask containing \(Spirulina\) extract  | 10±0.4                          |
| Face mask containing clindamycin           | 12±1.1                          |

The face mask without \(Spirulina\) did not show antibacterial properties. It was means \(S.\) \(platensis\) contains an active compound which could against \(P.\) \(acnes\). The presence of antibacterial activity was supported by active compounds contained in the \(Spirulina\). The zona of inhibition of face mask containing \(Spirulina\) biomass and extract (0.0035 g per well) was 10±0.4 mm, while zone of inhibition of face mask containing clindamycin (0.0001 g per well) was 12±1.1 mm. The concentration of active compounds provide different effect to antibacterial activity. The concentration of clindamycin in the face mask was less than extract, however its zona of inhibition was bigger, allegedly because clindamycin purely compared to crude extract. Crane et al (2013) reported that Clindamycin was active against many \(P.\) \(acnes\) strains, but some strains were highly resistant. In most reports, clindamycin and vancomycin are mentioned as the first drugs to consider for deep-seated \(P.\) \(acnes\) infections (Millett et al 2011). Generally, microalgae methanol extract containing flavonoids, terpenoids, phenolic and steroids show good antibacterial abilities (Amri et al 2017).

![Figure 3. Inhibition zone of \(Spirulina\) face mask. (A) face mask without extract, (B) face mask containing extract, (C) face mask containing clindamycin.](image)

4. Conclusion

\(S.\) \(platensis\) cultivated in modified media contain active compounds which are potential as ingredient of face mask. \(Spirulina\) face mask had colour of green, consistency of semisolid, pH value of 6, viscosity of 7306.7±9.2 cP, homogenous, spreadability of 1.1 cm and antibacterial activity against \(P.\) \(acnes\). Further studies required to understand the mechanism and the actual efficacy of \(Spirulina\).

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