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ANTIBACTERIAL ACTIVITY, TOTAL PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF A GREEN MICROALGA *Desmodesmus sp.* (U-AU2) FROM LOS BAÑOS, LAGUNA (PHILIPPINES)

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ABSTRACT – Methanolic extract of a colonial green microalga *Desmodesmus* sp. (U-AU2) isolated from a rock surface of a cement wall of a building found in Los Baños, Laguna (Philippines), was subjected to microtiter plate dilution assay against a wide spectrum of bacteria. U-AU2 exhibited pronounced activity against Gram-positive bacteria, *Staphylococcus aureus* with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 31.25 and 125.00 µg/ml, respectively. It was moderately active against *Listeria monocytogenes*, Methicillin-Resistant *S. aureus* and *Pseudomonas aeruginosa* (MIC = 250 µg/ml) as well as *Aeromonas hydrophila* (MIC = 1000 µg/ml). Minimum bactericidal concentration (MBC) of 1000 µg/ml was observed against *L. monocytogenes*, Methicillin-Resistant *S. aureus*, *A. hydrophila* and *P. aeruginosa*. On the other hand, no antibacterial activity was observed against *Enterobacter aerogenes*, *Salmonella typhimurium* and *Escherichia coli*. Phenolic content of the methanolic extract was determined using Folin-Ciocalteu reagent and found to have total phenolic content of 652.66 µg GAE/ml. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay and copper reduction antioxidant capacity (CUPRAC) assay. Relative antioxidant efficiency showed that U-AU2 exerted potent radical scavenging activity and high ability of reducing copper ions from Cu (II) to Cu (I) in a concentration dependent manner. The findings further revealed that the copper ion chelating ability as well as the radical scavenging activity of the extracts were dose-dependent and positively correlated to their phenolic content. The results of this study showed that U-AU2 could be used as alternative source of bioactive compounds for pharmaceutical industry.

Keywords: methanolic extract; phenolic content; antioxidant activity; antibacterial activity

INTRODUCTION

Recently, researchers agreed that the rate of discovery from traditional microbial drug producers like actinomycetes and hyphomycetes is decreasing. Because microalgae are largely unexplored, they represent hot spots for discovery of new drugs (Singh *et al.*, 2005). Microalgae are

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diverse group of photosynthetic microorganisms that exist as single cells or as loosely organized clumps of cells (colonies) and are found in both marine and freshwater ecosystems. The capability of microalgae to proliferate or survive over a broad range of environmental conditions results in the production of several secondary metabolites, which are of significant value in several biotechnological fields such as aquaculture, health, food and pharmaceutical industries (Andersen, 1996). Microalgae produce a number of structurally novel and biologically active secondary metabolites that act as chemical defense against herbivores, competition for space and predation. These metabolites are reported to have bacteriostatic, bactericidal, antifungal, antiviral, anti-inflammatory, and antitumor activity (Simić *et al*., 2012; Uma *et al.,* 2011). Biologically active compounds such as carotenoids, polyunsaturated fatty acids (PUFA), polyphenols, phycobilins, polysaccharides, vitamins, sterols, and other antioxidants are also present in microalgae (Choochote *et al.,* 2014). These compounds are largely unexplored natural sources of bioactive ingredients and are gaining much attention since they can lead to the discovery of new compounds or bioactivities. Microalgae with high contents of bioactive compounds as well as high growth rate in the natural environment have yet to be exploited for biotechnological purposes. Hence, the continuing focus on research about the isolation and characterization of such microalgae.

Several species of green microalgae have been proven to have antibacterial activity *in vitro* against both Gram-positive and Gram-negative bacteria. Strains of *Scenedesmus* (*S. obliquus*, and *S. quadricauda*) were reported to show antibacterial activities against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus subtilis* (Abedin and Taha, 2008; Guedes *et al*., 2011). In addition to this, Bhagavathy *et al.,* (2011) reported that various organic solvent extracts and purified pigments (carotenoid, chlorophyll) of *Chlorococcum humicola* inhibit the growth of *E. coli, Salmonella typhimurium, Klebsiella pneumoniae, Vibrio cholera, P. aeruginosa, S. aurues,* and *B. subtilis.* In a study made by Ghasemi *et al.,* (2007), aqueous, methanolic and hexanolic extracts of *Chlorella vulgaris* showed growth inhibitory effect against *S. epidermidis, S. aureus,* and *S. typhi.* Green microalgae serve as good sources of antibacterial substances that could have numerous applications in the pharmaceutical industry.

Antioxidants, either synthetic or natural, are substances that help in preventing oxidation and oxidative damage to cells by scavenging free (oxygen or hydroxyl) radicals such as superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH) and singlet oxygen ($^1O_2$) (Simić *et al*., 2012). The possibility of using microalgae as source of antioxidant compounds are supported by some earlier studies wherein successful isolation of various types of antioxidant compounds including lutein in *Muriellopsis* sp.; β-carotene in *Dunaliella salina*; lutein and zeaxanthin in *Scenedesmus almeriensis*; astaxanthin and lutein in *Chlorella zofingensis*; carotenoids in *Haematococcus pluvialis*; astaxanthin in *H. pluvialis* and *Chlorococcum* sp.; α-tocopherol in *Nanochloropsis oculata*; novel peptide (Leu-Asn-Gly-Asp-Val-Trp (702.2 Da)) in *C. ellispoidea*; and phenolic compounds (Phenol,2,4-bis (1,1-dimethylethyl) and Phenol,2,6-bis (1,1-dimethylethyl)-4-methyl) in *Synechocystis* sp. have been reported (Plaza, *et al.,* 2010; Ko, *et al.,* 2012; Choochote *et al.,* 2014). Researches on the antioxidant activity of microalgae are few, specifically regarding the relationship between their antioxidant capacity and phenolic content. Thus, it is valuable to assess the relationship between these two parameters and to identify some rich sources of antioxidants from microalgae (Li *et al*., 2007).

Desmodesmus spp. of the Chlorophyceae are cosmopolitan microalgae, occurring widespread mainly in the subtropical and tropical regions. These microalgae are considered to possess high adaptability and tolerance to severe conditions such as high temperature and desiccation but grows best at relatively high light intensity and moderate temperature. The cells of *Desmodesmus* spp. are greenish, enclosed within a common mucilaginous sheath and occur as either obovate or ellipsoidal and are heavily-granulated. These organisms are usually arranged in tetrads but can also be observed in single and double-celled forms. The colonial forms always have spiny projections that surround the whole cell surface. These forms were developed through production of daughter cells within mother cell (El Semary, 2011).

In this study, a local isolate of *Demodesmus* sp. (morphologically similar to *D. abundans*) was studied for its antibacterial activity, phenolic content and antioxidant activity. DPPH-radical scavenging activity and cupric ion reducing ability assays were utilized to characterize the antioxidant activity of the

green microalgae. To date, several studies (in other countries) reported about the bioactivity potential of several strains of *Desmodesmus* sp., but no reported data exist on its biological activity in the Philippines. Because of this, it is very much essential to explore the capabilities of local strains of this organism as source of bioactive compounds for biotechnological exploitation and application.

MATERIALS AND METHODS

Mass Cultivation of Algal Isolate

The Phycology Laboratory I, Institute of Biological Sciences (IBS), University of the Philippines Los Baños (UPLB), Philippines, provided the microalga *Desmodesmus* sp. U-AU2 (isolated and deposited by E.DLR. Arguelles) used in the present work. This organism was isolated from a greenish crust sample obtained on rock surface of a cement wall at IBS greenhouse. The microalgae was cultivated in large amounts to be used for screening of bioactive compounds. Initially, inoculum development was done in preparation for inoculation of three liters medium. The algal isolate was inoculated into nine sterile bottles containing 100 mL each of liquid BG-11 medium. The bottles were incubated for a period of two to four weeks and were exposed to indirect sunlight with light/dark cycle as that of a normal day/night cycle. After the incubation period, the culture was inoculated to three sterile 6-L glass bottles containing three liters of media (three 100 mL cultures per 6-L bottle). The algal biomass (cell count = 5.12×10^7) was harvested after 22 days (the stationary phase) and centrifuged at 10000 rpm and 4°C, for 10 min. The harvested microalgae were rinsed with distilled water. The preparation was centrifuged again and freeze-dried (using Virtis Freeze mobile 25 SL lyophilizer) for long-term storage.

Preparation of Algal Extract for Antimicrobial Screening

The powderized algal biomass was soaked in methanol (1g biomass: 20 ml methanol) overnight with stirring. The extract was filtered through glass funnel and Whatmann No. 1 filter paper. The resulting extract was concentrated to dryness in a rotary evaporator under reduced pressure (at 40°C) until a crude extract was obtained, and was conserved at 4°C.

Micro-dilution Antimicrobial Assay

The two-fold serial dilution technique was used to determine the minimum inhibitory concentration (MIC) of the test sample. Three Gram-positive bacteria (*Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus*, and *Listeria monocytogenes*), and five Gram-negative bacteria (*Aeromonas hydrophila, Pseudomonas aeruginosa, Enterobacter aerogenes, Salmonella typhimurium* and *Escherichia coli*) were used as test organisms. Bacteria were pre-cultured overnight with shaking in Luria Bertani (LB) medium at 37^o C.

In a 96-well microtiter plate, 100 µl of bacterial cultures ($1x\ 10^5$ cells/ml) were added to 100 µl of test sample prepared in various dilutions starting from 1000µg/ml down to 7.8125 µg/ml. Methanol was also included in the plate serving as the negative control. The seeded plate was covered and incubated overnight, after which the minimum inhibitory concentration (MIC) of the algal extract was noted. MIC is defined as the lowest concentration of the test sample (algal extract) that inhibited bacterial growth after a 12-h incubation period. At the end of the incubation period, the plates were evaluated for the presence or absence of microbial growth. The lowest concentration of the algal extract at which there was no apparent growth was considered as the MIC for the extract–microbe combination being examined. As for the controls, the MIC of streptomycin against each bacterial species was similarly determined.

Minimum Bactericidal Concentration (MBC)

The MBC were determined by inoculating one loop of sample in wells that showed no apparent growth from the MIC assays onto nutrient agar and tryptic soy agar plates. The plates were incubated at 30°C, 24 hours and were examined for colony growth or lack of growth for each dilution

subculturing. No bacterial growth indicated that the algal extract was bactericidal at that particular dilution. Growth indicated that the sample was bacteriostatic at that dilution. The lowest concentration displaying no visible growth on agar subculture was considered as MBC value.

Determination of Total Phenolic Content

The total phenolic content was determined by Folin-Ciocalteu method according to the procedure of Nuñez Selles *et al.,* (2002). The sample was diluted with water and to 0.5 ml of the diluted sample, 0.5 ml of Folin-Ciocalteu's phenol reagent (Sigma-Aldrich) and 0.5 ml of 10% Na_2CO_3 were added. After standing for 5 min, 5 mL of sterile distilled water were added. The absorbance readings were measured and recorded at 720 nm (Shimadzu UV-1601 spectrophotometer) with water plus reagent as blank sample. Total phenolic content was computed in a standard curve with gallic acid as reference phenol. The results were expressed as gallic acid equivalents (GAE) since the total phenolic content was determined using the equation acquired from a standard gallic acid calibration curve.

DPPH Radical Scavenging Assay

The synthetic antioxidants butylated hydroxyl-anisole (BHA) and butylated hydroxyl-toluene (BHT) were used as reference antioxidants. Briefly, 100 µl of aliquot of test samples was added to 5.0 ml of 0.1 mM DPPH methanolic solution. The mixture was vortexed and then left to stand at room temperature for 20 min. The absorbance of all the algal sample solutions was measured at 517 nm. The scavenging effect (%) was calculated by using the formula given by Ribeiro *et al.,* (2008).

$$\textbf{Inhibition (\%)} = [(\text{A control} - \text{A sample})/\text{A control}] \times 100$$

Copper Reduction Antioxidant Capacity (CUPRAC) Test

The cupric ion reducing capacities of the algal extract was determined according to Alpinar *et al.,* (2009). For this, 1mL each of 0.01 M $CuCl_2$, 0.0075M neocuproine, and Ammonium acetate ($C_2H_3O_2NH_4$) buffer (1M, pH 7.0) solutions were sequentially added into a sterile test tube. Then, 0.5mL of the algal extract at different concentrations (5, 10, 15, 20, and 25 µg GAE/ml) was mixed, and the total volume was brought up to 4.1mL with sterile distilled water. After 30min incubation at room temperature, the mixture absorbance was measured at 450 nm and recorded against a blank.

Statistical analyses

All data were presented as means ± standard deviations (mean ± SD) of three parallel measurements. Calculation of linear correlation coefficient and correlation analysis were carried out using MS Office Excel 2007.

RESULTS AND DISCUSSION

The IBS-UPLB provided the microalga *Desmodesmus* sp. U-AU2 used in the study. The organism was isolated from a greenish crust sample obtained on rock surface of a cement wall at IBS greenhouse. The cells of this isolate is characterized by being greenish in color, enclosed within a common mucilaginous sheath and occur as either obovate or ellipsoidal and are heavily-granulated. Usually the cells are arranged in tetrads but can also be observed in single and double-celled forms. The colonial forms of this organism always have spiny projections that surround the whole cell surface.

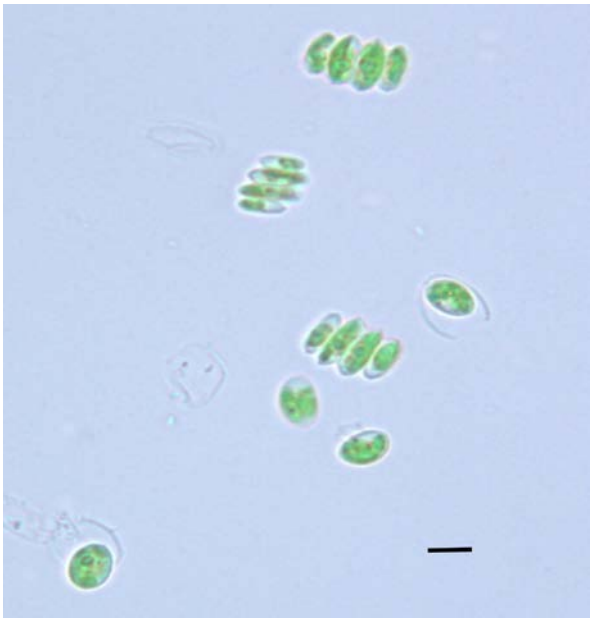

Figure 1. Photomicrograph of *Desmodesmus* sp. (1000x). Scale bar = 5 µm.

Antibacterial Screening

Microalgal strain *Desmodesmus* sp. (U-AU2) was grown autotrophically (using an improvised laboratory scale photobioreactor) in batch culture using BG 11 medium. Methanolic extract of U-AU2 was subjected to antimicrobial assays against a wide spectrum of microorganisms. U-AU2 exhibited pronounced activity against Gram-positive bacteria, *Staphylococcus aureus* with MIC and MBC of 31.25 and 125.00 µg/ml, respectively. It was moderately active against *Listeria monocytogenes,* Methicillin-Resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* (MIC = 250 µg/ml) as well as *Aeromonas hydrophila* (MIC = 1000 µg/ml). Minimum bactericidal activity of 1000 µg/ml was observed against *Listeria monocytogenes,* Methicillin-Resistant *S. aureus, Aeromonas hydrophila* and *P. aeruginosa*. The antibacterial activity of the algal extract was compared to streptomycin (standard antibiotic). In a negative control, methanol had no inhibitory effect on the tested bacterial pathogen. Methanol is considered one of the frequently used solvents for determining MIC of natural antibacterial compounds and are considered as solvent that evidently do not affect the bacterial growth significantly since it is not toxic in minimal amounts (Wadhwani, *et al.*, 2008).

Table 1. Antibacterial activity of *Desmodesmus sp.* (U-AU2)

TEST ORGANISM	MINIMUM INHIBITORY CONCENTRATION (µg/ml)	MINIMUM BACTERICIDAL CONCENTRATION (µg/ml)
Gram-positive bacteria		
Staphylococcus aureus	31.25	125.00
Listeria monocytogenes	250.00	1000.00
Methicillin-Resistant *Staphylococcus aureus*	250.00	1000.00
Gram-negative bacteria		
Pseudomonas aeruginosa	250.00	1000.00
Aeromonas hydrophila	1000.00	1000.00
Escherichia coli	>1000.00	ND
Enterobacter aerogenes	>1000.00	ND
Salmonella typhimurium	>1000.00	ND

*ND = Not Determined

In the present study *Desmodesmus* sp. (U-AU2), when extracted with methanol showed significant activity against some of the bacterial strains tested. The result of the present study is consistent with earlier reports as methanol extracts of U-AU2 showed to be relatively more potent in displaying antibacterial effect. Methanolic extracts of a cyanobacteria (*Microchaete tenera*) and two green algae (*Nitella tenuissima* and *Sphaeroplea annulina*) when tested for in vitro antibacterial activity averse to four bacterial strains, exhibited notable effect against *Pseudomonas aeruginosa* (Prashantkumar, *et al.,* 2006). *Pseudomonas aeruginosa* used in the present study was found to be inhibited by the algal extracts.

Desmodesmus sp. has been documented to have a wide array of antibacterial properties when tested against various pathogenic strains such as *P. aeruginosa, S. aureus* and *E. coli.* A study made by El Semary, 2011 showed that fractions of methanolic extracts of *Desmodesmus sp.* exhibited the high antimicrobial activities against pathogenic bacterial strains including multi-drug resistant *P. aeruginosa, E. coli, S. aureus and B. subtilis.* This result is similar to this study since the microalgal extract showed no activity against *S. typhimurium* but showed a strong activity against *S. aureus* and Methicillin-resistant *S. aureus*. A study made by Desbois *et al.,* (2008) proved that a polyunsaturated fatty acid hexdecatrienoic

acid n4 (in the methanolic extract) showed antimicrobial activity against both Gram-positive and Gram-negative bacteria and is highly active against multidrug resistant *S. aureus*. Turney *et al.* (2006) also indicated that antimicrobial activity can also be associated to volatile compounds (such as hydrogen peroxide, terpenoid, and bromoether, volatile fatty acids compounds). The most common compounds in methanol extract were plasticizer compound, phenols, flavonoid and phytol (acyclic diterpene alcohol), and in acetone extract were phytol, plasticizer compound, ester, and alkenes (Turney, *et al.,* 2006).

Study results showed that methanolic extract of microalgae have effects on the growth of some of Gram-positive and Gram-negative bacterial species. Generally, pathogenic bacteria have varying responses and sensitivity to microalgae methanolic extracts. In most cases, Gram positive would respond more to these extracts than the Gram-negative bacteria. Such difference can be due to genetic or chemical structure of microorganisms and the nature of their cell membrane. Active compounds are typically less effective against Gram-negative bacteria because of their composite, multilayered cell wall structure – which makes penetration of bioactive compound more difficult (Amaro *et al.,* 2011). This explains why the antibacterial activity of the supernatant (methanolic extracts) is more potent against Gram-positive than Gram-negative bacteria. Gram-positive bacteria contains very high level (90-95%) of peptidoglycan and contains phospholipids (5-10%) and lipopolysaccharides, making it suitable medium to entrance and reaction of the antimicrobial factors inside the Gram-positive cell destroying the protein biosynthesis units (DNA and RNA) and cell membrane. On the other hand, the membrane of Gram-negative bacteria has two layers, the outer and inner membranes that are separated by periplasmic space. The outer membrane consists of phospholipids, lipoprotein and mucopolysaccharides and the inner membrane consists of peptidoglycan (glycopeptide) (5-10%). The structural feature of the said bacteria is not suitable for the entrance of antimicrobial agents and thus reduces of its effects on the bacteria (Al-Samary, 1999).

Several important factors such as temperature of incubation, strain selection, incubation period, medium constituents, pH of the culture medium and light intensity can influence the antimicrobial agent production of certain microorganism (Ördög, *et al.,* 2004). Strain selection is a very important factor, as biological activity differs among strains of one species. Changes in growth conditions of microalgae do affect biological activity, as there are optimal conditions for the synthesis of secondary metabolites. These conditions under which the different secondary metabolites are synthesized by an organism must be known in order to maximize the production of the beneficial secondary metabolites (Ördög, *et al.,* 2004) For example, the optimal temperature for growth and mass production of an antimicrobial substance in *Synechococcus leopoliensis* is 35^{o}C (Noaman *et al.,* 2004). Extracellular filtrates from *Calothrix* sp. and *Anabaena* sp., grown at the most suitable conditions for growth at temperature (40±2°C) and light intensity (90–100 µmol photons m^{-2}. s^{-1}) show the potent activity against plant pathogen *Rhizoctonia bataticola* and *Pythium debaryanum* (Radhakrishnan *et al.,* 2009). In this study, such modifications in the cultivation conditions were not done. The microalgal cells were grown in a rich culture media (without limiting any chemical components) and were harvested at stationary phase of growth.

Total Phenolics and Antioxidant Activity

Total phenolic content was determined using Folin-Ciocalteu reagent in terms of gallic acid equivalents (GAE). Antioxidant activity was evaluated using diphenyl- 1,2-picryl hydrazyl (DPPH) free radical scavenging activity assay and reduction of copper ions using the CUPRAC assay. Total phenolic content of the methanolic extract of U-AU2 was observed to be 652.66±0.042 µg GAE /ml.

DPPH Radical Scavenging Assay

The antioxidant activities of the methanolic algal extracts were measured using two assays: DPPH free-radical scavenging assay and cupric ion reducing capacities (CUPRAC assay). The study used DPPH scavenging assay to test the capacity of the antioxidative compounds acting as proton radical scavengers or hydrogen donors. Highly antioxidative substances will cause the change from the purple chromogen radical to the pale yellow hydrazine (Goh *et al.,* 2010). Low absorbance readings correlates to

high amount of scavenged free radicals by the antioxidants. Methanolic extract of the microalgae was further analyzed to check its scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH). Relative antioxidant efficiency is presented in Figure 2. *Desmodesmus* sp. (U-AU2) exerted radical scavenging activity showing inhibition percentage that is concentration dependent. The percentages of antioxidant property of the methanolic extract of U-AU2 at concentrations from 5-25 µg GAE range from 7.34-22.19%. Among the various concentrations of methanolic extract of U-AU2 used, 25µg GAE had the strongest scavenging ability while 5µg GAE had the lowest. The results exhibited that the scavenging activity increases when the concentration of the algal extracts is increased. High percent DPPH radical inhibition of the extract corresponds to high phenolic content on the algal extract. On the other hand, algal extract concentration with low DPPH radical percent inhibition contains relatively low phenolic content.

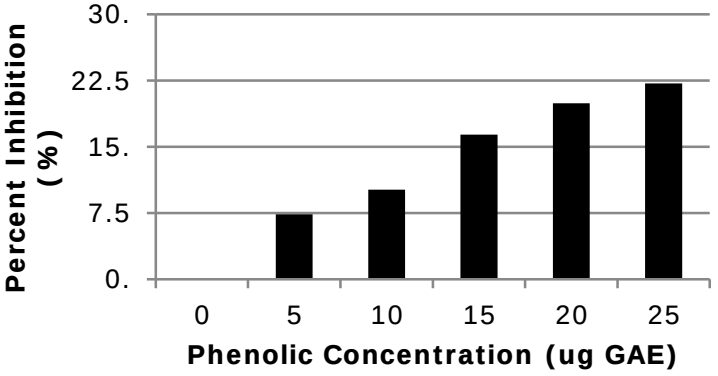

Figure 2. Scavenging effects on DPPH free radical by various concentrations of polyphenols extracted from *Desmodesmus sp* (U-AU2).

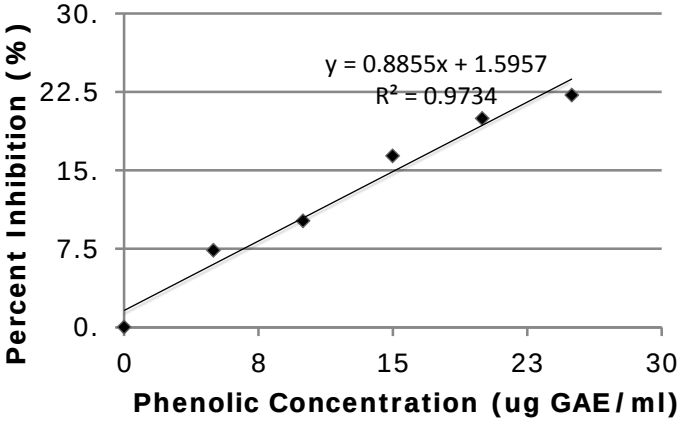

Figure 3. Simple regression correlation between total phenolic content and total antioxidant activity using DPPH Radical Scavenging Assay of *Desmodesmus* sp. U-AU2.

The correlation coefficient (R^2) between antioxidant activity and total phenolic content of U-AU2 using DPPH scavenging assay is shown in Figure 3. Total phenolic content in crude extract of the microalgae is highly correlated to the antioxidant activity (DPPH radical scavenging assay) and the study agrees with the earlier studies (Sushanth and Rajashekhar (2015); Shetty and Sibi (2015)). Strong positive correlation coefficients were observed between total phenolics and DPPH radical scavenging assay for U-AU2 having value of (R^2=0.973). The result of this study shows that phenolic compound enhances the antioxidant properties of the microalga.

CUPRAC Assay

Reducing power of the microalgal extract on copper ions using the CUPRAC assay is shown in Figure 4. CUPRAC assay is a method based on reduction of Cu (II) to Cu (I) by antioxidants. Elevated absorbance readings indicated higher reducing ability of the methanolic extract. All of the samples showed the ability of reducing copper ions from Cu (II) to Cu (I) in a concentration dependent manner. Highest reducing activity was observed at 25 µg GAE/ml of phenolics in microalgal extract when compared to the lower concentration.

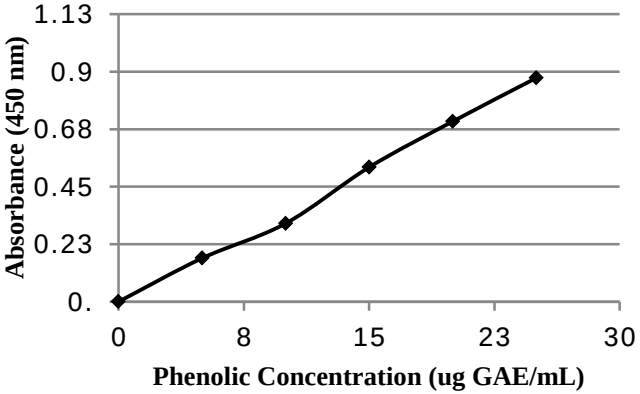

Figure 4. Reducing power of various concentrations of polyphenols extracted from *Desmodesmus* sp (U-AU2) using CUPRAC assay.

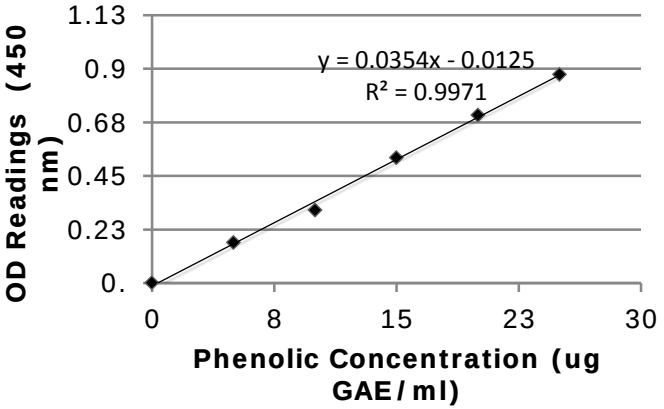

Figure 5. Simple regression correlation between total phenolic content and total antioxidant activity CUPRAC Assay of *Desmodesmus* sp. U-AU2.

At a concentration of 5µg, 10µg, 15µg, 20µg and 25µg of *Desmodesmus* sp. (U-AU2) the absorbances were 0.17, 0.306, 0.526, 0.705, and 0.875 respectively. These results are similar to those obtained from the DPPH assay in which 25µg showed the highest total antioxidant capacity (TAC). The results showed that the antioxidant activity was increased when the concentration of the extract was increased.

The strong correlations (Figure 5) between the result using the CUPRAC assay of measuring antioxidant capacity and the total phenolic content (R^2 = 0.9971) proved that phenolic compounds contribute to the antioxidant activities of these microalga, and therefore could play an important role in the beneficial effects of these alga. In addition, this organism can create diverse kinds of other antioxidants such as pigments, polyunsaturated fatty acids and polysaccharides. Several studies show that a wide range of phenolics exists in microalgae, but further identification of phenolic components is necessary to get more appropriate understanding regarding correlation between phenolics and antioxidant activity (Sushanth and Rajashekhar, 2015).

Antioxidants can be categorized based on their action such as free radical scavenger, metal chelater and oxygen scavenger (Tiwari 2001; Balanquit, *et al.,* 2015). The results of the present study suggested that the algal extracts could inhibit oxidation through the metal chelation mechanism and free radical scavenging. This antioxidant activity can be attributed to phenolic compounds as well as other phycochemical compounds such as carotenoids, polyunsaturated fatty acids (PUFA) and polysaccharides present in the algal extract. These compounds can act as natural antioxidants even though the phenolic contents in algal cells are low. Factors such as polarity of solvents and extraction methods for polyphenols must also be taken into consideration in order to extract the phenolic compounds of interest in the sample. The choice of solvent used for the extraction of phenolic compounds depends on its solubility property (Aili Zakaria, *et al.,* 2011). Phenolic compounds are commonly more soluble in polar organic solvents than in water. The preferable solvents that were commonly used were aqueous mixtures of methanol, ethanol, and acetone. Phenolic compounds extracted may also differ for different species, extraction methods, and solvents used (Aili Zakaria, *et al.,* 2011). These factors must be considered in further works on antioxidant potentials of Philippine microalgae.

CONCLUSION AND RECOMMENDATION

Microalgae and cyanobacteria are a source of many biologically functional substances that deserve attention because of the many health benefits they provide. The study illustrated for the first time the antibacterial activity, antioxidant capacity and phenolic content of a green microalga *Desmodesmus* sp. obtained from Los Baños, Laguna, Philippines. Study results showed that methanolic extract of the alga has antibacterial effect on the growth of some Gram-positive and Gram-negative bacterial species. Further studies on the identification of the active component in the extract as well as the mechanism of action against bacterial cells are necessary to evaluate its potential use in the pharmaceutical industry. The correlation coefficient between the antioxidant capacities and the phenolic contents was significant, indicating that phenolic compounds were a major contributor to the antioxidant capacities of the microalga. Identification of phenolic substances from these microalgae is required to evaluate whether these microalgae contains novel phenolic compounds that are not known from terrestrial plants.

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STATEMENT OF AUTHORSHIP

The senior author prepared the conceptual framework and conducted the experiments and undertook the preparation of the manuscript. The second, third and fourth authors made important recommendations in the conduction of the research, reviewed and finalized the manuscript.

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