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ORIGINAL ARTICLE / ÖZGÜN MAKALE

CHEMICAL COMPOSITION AND *IN VITRO* ANALYSIS OF ANTIBACTERIAL ACTIVITY OF *ACUTODESMUS DIMORPHUS* (TURPIN) P.M. TSARENKO (SCENEDESMACEAE, CHLOROPHYTA)

ACUTODESMUS DIMORPHUS (TURPIN) P.M. TSARENKO (SCENEDESMACEAE, CHLOROPHYTA)' NIN KİMYASAL BİLEŞİMİ VE ANTİBAKTERİYEL AKTİVİTESİNİN İN VİTRO ANALİZİ

Eldrin DLR. ARGUELLES[1]*

[1]Philippine National Collection of Microorganisms (PNCM), National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippine Los Baños, College, 4031, Laguna, Philippines

ABSTRACT

Objective: *Green microalgae are fast growing organisms and are known to have diverse bioactive compounds and biomolecules. The chemical composition and antibacterial activities of a green microalga, Acutodesmus dimorphus BIOTECH 4039 were studied.*
Material and Method: *Dried algal biomass of A. dimorphus was subjected to proximate and elemental composition analysis. Methanolic extract of A. dimorphus was used to determine the total phenolic content (TPC) and antibacterial activity of the microalga. TPC was measured using the Folin–Ciocalteu method. On the other hand, the antibacterial activity against medically important bacterial pathogens (Staphylococcus epidermidis BIOTECH 10098, penicillin acylase-producing Bacillus cereus BIOTECH 1509, Listeria monocytogenes BIOTECH 1958, Methicillin-Resistant Staphylococcus aureus BIOTECH 10378, Pseudomonas aeruginosa BIOTECH 1824, penicillin acylase-producing Escherichia coli BIOTECH 1634, and Serratia marcescens BIOTECH 1748) was done using modified Kirby-Bauer diffusion method.*
Result and Discussion: *A. dimorphus contained high amounts of protein, ash, and lipid with percent composition of 43.19 ± 0.13, 26.92 ± 0.01, and 14.17 ± 0.04, respectively. The elemental nutrient composition of the algal biomass was observed to be in a decreasing order of Ca > Mg > K > Mn > Na > Fe > Zn > Pb > Cu > Cd >Cr. In addition, A. dimorphus has a TPC of 5.34 ± 0.09 mg GAE/g. Potent antibacterial activities of A. dimorphus extract were observed against Methicillin-*

* **Corresponding Author / Sorumlu Yazar:** Eldrin DLR. Arguelles
e-mail / e-posta: edarguelles@up.edu.ph, **Phone / Tel.:** +63495362721

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Resistant Staphylococcus aureus, S. epidermidis, penicillin acylase-producing Bacillus cereus with zones of inhibition of 15.1 ± 0.3 mm, 13.5 ± 0.1 mm, and 6.82 ± 0.7 mm, respectively. The study shows the use of A. dimorphus as good alternative source of important compounds and microelements that can be use in industrial and pharmaceutical application.
Keywords: *Antibacterial activity, chemical composition, microalgae, Philippines*

ÖZ

Amaç: *Yeşil mikroalgler hızlı büyüyen organizmalardır ve çeşitli biyoaktif bileşiklere ve biyomoleküllere sahip oldukları bilinmektedir. Yeşil bir mikroalg olan Acutodesmus dimorphus BIOTECH 4039'un kimyasal bileşimi ve antibakteriyel aktiviteleri incelenmiştir.*
Gereç ve Yöntem: *A. dimorphus'un kurutulmuş alg biyokütlesi proksimate ve elemental kompozisyon analizine tabi tutulmuştur. A. dimorphus'un metanolik ekstresi, mikroalglerin toplam fenolik içeriğini (TPC) ve antibakteriyel aktivitesini belirlemek için kullanılmıştır. TPC, Folin-Ciocalteu yöntemi kullanılarak ölçülmüştür. Öte yandan, tıbbi açıdan önemli bakteriyel patojenlere (Staphylococcus epidermidis BIOTECH 10098, penisilin asilaz üreten Bacillus cereus BIOTECH 1509, Listeria monocytogenes BIOTECH 1958) karşı antibakteriyel aktivite, Methicillin-Resistant Staphylococcus aureus BIOTECH 10378, Pseudomonas aeruginosa BIOTECH 1824, penicillin acylase-producing Escherichia coli BIOTECH 1634 ve Serratia marcescens BIOTECH 1748) modifiye Kirby-Bauer difüzyon yöntemi kullanılarak yapılmıştır.*
Sonuç ve Tartışma: *A. dimorphus, sırasıyla 43.19 ± 0.13, 26.92 ± 0.01 ve 14.17 ± 0.04 yüzde bileşimiyle yüksek miktarda protein, kül ve lipid içermektedir. Alg biyokütlesinin elementel besin bileşiminin azalan bir sırayla Ca > Mg > K > Mn > Na > Fe > Zn > Pb > Cu > Cd >Cr olduğu görülmüştür. Ayrıca, A. dimorphus 5.34 ± 0.09 mg GAE/g TPC değerine sahiptir. A. dimorphus ekstraktının Metisiline Dirençli Staphylococcus aureus, S. epidermidis, penisilin asilaz üreten Bacillus cereus'a karşı sırasıyla 15.1 ± 0.3 mm, 13.5 ± 0.1 mm ve 6.82 ± 0.7 mm inhibisyon zonları ile güçlü antibakteriyel aktiviteleri gözlenmiştir. Bu çalışma, A. dimorphus'un endüstriyel ve farmasötik uygulamalarda kullanılabilecek önemli bileşikler ve mikro elementler için iyi bir alternatif kaynak olarak kullanılabileceğini göstermektedir.*
Anahtar Kelimeler: *Antibakteriyel aktivite, Filipinler, kimyasal bileşim, mikroalg*

INTRODUCTION

Green microalgae are named for their grass-green chloroplasts with cell walls that are mostly cellulosic and may exist in several forms such as unicellular, filamentous, colonial and sheet-like. These microalgae are fast growing and are known to have diverse bioactive compounds and macromolecules that are yet to be discovered for biotechnological use, and the characterization as well as isolation of microalgae with the potent bioactive compounds remain the focus of continuing research [1,2]. The capability of several microalgal strains to survive and thrive in extreme environmental stresses results in the formation of a variety of secondary bioactive metabolites, important in agriculture and food industries [1,3]. These active metabolites are produced by these algae to maintain cell signaling pathways and constant intracellular membrane functions in response to sudden environmental changes.

Microalgal biomass together with algae-derived compounds has a vast potential use, from aquaculture and animal feed formulation to nutritional products for human application. Bioactive substances such as fatty acids, carotenoids, phycobilin, vitamins, polysaccharides, and sterols, are found in microalgal biomass [4-6]. Previous studies documented the successful production of several bioactive compounds from a number of economically-important microalgal species, including β-carotene and other carotenoids in *Dunaliella salina*; lutein in *Muriellopsis* sp.; zeaxanthin and lutein in *Scenedesmus almeriensis*; polyphenolic compounds and chlorophyll in *Synechocystis* sp.; lutein and astaxanthin in *Chlorella zofingiensis*; carotenoids and astaxanthin in *Haematococcus pluvialis* and *Chlorococcum* sp.; carotenoids in *Nanochloropsis oculata;* and bioactive peptide in green microalga, *Chlorella ellipsoidea* [4-6]. Bioactive metabolites aid microalgal cells in their ecological interactions with their environment, including cell to cell signaling and protection against competitors and predators [1,4]. Metabolites such as phenolics, fatty acids, glycolipids, diketone, terpenes, and alkaloids derived from green microalgae are reported to exhibit antibacterial activities [1,4,8]. These metabolites have been a valuable renewable

source in the development of novel pharmaceuticals, such as anti-cancer, antibiotic, and anti-inflammatory drugs. Microalgae serve as a reliable and good source of bioactive products since it is possible to cultivate these organisms in large scale using bioreactors and isolation of the target bioactive compounds are quite easy as compared to land plants. In addition, growth can be controlled (using culture media) so that the harvested algal biomass does not contain harmful substances such as pesticides and herbicides [6-8].

Acutodesmus dimorphus is a freshwater green microalga characterized by having uninucleated and spindle-shaped cells that forms a coenobia with linear chain of cells (2-4 cells). Previous studies documented that this microalga could produce bioactive compounds (such as gibberillins, cytokinins and auxins) which can be harnessed as biostimulants in biofertilizer formulation [3]. In addition, chemical composition of species belonging to the genera *Acutodesmus* were documented to possess high concentration of protein (about 30-40%) in their dried biomass showing the potential of these microalgae as alternative source of single cell proteins for food and feed application [3,5,6]. However, studies on the antibacterial activities of *A. dimorphus* are non-existent, particularly regarding the correlation of chemical constituents of the extract and its antimicrobial properties. Thus, it is necessary to search for novel sources of bioactive compounds from *A. dimorphus* as well as other green microalgae and assess their biological activities [6-8]. This study was done to assess the chemical composition and evaluate the antibacterial (using Kirby-Bauer inhibition assay) activity of a freshwater green microalgae, *A. dimorphus*.

MATERIAL AND METHOD

Microalgal Culture and Mass Production

The green microalga, *A. dimorphus* BIOTECH 4039 was obtained from a culture collection from the University of the Philippines Los Baños at PNCM-BIOTECH. Initially, 100 ml of *A. dimorphus* culture was transferred into three 1 L borosilicate flasks with BG 11 culture medium [9]. The components as well as the media were all sterilized at 15 psi for 15 min. Mass production of *A. dimorphus* was done for 16 days under 12:12 light condition (light intensity of white lamps is 120 µmol photons m^{-2} s^{-1}) and kept at 24 ± 2 °C. The culture set up was bubbled continuously with filtered air from an air pump with a superficial gas velocity of 300 ml min^{-1}. The biomass was collected via centrifugation for 10 min at a speed set at 10,000 rpm. The algal pellet was freeze-dried using a lyophilizer (Virtis Freeze mobile 25 SL) to obtain dried biomass [9].

Preparation of Microalgal Extract

The algal extract was prepared by subjecting 1 gram of freezed-dried biomass of *A. dimorphus* in 20 ml methanol (continuous stirring for 1 hr) placed in an ultrasonic bath for 30 minutes. The reaction mixture was centrifuged for 20 min at a speed of 12,000 rpm at room temperature. The crude algal extract was further concentrated via a rotary evaporator under reduced pressure set at 40°C and was kept under refrigerated condition (4°C) before use in the biological assays done in the study [8,9].

Proximate Composition Analysis

Moisture Content

About 3 grams of well mixed *A. dimorphus* biomass was weighed in tared evaporating dish. The alga was placed in an oven, set at 105 ^{o}C for at least 5 hours. It was then placed in a desiccator, allowed to cool to room temperature, and weighed. The sample was returned to the oven for another 30 min, cooled, and reweighed. The procedure was repeated until consecutive weighing does not differ by more than 0.001 gram [2,7].

Ash Content

The *A. dimorphus* biomass was ignited in a muffled furnace at 550^{o}C for 2 hours. It was then cooled to 50^{o}C before placing in a desiccator. The sample was cooled to room temperature and weighed;

re-ignited repeatedly for 30 minute intervals until there was no more loss in weight [9,10]. The ash was calculated as follows:

$$\textbf{Ash Content}\ (\%) = \left(\frac{\textbf{Weight}_{ash}}{\textbf{Weight}_{sample}}\right) \times \mathbf{100}$$

Fat Content (Soxhlet Method)

About one gram of *A. dimorphus* biomass was weighed in a thimble made of filter paper and dried in an oven for 2 hours. The treated *A. dimorphus* biomass was transferred in the extractor (using tared Soxhlet flask) and extracted using ether for 16 hours. After extraction, the sample was removed and the solvent recovered. The Soxhlet flask containing the lipid was dried in a hot plate for 5 min or until the solvent were removed, then cooled and weighed [7,9,10]. Crude fat was calculated as follows:

$$\textbf{Lipid Content}\ (\%) = \left(\frac{\textbf{Weight}_{lipid}}{\textbf{Weight}_{sample}}\right) \times \mathbf{100}$$

Crude Fiber Content (Weende Method)

About 0.3 gram of fat-free *A. dimorphus* biomass was weighed using a 500 ml Erlenmeyer Flask and 200 ml of H_2SO_4 (boiling condition) was added in the container. The flask was attached to a condenser and heated for exactly for 30 min. The flask was rotated frequently to ensure that the materials on the sides of the flask are in contact with the solution. After 30 min, the mixture was immediately filtered using a linen cloth in a stemless funnel and immediately washed with hot distilled water until washings are no longer acid. The residue in cloth was washed back to Erlenmeyer flask using boiling 200 ml NaOH. The flask is attached to a condenser and boiled again for 30 min. The residue was immediately filtered thru cloth in a funnel, washed with sterile distilled water, and transferred quantitatively back to flask. The residue was filtered through gooch crucible prepared with a thin layer of asbestos. The crucible and contents were dried at 110°C to constant weight and ignited at 600°C until carbonaceous matter has been consumed. The loss in weight was reported as crude fiber [7,9,10].

Crude Protein (Kjeldahl) Method

Protein content was obtained by analysis of total nitrogen using the conventional Kjeldahl method and multiplying the amount of total nitrogen by a specific protein factor suitable for the sample. Protein was expressed in g per 100 g edible portion [7,9,10].

Carbohydrate or Nitrogen Free extract Content

Carbohydrate content was calculated by subtracting the sum of crude fat, moisture, ash, crude fiber and crude protein from 100. Zero value was assigned to carbohydrates if the sum of water, protein, fat and ash is more than 100 [7,9,10].

$$\%\textbf{Carbohydrate} = \mathbf{100} - (\%\textbf{Moisture Content} + \%\textbf{Protein} + \%\textbf{Fat} + \%\textbf{Ash})$$

Elemental Composition Analysis

The biomass of *A. dimorphus* was treated to dry ashing using the standard Association of Official Analytical Chemists (AOAC) methods [7,10]. Initially, 1 g of *A. dimorphus* biomass was placed in a crucible and dried at 550°C for 5 h using a muffle furnace. The drying method was repeated until a grayish or white residue was observed and the difference in the weight of the sample is less than 0.05%. The residue was dissolved in 10 ml of concentrated HCl (1:1 ratio) and by heating slowly the reaction mixture. The solution was shortly placed to heat (hot plate with a temperature of 100°C) to dissolve further the remaining ash. The collected solution was cooled and filtered (using a Whatman filter paper) and transferred in a flask. Quantification and detection of sodium, calcium, magnesium, manganese,

zinc, potassium, chromium, cadmium, iron, lead, and copper using an atomic absorption spectrophotometer Perkin Elmer AAnalyst 400 [7].

Determination of Total Phenolic Content (TPC)

The TPC of *A. dimorphus* was estimated using the methods done by Nuñez-Selles et al. [11]. Briefly, about 0.5 ml of *A. dimorphus* crude extract was added with 0.5 ml of Folin-Ciocalteau's reagent and 0.5 ml 10% sodium carbonate solution for one minute. The mixture was kept for 5 min at normal room temperature. The volume of the reaction mixture was adjusted to 5 ml using sterile distilled water. Optical density (absorbance reading) of the reaction mixture was measured using an Ultraviolet-Visible spectrophotometer at a wavelength of 720 nm. The TPC was given as milligram of gallic acid equivalent (GAE) per gram (calibration curve equation: $y = 0.06415x - 0.0140$, $R^2 = 0.9978$) [2,11].

Antibacterial Activity

A. dimorphus crude extract was tested against four Gram-positive bacteria (*Staphylococcus epidermidis* BIOTECH 10098, penicillin acylase-producing *Bacillus cereus* BIOTECH 1509, *Listeria monocytogenes* BIOTECH 1958, and Methicillin-Resistant *Staphylococcus aureus* (MRSA) BIOTECH 10378) and three Gram-negative bacteria (*Pseudomonas aeruginosa* BIOTECH 1824, penicillin acylase-producing *Escherichia coli* BIOTECH 1634, and *Serratia marcescens* BIOTECH 1748) using cylinder cup assay (Modified Kirby-Bauer Method). All bacteria were pre-cultured using Luria Bertani (LB) broth medium and incubated for 24 hours at 37°C under shaking condition. The viability and purity of each bacteria were regularly monitored by doing regular morphological characterization and biochemical tests (Gram staining, coagulase test, starch hydrolysis test, oxidase test, methyl red/ Voges-Proskauer (MRVP) test, citrate utilization test, nitrate and urease test) [2,7,9].

Cylinder cup assay (Modified Kirby-Bauer diffusion method) was used to check the antimicrobial activity of the algal extract [2,9,12]. Initially, 10 ml of sterile Mueller-Hilton agar was placed in sterile plates and allowed to solidify. Five milliliters of top agar with 0.5 ml of bacterial inoculum (cell density of $1x\ 10^6$ cells/ml) was then added. Bacterial density used in top agar was adjusted to equal turbidity of 0.5 McFarlands. Four cylinder cups were placed on the agar surface. Two cylinder cups were filled with 0.1 ml of *A. dimorphus* extract while the other two cups were each filled with 0.1 ml of sterile distilled water (negative control) and 0.1 ml 1000 ppm tetracycline (positive control). Each plate was then incubated at 35°C for 24 hours [2,9,12]. After the incubation period, zones of inhibition were measured for *A. dimorphus* extract as well as the standard antibiotic and are expressed as activity percentage. Antibacterial activity of *A. dimorphus* extract was classified as weak (inhibition zone < 50%), moderate (inhibition zone 50-70%), or strong (inhibition zone ≥ 70%) [12].

$$\textit{Antibacterial Activity}\ (\%) = \frac{A}{B}\ x\ 100$$

Where: A = clear zone of *A. dimorphus* extract (mm) and B = clear zone of antibiotic (mm)

Statistical Analyses

The data from the different assays and analysis are given as means ± standard deviations of three experimental replicates and was computed using MS Office Excel 2019.

RESULT AND DISCUSSION

Proximate Composition Analysis

Proximate composition is a method used to quantitatively quantify the nutritional component of a sample. The method is composed of determining ash, moisture, crude protein, crude fiber, and crude fat. Even though proximate analysis does not provide a complete nutritional assay, the method is easy to conduct and is considered as an inexpensive assay to assess the nutritional quality of a sample. Thus, determining the chemical composition of microalgae will provide valuable information on the potential of a sample (eg. algal biomass) for food and industrial application [13].

The biomass of *Acutodesmus dimorphus* BIOTECH 4039 contained high amounts of protein, ash, and crude fat (lipid) with percent composition of 43.19 ± 0.13, 26.92 ± 0.01, and 14.17 ± 0.04, respectively (Table 1). The result observed in this study is similar to earlier studies documenting that proximate composition of different green microalgal strains reported protein as the most copious biomolecule of the alga making up to 70% of the overall dry weight biomass [14,15,16]. High concentration of protein was observed in *A. dimorphus* biomass showing the potential of this alga as alternative source of single cell proteins for food and feed application. Protein is typically the major biochemical composition of microalgae that is affected by growth medium and growth stage as what was observed in this study. Rapidly growing microalgal cells (cells that are in exponential phase of their life cycle) contains high amounts of protein as well as low carbohydrate content. However, when cells enters the stationary phase, majority of the carbon (in the algal cell) is being used to generate lipids [17]. The ash content of *A. dimorphus* was greater than those documented for *Phaeodactylum tricornutum* (17.0%), *Nannochloropsis granulata* (6.70%), *Scenedesmus quadricauda* (25.77%), and *Neochloris oleoabundans* (16.70%) [9, 15]. The ash content of *A. dimorphus* shows that high mineral content is present in the algal biomass. Crude fat includes true fats and various lipid substances such as fatty acids, lecithin and pigments extracted from microalgae by Soxhlet method using ether as solvent [10]. In this study, crude fat content of *A. dimorphus* is higher as compared to those observed by Tibbets [15] from *Tetraselmis chuii* (12.3%) and *Porphyridium aerugineum* (13.7%) grown in photobioreactors. Variation in the amount of cellular lipid are correlated to algal growth stage. Overall, total fatty acids increase with late algal growth stage (especially at stationary phase of growth where lipid accumulation in algal cells is observed). And also, under stressful conditions several strains of microalga change their biosynthetic pathways in generation of neutral lipids (about 20–50% dry cell weight), mostly in the form of triacylglycerol, allowing microalgae to tolerate unfavorable growth conditions [18]. The current study also showed that dominant components of *A. dimorphus* biomass aside from ash, protein and lipids were moisture (4.15 ± 0.11%), carbohydrates (6.45 ± 0.02%), and crude fiber (5.12 ± 0.17%). The chemical composition of *A. dimorphus* differs when compared to other microalgal species [2,9]. Differences on the proximate composition of microalgae may be due to variations in culture growth condition, by which these microalgae are signaled to stimulate or inhibit the metabolic formation of several important biochemical components.

Table 1. Proximate analysis composition of *Acutodesmus dimorphus*.

Proximate composition	Percent composition (%)
Ash Content	26.92 ± 0.01
Moisture Content	4.15 ± 0.11
Carbohydrate	6.45 ± 0.02
Crude Fiber	5.12 ± 0.17
Crude Fat	14.17 ± 0.04
Crude Protein	43.19 ± 0.13

Elemental Composition Analysis

Assessment of minerals present in algal biomass shows important information of the nutrient composition of the alga. Ash is simply the total mineral content of the microalgal sample. Naturally, ash from combustion of algal biomass is composed of high concentration of elemental nutrients, such as calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), and sodium (Na), as well as heavy metals. Among other heavy metals (mineral elements), chromium (Cr), zinc (Zn) arsenic (As), manganese (Mn), copper (Cu), cobalt (Co), nickel (Ni), and molybdenum (Mo) are the trace and minor elements that exists at varying concentrations in microalgae biomass [13].

The average concentration of each mineral components in *A. dimorphus* is presented in Table 2. The elemental composition and distribution in *A. dimorphus* biomass were noted to be in a decreasing order of Ca > Mg > K > Mn > Na > Fe > Zn > Pb > Cu > Cd >Cr. *Acutodesmus dimorphus* has high

amounts of minerals (calcium, magnesium, potassium, and manganese), like those documented for *Scenedesmus* sp., *Navicula* sp., *Oscillatoria* sp., *Spirulina* sp., and *Scenedesmus quadricauda* [9,14]. Calcium (10,271.11 ± 9.01 ppm) is the dominant microelement in *A. dimorphus* biomass, followed by magnesium (9,934.32 ± 121 ppm), potassium (7,103 ± 0.61 ppm), and manganese (9,870.42 ± 1.03 ppm). This observation may be due to the influence of the concentration of inorganic compounds and salts presents in the culture medium where the algae grow. Microelements such as copper, zinc, iron, cadmium, and lead are also present in *A. dimorphus* biomass which is below the toxicity limits. Thus, confirming the potential application of this microalgae as good source of important microelements that can be use in industrial and pharmaceutical application. Other important micro-elements like calcium, iron, and potassium were also observed in high amounts showing the potential use of *A. dimorphus* as additives to improve food quality needed to address malnutrition that is timely and relevant to several countries in the world [1,2,14].

Table 2. Concentrations and composition of micro and macro-elements of *Acutodesmus dimorphus*.

Microalga	Elemental Parameter* (in ppm)										
	Ca	**Mg**	**Na**	**Mn**	**K**	**Fe**	**Zn**	**Cu**	**Pb**	**Cr**	**Cd**
Acutodesmus dimorphus	10,271.11 ± 9.01	9,924.32 ± 121	3,197 ± 102	9,870.42 ± 1.03	7,103 ± 0.61	398.44 ± 1.94	3.15 ± 0.31	1.16 ± 0.01	1.32 ± 0.13	0.89 ± 0.05	0.97 ± 0.03

* All experimental datas are expressed as mean ± standard deviation (n = 3)

Total Phenolic Content

Phenolic compounds (such as gallic acids, kaempferol, and quercetin) are group of chemical substances commonly found in several microalgal species. These metabolites are known for its bioactive properties such as antimicrobial, cytotoxicity and antioxidant properties which can be used as unique sources of bioactive ingredient for generation and synthesis of new drugs [1,20]. The total phenolic content (TPC) of *A. dimorphus* is 5.34 ± 0.09 mg GAE/g extract. It is greater than those previously reported phenolic contents of microalgae such as *Botryococcus braunii, Chaetoceros calcitrans, Chlorella vulgaris, Haematococcus pluvialis, Nannochloropsis ornata, Neochloris oleoabundans, Parachlorella kessleri, Phaeodactylum tricornutum, Porphyridium cruentum, Tetraselmis suecica,* and *Scenedesmus obliquus* with TPC of 1.99 ± 0.17, 1.84 ± 0.11, 1.47 ± 0.16, 1.23 ± 0.06, 2.04 ± 0.35, 3.73 ± 0.24, 1.38 ± 0.16, 3.75 ± 0.46, 1.71 ± 0.57, and 1.94 ± 0.16 mg GAE/g, respectively [19]. However, Safafar et. al. [20] showed that biomass of *Desmodesmus* sp., *Nannochloropsis salina, Nannochloropsis limnetica,* and *Chlorella sorokiniana* have higher TPC than *A. dimorphus*, which are 7.72 ± 0.08, 6.45 ± 0.25, 5.78 ± 0.12, and 5.86 ± 0.06 mg GAE/g, respectively. Variations in the TPC values among different species of microalgae is dependent on several factors such as solvent type, polarity of extractant (solvent), and solubility of algal phenolic compounds [11,12].

Antibacterial Activity

Microalgae are novel sources of secondary metabolites for pharmaceutical application. Bioactive metabolites produced by different strains of green microalgae include carotenoids, phenolic compounds, alkaloids, terpenes, fatty acids, and other small bioactive metabolites [21]. These intracellular and extracellular metabolites can serve as bioactive substances which are reported to have anticancer, antibacterial, antioxidant, and antiviral properties [21,22,23]. *A. dimorphus* exhibited antibacterial activities against *S. epidermidis,* penicillin acylase-producing *B. cereus,* and Methicillin-resistant *S. aureus* (MRSA). Potent antibacterial activities of *A. dimorphus* extract were observed against MRSA and *S. epidermidis* with zones of inhibition of 15.1 ± 0.3 mm and 13.5 ± 0.1 mm, respectively (Table 3 and Figure 1). On the other hand, *A. dimorphus* extract showed a weak antibacterial activity against penicillin acylase-producing *Bacillus cereus* with zone of inhibition of 6.82 ± 0.7 mm. The findings of this investigation are comparable to that observed by Alsenani et. al. [24] wherein methanol and ethanol extracts of *Isochrysis galbana* and *Chlorella* sp. showed antibacterial activities against *Staphylococcus*

aureus and *S. epidermidis* with zones of inhibition of 16.67 ± 0.58 mm and 18.33± 0.55 mm, respectively. In addition, Smith et al. [25,26] reported that fatty acids (palmitoleic acid eicosapentaenoic acid (EPA), and hexadecatrienoic acid) from *Phaeodactylum tricornutum* showed antagonistic activity towards MRSA [26]. The potent antibacterial properties of *A. dimorphus* extract against bacterial pathogens may also be due to metabolites such as phenolic compounds, alkaloids, gallic acids, and terpenoids that are present in microalgal cells [26,27,28]. To the best of our knowledge, this study is the first to report the antibacterial activities of *A. dimorphus* against *S. epidermidis*, penicillin acylase-producing *Bacillus cereus*, and MRSA.

Table 3. Antibacterial activities of *Acutodesmus dimorphus* extract.

Sample	Antibacterial Activity[a]						
	Gram-positive bacteria				Gram-negative bacteria		
	***Staphylococcus epidermidis* BIOTECH 10098**	**Penicillin acylase-producing *Bacillus cereus* BIOTECH 1509**	***Listeria monocytogenes* BIOTECH 1958**	**Methicillin-Resistant *Staphylococcus aureus* BIOTECH 10378**	***Pseudomonas aeruginosa* BIOTECH 1824**	**Penicillin acylase-producing *Escherichia coli* BIOTECH 1634**	***Serratia marcescens* BIOTECH 1748**
***Acutodesmus dimorphus* BIOTECH 4039**	13.5 ± 0.1 (79.88***)	6.82 ± 0.7 (33.43*)	-	15.1 ± 0.3 (88.30***)	-	-	-
Tetracycline[b]	16.9 ± 0.7	20.4 ± 0.2	15.8 ± 0.3	17.1 ± 0.4	19.7 ± 0.1	18.5 ± 0.2	15.7 ± 0.9

Note: [a]Antibacterial activity percentage (%) of algal extracts in contrast to tetracycline: inhibition zone (mm) of algal extract/ inhibition zone (mm) of antibiotic (tetracycline): *** strong inhibition (≥ 70%), **moderate inhibition (50-70%), and *weak inhibition (< 50%) against bacterial pathogen. [b]positive control (antibiotic). (-) no zone of inhibition.

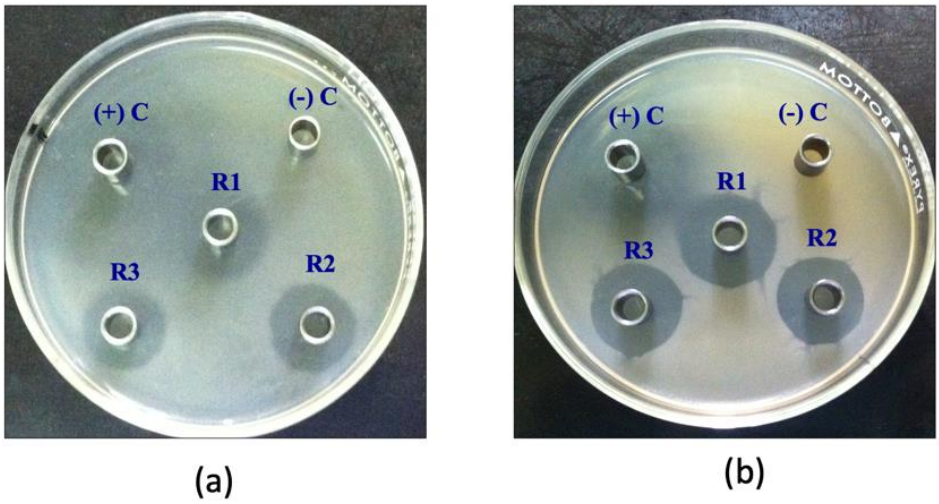

Figure 1. Zones of inhibition exhibited by *A. dimorphus* methanol extract against (a) *Staphylococcus epidermidis* and (b) Methicillin-Resistant *Staphylococcus aureus* growth. (+C) tetracycline/positive control, (-C) methanol/negative control, (R1, R2, R3) replicates 1-3.

The study also documented that *A. dimorphus* methanol extract do not have antibacterial activities against *Pseudomonas aeruginosa*, penicillin acylase-producing *Escherichia coli*, and *Serratia marcescens* (Table 3). Generally, methanolic extract of *A. dimorphus* is considered more effective in inhibiting Gram-positive strains of bacteria than Gram-negative strains. Variations in the antagonistic activities of *A. dimorphus* extract may be due to cell wall differences among the groups of bacteria.

Gram-negative bacteria have multilayered cell walls that can serve as an additional protection against bioactive substances present in *A. dimorphus* extract [1,2,27,28,29]. Additional experimental research that will concentrate on the isolation and identification of the bioactive compounds is recommended. Also, an *in vivo* toxicity assays should be conducted to further prove the safety and effectivity of *A. dimorphus* extract for industrial application.

In conclusion, *Acutodesmus dimorphus* biomass is composed mainly of proteins, lipids, and ash. Also, high amounts of phenolic compounds as well as other important minerals such as calcium, magnesium, potassium, and manganese are present in *A. dimorphus* biomass. In addition, potent antibacterial activities against *Staphylococcus epidermidis*, Methicillin-resistant *S. aureus*, and penicillin acylase-producing *Bacillus cereus* were also exhibited by the algal extract. The results of this assay is the first report that documents the antibacterial activities of *Acutodesmus dimorphus* in opposition to these important bacterial pathogens. Thus, confirming the potential application of this microalgae as good alternative source of important compounds and microelements that can be use in industrial and pharmaceutical application.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The author declares that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The author declares that the ethics committee approval is not required for this study.

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