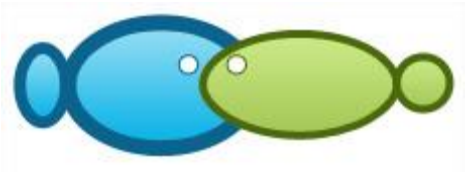

Antioxidant and α-glucosidase inhibitor activity characterization of bioactive components from *Parachlorella kessleri* AUP5

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Abstract. A spherical green microalga, namely *Parachlorella kessleri* AUP5, was isolated from freshwater ponds of Andalas University, Padang, Indonesia and further organic solvents extracts were realised to study antioxidant and α-glucosidase inhibitor activities of this microalga. The studies demonstrated radical scavencing 2,2-diphenyl-1-picrylhydrazyl (DPPH) metahnol extract was found to be 54.64 ± 0.8% at 500 μg ml^{-1} with a total phenolic content of 84.22 mg (GAE) g^{-1}. In the α-glucosidase screening inhibition assay of *Parachlorella kessleri* AUP5, hexane extract was found to be 91.97±0.29 % which was the highest α-glucosidase inhibition followed by ethyl acetate and methanol extract in amount of 84.37±1.89 % and 84.27±0.89 %, at concentration of 250 μg ml^{-1} respectively. Hexane extracts were subsequently characterized using gas chromatography–mass spectrometry for identifying bioactive compounds. The former was found to be enriched with 2,6-Octadien-1-ol, 3,7-dimethyl-acetate (E) (37.53%), 9,12-Octadecadienoic acid (29.25%), and tetradecanoic acid (7%). The presence of these bioactive compounds in *Parachlorella kessleri* AUP5 might make it beneficial as a source of α-glucosidase inhibitor agents. Thus, our findings indicate that freshwater microalgae, namely *Parachlorella kessleri* AUP5 has potential to be a source of promising antioxidant and antihyperglycemic therapeutic agents.
Key Words: microalgae, identification, phenolic.

Introduction. Microalgae are photosynthetic microorganisms which are a potential source of bioactive compounds that can be used for various purposes in pharmaceuticals, cosmetics, bioenergy and bioremediation (Dębowski et al 2020; Perdana et al 2021; Zhang et al 2014). The properties of natural bioactive compounds from microalgae which have made them promising sources for discovering natural compounds are proteins, fatty acids, carbohydrates, chlorophylls, phenolic compounds, and carotenoids (Fu et al 2017; Kreckhoff et al 2019; Wali et al 2020). These bioactive compounds may have great potential for therapeutic activities such antioxidant and cytotoxic activities (Hussein et al 2019; Rodrigues et al 2012), antifungal (Nainangu et al 2020), anti-inflammatory (Lauritano et al 2016), and antidiabetic activity (Kawee-Ai et al 2019). Microalgae grow rapidly and do not compete directly with crops for agricultural land in the process of cultivation (Nuhma et al 2021; Santhakumaran et al 2020). Therefore, efforts to study microalgae as a source of bioactive compounds for pharmaceuticals discovery became an important research direction.

In the last decades, increased attention for microalgae-based pharmaceutical and nutraceutical products has been paid to the commercial and industrial potential of microalgae (Qamar et al 2021; Musifa et al 2023). Several studies reported, microalgae like *Chlorella* sp., *Nannochloropsis* sp., and *Tetraselmis* sp. have the ability to produce valuable secondary metabolites especially for aquaculture applications (Islam et al 2022). Microalgae *Scenedesmus rubescens* showed a great promise for antioxidants and polyphenolic compounds production, at around 48.57 ± 3.99 mg gallic acid equivalent (GAE) g^{-1} (Morowvat & Ghasemi 2016). In addition, apart from being a source of

bioactive compounds such polyphenols, microalgae and cyanobacteria also produce carotenoid and chlorophyll that has attracted the interest of food industries and related sectors due their ability as antioxidant and food additives (Almendinger et al 2021).The antioxidant capacity of biomass and extracts of microalgae works not only because of the presence of one compound, also because of the presence of a mixture of different antioxidant aromatic compounds such aromatic compounds, carotenoids, and aromatic amino acids (Goiris et al 2012).

Microalgae *Parachlorella* sp. is a chlorophyte with the ability to accumulate amounts of lipids and carotenoid content, which eventually have made this microalga a functional food and nutraceutical (Heo et al 2018; Juárez et al 2011; Shaikh et al 2019). This microalga was also reported to contain high amount of protein and carbohydrates in the addition of molasses to culture during cultivation (Juárez et al 2011; Piasecka et al 2019). Several studies reported this microalga has higher biomass productivity and chemical composition such as chlorophyll, carotenoids, carbohydrates and lipids, which determines this microalgae to have good antioxidant properties (Sharma et al 2019). However, the phytochemical compositions, as well as α-glucosidase inhibitor activity and antioxidant activities of this microalga haven't been studied enough.

In the present study, a green microalga was isolated, and the species was identified as *Parachlorella kessleri* AUP5. Consequently, organic solvent extraction of this isolated microalgae was carried out to evaluate its bioactive compounds, and further investigation for any potential for antioxidant and α-glucosidase inhibitory activities of the extracts was conducted. After all, the bioactive compounds present in the most potent extracts were identified.

Material and Method

Isolation, identification and cultivation of microalgae. A water sample was collected in sterile glass bottles from the water surface of the pond in Padang City (West Sumatera), around Andalas University, Indonesia, in 2019. The sample was brought to the laboratory and inoculated into sterilized Bold's Basald medium (BBM) at room temperature (25 ± 2°C) for 1 week under fluorescent lamps (±2200 lux Philips, Indonesia). Bold basal medium (BBM) that was used to maintain the microalgal culture had the following composition: $NaNO_3$ – 0.25 g L^{-1}, KH_2PO_4 – 0.175 g L^{-1}, K_2HPO_4 – 0.075 g L^{-1}, NaCl – 0.025 g L^{-1}, $FeSO_4 \cdot 7H_2O$ – 0.00498 g L^{-1}, $CaCl_2 \cdot 2H_2O$ – 0.025 g L^{-1}, $Na_2EDTA \cdot 2H_2O$ – 0.01 g L^{-1}, $MgSO_4 \cdot 7H_2O$ – 0.075 g L^{-1}, H_3BO_3 – 0.00805 g L^{-1}, and 1 mL of trace elements solution consisting of $Co(NO_3)_2 \cdot 6H_2O$ – 0.0494 g L^{-1}, $MnCl_2 \cdot 4H_2O$ – 1.81 g L^{-1}, $ZnSO_4$ – 0.222 g L^{-1}, $NaMoO_4 \cdot 5H_2O$ – 0.39 g L^{-1}, $CuSO_4 \cdot 5H_2O$ – 0.079 g L^{-1}, $H_3BO_3 \cdot 2H_2O$ – 2.86 g L^{-1}, and a pH of 6.8 (Aburai et al 2013). After an enrichment step, sample cultures were used for further isolation using serial dilution combined with micropipette washing technique method and purified microalgae was monitored on optical microscope (1000X magnification). The isolated microalga was further grown in BBM, at room temperature (25 ± 2°C) under fluorescent lamps (±2200 lux Philips, Indonesia). Microalgae growth was evaluated daily from the optical density (OD) measurements at 680 nm with UV-VIS spectrophotometer (Genesys 20) (Ricken et al 2019; Sekatresna et al 2016). The microalgae were morphologically identified using an optical microscope (1000X magnification) and purified microalgae culture was centrifuged at 13000 rpm, for 1 ml aliquot of the cultures, for 10 min at room temperature. The microalgae pellet was washed twice with sterile deionized water and stored in the freezer at -20°C before using it in DNA extraction for molecular identification.

PCR amplification of 18S rRNA genes. Genomic DNA of purified microalgae sample was extracted and purified using a DNA purification Dneasy® Tissue Kit (Qiagen Sciences, Valencia, Calif., Md, USA), according to the manufacturer's protocol. Briefly, 10 mL of microalgae culture was centrifuged at 14000 rpm. The resulting pellet was dissolved in 400 µL buffer AP1 and 4 µL RNAse, then vortexed and incubated at 65°C for 10 minutes then, 130 µL buffer P was added. The mixtures were incubated for 5 min with ice cubes, then centrifuged at 14000 rpm for 5 minutes to get the filtrate. After that, the

filtrate was put into Qiashedder spin column and centrifuged for 2 minutes at 14.000 rpm. The pellet was transferred to a new tube and 150 µL of buffer AW1 were added. DNA mixture was transferred into a DNeasy mini spin column and centrifuged for 1 minute at 8000 rpm. The filtrate was removed, and the matrix transferred to a new column tube. A total of 500 µL buffer AW2 was added to the tube and centrifuged for 1 minute at a speed of 8000 rpm. Then the filtrate was discarded and 500 µL of buffer AW2 was added then centrifuged at 14000 rpm for 2 minutes. To the DNA trapped in the matrix, 100 µL of buffer AE was added, which was heated and incubated for 5 minutes at room temperature and centrifuged for 1 minute at 8000 rpm. This process was repeated twice. Furthermore, a qualitative test was carried out on the resulting DNA isolation (Quick-Start Protocol Qiagen) (Sekatresna et al 2016; Suarez-montes et al 2022).

Phylogenetic analysis. Microalgae DNA was isolated from pure culture and polymerase chain reaction (PCR) amplification of almost the entire length of 18S rDNA fragment (Elshobary et al 2020). The PCR reaction was performed using a pair of eukaryotic 18s rDNA primers with a forward primer pair of 5'-CCTGGTTGATCCTGCCAG-3' and reverse 5' TTGATCCTTCTGCAGGTTCA 3'. The Polymerase Chain Reaction (PCR) contained 50 ng microalgal genomic DNA (template), along with 0.4 µM of each primer, 1x Go Taq Green PCR master mix (Promega), and the final volume was adjusted to 25 µL by nuclease-free water. The 18S rDNA amplification was carried out in a PCR BIO-RAD C1000 Thermal Cycler apparatus. The PCR cycling conditions performed as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 s, primer annealing at 56.3°C for 30 seconds, primer extension at 72°C for 2 minutes, and final extension at 72°C for 7 minutes. Final PCR products (~1700 bp) were loaded into 1.5% (w/v) agarose gel stained with ethidium bromide, separated by electrophoresis, and viewed using a gel documentation system (The Biorad Gel Doc XR+ Imaging System). The pure PCR product was shipped to Korea for sequencing. The results of sequencing were compared with GenBank data using the basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/blast) (Sayers et al 2022). The aligned sequences were submitted to distance estimation, and the phylogenetic tree was built applying Geneious Tree Builder according to Neighbor-Joining (NJ) method using MEGA 6.0 Software (Kusnanda et al 2023; Ricken et al 2019).

Preparation of solvent extracts. Ten grams the microalgae biomass was extracted with 50 mL of methanol, ethyl acetate, and n-hexane by maceration in dark bottles at room temperature for 48 h. Then, microalgae extracts were centrifuged at 3500 rpm for 10 min and filtered through filter paper. The organic solvent extracts were removed by evaporation to obtain the crude extracts of microalgae. Further, these microalgae extracts were stored and kept at -20°C in the freezer, in the dark, before being used for total phenolic compound (TPC), antioxidant activity and α-glucosidase inhibitor activity determination (Almendinger et al 2021; Tolpeznikaite et al 2021).

Total phenolic content (TPC). Total phenolic content (TPC) in each microalgae extract was evaluated using Folin-Ciocalteau (FC) method, according to the method by Maadane et al (2015) and Morowvat and Ghasemi (2016) research with slight modifications using gallic acid as a standard. Briefly, 0.2 mL of the microalgae extracts (1000 µg mL^{-1}) was mixed with 1 mL of 10% aqueous Folin-Ciocalteu solution, stirred and left for 5 min, followed by adding 0.8 mL sodium carbonate (20% in distilled water). The mixture was further incubated at room temperature for two hours in the dark and the absorbance was measured at a wavelength of 750 nm by using a UV–Vis spectrophotometer (Shimadzu UV–Vis 1280) with methanol as the blank. TPC was expressed in mg GAE mg g^{-1} extract weight (EW) using a gallic acid calibration curve.

Antioxidant activity. The antioxidant potential of each sample was assessed at concentrations of 250-500 µg mL^{-1}. To 3 mL of each microalgae extract were added 1 mL of 60 mM DPPH solution and the mixture was incubated for 30 min at room temperature. The absorbance was then measured at a wavelength of 517 nm using spectrophotometer

(Shimadzu UV–Vis 1280). Ascorbic acid (Supelco, USA) was used as a positive standard at concentration of 7.5 µg mL^{-1}. The radical inhibitory activity was calculated using the following equation:

$$\text{Radical Scavengy Activity}(\%) = [\frac{\text{Absorbance sample} - \text{Absorbance control}}{\text{Absorbance Sample}}] \times 100$$

where Absorbance sample is the absorbance of the sample and Absorbance control is the absorbance of the control (Wairata et al 2021).

Inhibition of α-glucosidase activity. The inhibitory activity of α-glucosidase was determined according to the modified method of Salehi et al (2013). The mixture contained 20 µl α-glucosidase (0.5 unit mL^{-1}), 120 µl of 0.1 M phosphate buffer (pH 6.9) and 10 µl of microalgae extract at concentrations of 125-250 µg mL^{-1}. The mixed solution was incubated in 96-well plates at 37°C for 15 min. After pre-incubation, the enzymatic reaction was initiated by adding 20 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) and the reaction mixture was incubated for another 15 min at 37°C. The reaction was terminated by adding 80 µl of 0.2 M sodium carbonate solution and then absorbance reading was recorded at 405 nm by microplate reader (Spectro Star, BMG Labtech). The reaction system without microalgae extracts was used as control and the inhibitory rate of sample on α-glucosidase was calculated by the following formula:

$$\text{Enzim Inhibition}(\%) = [\frac{\text{Absorbance sample} - \text{Absorbance control}}{\text{Absorbance Sample}}] \times 100$$

where absorbance sample is the absorbance of all the reaction agents and microalgae extract, absorbance control is the absorbance of negative control consisting of all the reaction agents and dimethyl sulfoxide (DMSO) solution, except the microalgae extract.

GC-MS analysis of Parachlorella kessleri AUP5 extracts. To prepare the samples, the best crude extracts of *Parachlorella kessleri* AUP5 (10 mg) that have potential for α-glucosidase activity was examined for its phytoconstituents using a gas chromatography-mass spectrometer (GC-MS) (GCMS-QP2020; Shimadzu, Japan). The microalgae *Parachlorella kessleri* AUP extracts were analysed using gas chromatography-mass spectrometry (GCMS-QP2020; Shimadzu, Japan) equipped with (HP-5MS) capillary column (30 m × 30 m × 0.25 mm × 0.25 µm). The temperature gradient of the GC oven was maintained at an initial temperature of 60°C, was increased to 250°C at a rate of 2°C/min for 90 minutes. Helium was used as a carrier gas, at a constant flow of 0.9 mL/min, and an injection volume of 2 µL (split ratio of 10:1). The identification of unknown compounds was based on comparing their retention time (RT) relative to those of the known compounds and by matching spectral peaks available at Mass Spectral Library (Chaidir et al 2021; Elshobary et al 2020).

Results

Morphological features of Parachlorella kessleri microalgae. The isolated microalgae are unicellular microalgae, round in shape, and green in colour (Figure 1). Based on morphological characters, the isolated algae were as expected for *Parachlorella* sp. and belong to the Chlorophyta phylum (green microalgae). Microalgae morphological analysis for identification is often inaccurate, and it makes difficult to identify microalgae species accurately. Therefore, total DNA of microalgae was isolated, PCR-amplified rDNA (18s rDNA) was measured and phylogenetic tree provided better identification (Zhao et al 2020; Chaidir et al 2017).

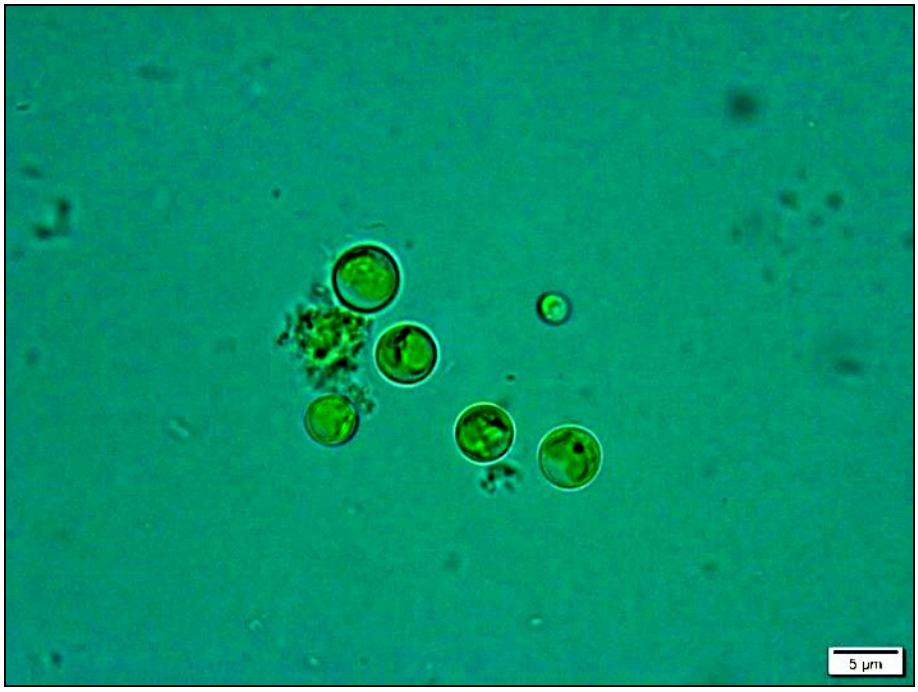

Figure 1. Light microscopic images of cell morphology of the isolated microalgae (1000X magnification).

Strain identification and similarity analysis. The use of DNA barcodes has become important in the identification process based on sequence comparisons against DNA databases (Hadi et al 2016). The microalgal DNA was extracted using DNA Extraction Kits (QIAGEN) and subjected directly to PCR amplification using universal 18S rDNA primers. The DNA sequences were analyzed using Basic Local Alignment Search Tool (BLAST) and phylogenetic trees to determine the microalgae identity. PCR amplification was immediately performed using the extracted microalgal DNA obtained after the treatments. According to BLAST analysis of corresponding sequences, the putative sequence of the isolated microalgae AUP5 strains showed 99% similarity with species characterized previously as *Parachlorella kessleri*. These results were supported using the results from the construction of the phylogenetic tree (Figure 2).

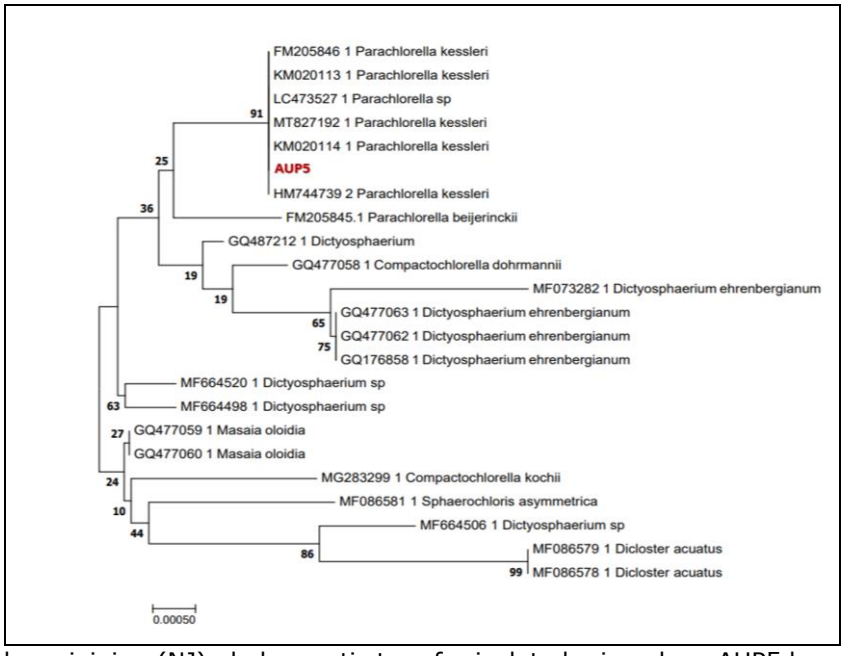

Figure 2. Neighbour joining (NJ) phylogenetic tree for isolated microalgae AUP5 based using 18S rRNA sequences (generated using MEGA software).

Total phenolic content and antioxidant activity. The n-hexane, ethyl acetate and methanol extracts of microalgae were evaluated for phenolic content. The total phenolic content (TPC) of the microalgae *Parachlorella kessleri* AUP5 extracts in Figure 3 with the methanol extract of showing the highest TPC 84.22 mg (GAE) g^{-1} extract followed by, ethyl acetate extract 49.47 mg (GAE) g^{-1} extract, while the n-hexane extract showed the lowest phenolic content 36.86 mg (GAE) g^{-1} extract.

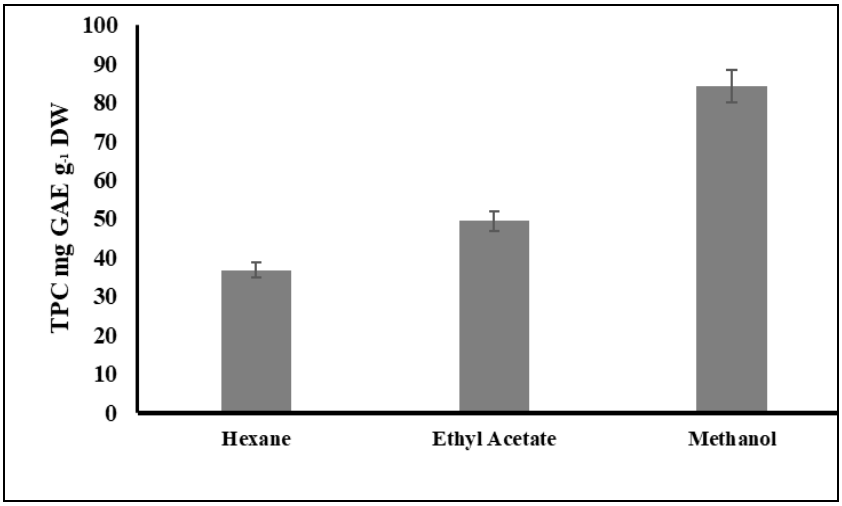

Figure 3. Total phenolic content of microalgae *Parachlorella kessleri* AUP5 extracts (means ± SEMs, n = 3).

Antioxidant activity. The percentage of 2,2-diphenyl-1-picrylhydrazyl DPPH radical scavenging activity of extracts is showed in Figure 4. At increasing concentrations from 250 to 500 µg mL^{-1}, all organic solvent extracts showed the free radical scavenging activity in the range of 7.48±0.0.67% to 54.64±0.8%. On concentration of 500 µg mL^{-1} methanolic extract gave the highest antioxidant capacity with amount 54.64±0.8% followed by ethyl acetate and hexane extracts with amounts of 27.86±00.96% and 10.38±0.0.4% respectively.

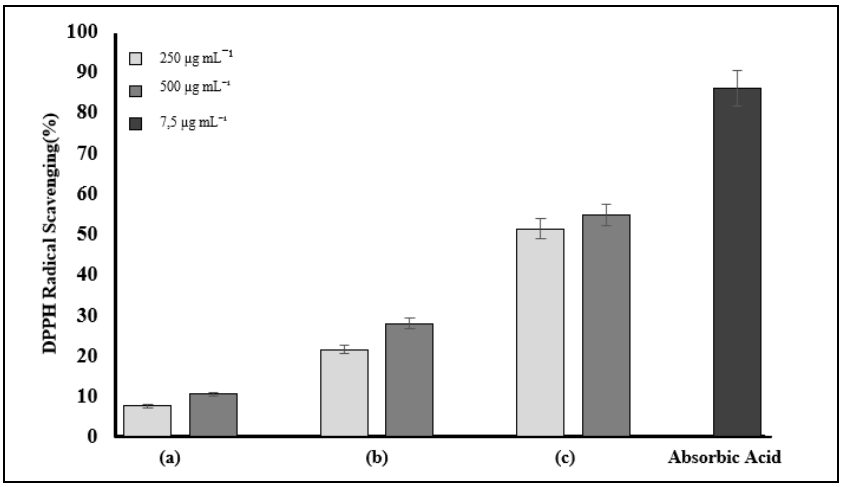

Figure 4. Antioxidant DPPH radical scavenging of *Parachlorella kessleri* AUP5 microalgae (a) hexane, (b) ethyl acetate and (c) methanol extracts (means ± SEMs, n = 3).

Inhibition of α-glucosidase activity of Parachlorella kessleri extracts. α-glucosidase inhibition activity of organic solvents extracts from *Parachlorella kessleri* AUP5 showed that crude extract from all extract solvents was able to inhibit the α-glucosidase enzyme. On concentration of 250 µg mL^{-1}, the greatest inhibition activity of organic solvent extracts was recorded in hexane with the percentage of the inhibition at 91.87%, followed by ethyl acetate and methanol extracts with amounts of 84.37±1.89% and 84.27±0.89% respectively.

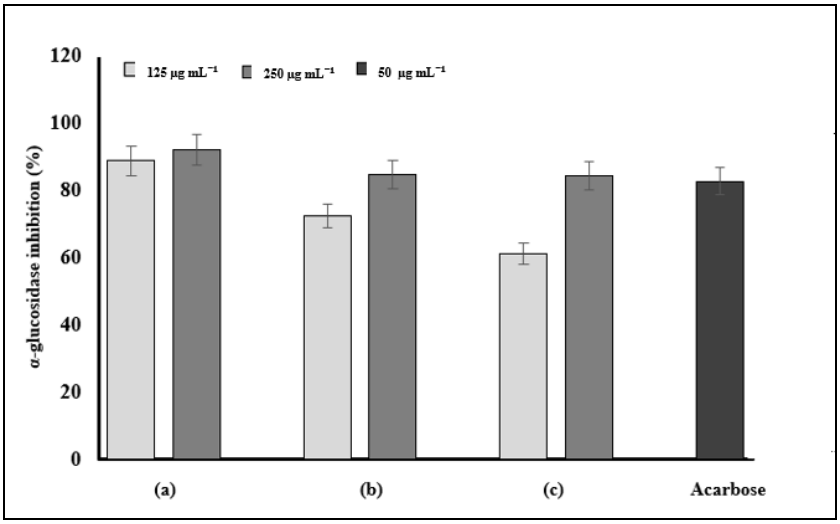

Figure 5. α-glucosidase inhibition activity of microalgae *Parachlorella kessleri* AUP5 microalgae (a) hexane, (b) ethyl acetate and (c) methanol AUP5 extracts (means ± SEMs, n = 3)

Chemical compositions of Parachlorella kessleri AUP5. The qualitative preliminary phytochemical screening of hexane extract of *Parachlorella kessleri* AUP5 was determined using GC–MS and revealed the presence of 9 total bioactive compounds (Figure 6 and Table 1). The major compounds included 2,6-Octadien-1-ol,3,7-dimethyl-acetate (E) (37.53%), 9,12-Octadecadienoic acid (29.25%), and tetradecanoic acid (7%).

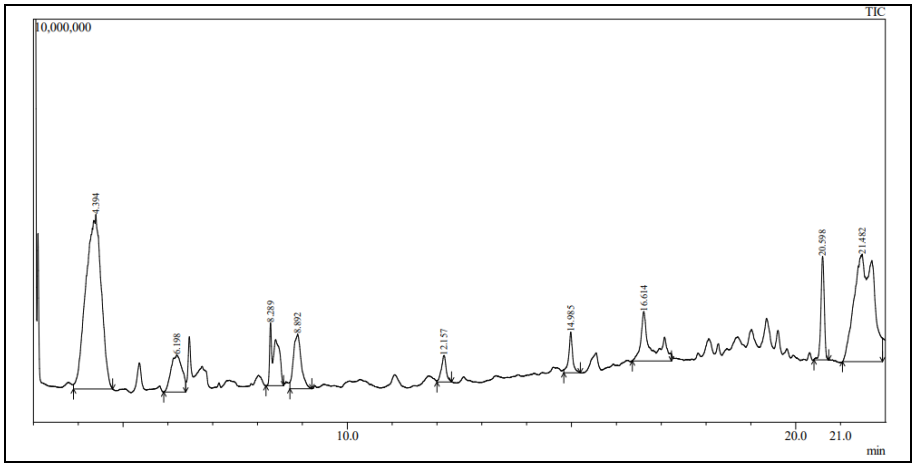

Figure 6. GC–MS chromatogram of hexane extract of *Parachlorella kessleri* AUP.

Table 1

Chemical constituents of *Parachlorella kessleri* AUP5 hexane extract

No	*RT*	*Compound name*	*Percent area (%)*	*Molecular formula*	*Molecular weight (g/mol)*
1	4.39	2,6-Octadien-1-ol,3,7-dimethyl-acetate (E)	37.53	$C_{12}H_{20}O_2$	196.29
2	6.19	Caryophyllene	5.71	$C_{15}H_{24}$	204.35
3	8.28	Unidentified	5.85	-	-
4	8.89	Naphthalene	5.83	$C_{10}H_8$	204.351
5	12.15	Dodecanoic acid	1.76	$C_{12}H_{24}O_2$	200.32
6	14.98	Heptadecane	2.17	$C_{17}H_{36}$	240.5
7	16.16	Tetradecanoic acid	7.00	$C_{14}H_{28}O_2$	228.37
8	20.59	Hexadecanoic acid	4.91	$C_{16}H_{32}O_2$	256.42
9	21.48	9,12-Octadecadienoic acid	29.25	$C_{18}H_{32}O_2$	280.4

Discussion. Isolate microalgae were microscopically identified by using an optical microscope that can be seen in Figure 1. It can be seen that this is a unicellular microalgae, and green colored. Based on molecular identification analysis of the amplified sequences of the 18S rDNA, isolated microalgae was identified as *Parachlorella kessleri* by BLAST/NCBI, with high 90% similarity to species of the Chlorophyta phylum. Several studies reported this microalgae was mostly found in freshwater sources. Based on morphological characters, the isolated algae were as expected for *Parachlorella* sp. and belong to the Chlorophyta phylum (Juárez et al 2011; Shaikh et al 2019).

In this study, the process of extracting biomass from *Parachlorella kessleri* AUP5 was carried out by individual extraction using different organic solvents based on their polarity that provide a higher efficacy for extracting compounds from microalgae (Elshobary et al 2020). The hexane, ethyl acetate and methanol extracts of microalgae were evaluated for phenolic content. The total phenolic content (TPC) of the microalgae *Prachlorella kessleri* AUP5 extracts shown in Figure 3, with the methanol extract showing the highest TPC of 84.22 mg (GAE) g^{-1} extract, while the hexane extract showed the lowest phenolic content of 36.86 mg (GAE) g^{-1} extract. This result showed higher values than the study from Haoujar et al (2022), which reported that microalgae *Phaeodactylum tricornutum* with TPC of 39.68±0.59 mg (GAE) g^{-1} extract, *Nannochloris* sp. with 33.54 ±0.75mg (GAE) g^{-1} extract, and *Tetraselmis suecica* with 28.42±1.18mg (GAE) g^{-1} extract. Moreover, when compared with other studies described in the literature, the extracting solvents used such as ethanol, methanol and water might have the potential to dissolve polyphenols more than other solvents to evaluate total phenolic content from microalgae extract (Andriopoulos et al 2022; Banskota et al 2019; Morowvat & Ghasemi 2016).

Based on the test results of screening inhibition of α-glucosidase of microalgae extracts, it is known that the hexane extract of *Parachlorella kessleri* AUP5 has the greatest inhibition activity (Figure 5), with the percentage of the inhibition found at 91.87%, compared to the other extracts of ethyl acetate and methanol with amounts of 84.37±1.89% and 84.27±0.89% respectively. Some metabolites from natural resources have been evaluated for the inhibition of α-glucosidase. The inhibitory potential of these extracts was higher than that of the reference antidiabetic acarbose, which was about 82.49±1.6% for α-glucosidase. These result are better than previous studies that reported methanol extracts of *Chlorella* sp., *Nannochloropsis* sp. and *Porphyridium* sp. showed potential inhibition to α-glucosidase in the range of 7.29% to 12.55% (Pratiwi & Husni 2021; Priatni et al 2021). Regarding the antidiabetic potential, the hexane extracts obtained from *Parachlorella kessleri* AUP5, were further analysed for bioactive compounds using GC-MS. The GC-MS chromatogram of hexane etract from *Parachlorella kessleri* AUP5 revealed nine major compounds have been identified. In our study, we observed that the bioactive compounds consist of bioactive metabolites from fatty acids, with the major components being 2,6-Octadien-1-ol,3,7-dimethyl-acetate (E) (37.53%), 9,12-Octadecadienoic acid (29.25%), and tetradecanoic acid (7%). Several studies reported also that mostly hexane extract from microalgae contains fatty acids and terpenoid compounds (Andriopoulos et al 2022; Yun et al 2020). Microalgae extracts of *Chlorella pyrenoidosa* (CP55) contained high values of polyunsaturated fatty acids, effective as a natural food source for preventing diabetes (Wan et al 2019). There are also studies that reported that bioactive compound methyl ester, such as octadecadienoic acid, exhibited as in vitro inhibitory enzyme against protein tyrosine phosphatase 1B(PTP1B), has good potential for treating diabetes (Li et al 2012). Mostly research that use lipophilic and pigment extraction using non polar solvents showed highest potential as antimicrobials, such as microalgae *Oscillatoria* sp. SSCM01, *Phormidium* sp. SSCM02 (Schuelter et al 2019; Nainangu et al 2020) and *Chlorococcum minutum* (Elshobary et al 2020). Additionally, the results showed that the potential from organic solvents extraction of microalgae *Parachlorella kessleri* AUP5 is of natural bioactive compounds, with antioxidant and antidiabetic properties.

Conclusions. Based on 18S rDNA sequence analyses, the isolated microalgae species was *Parachlorella kessleri* AUP5. The hexane extract showed the best α-glucosidase

inhibitor activity, while the methanol extract had a higher antioxidant activity due to the higher presence of phenolic contents than other extracts. Bioactive compounds found in hexane extracts, which were determined using GC-MS, showed 2,6-Octadien-1-ol,3,7-dimethyl-acetate (E) (37.53%), 9,12-Octadecadienoic acid (29.25%), and tetradecanoic acid (7%) as major compounds in microalgae *Parachlorella kessleri* AUP5 hexane extracts. The presence of numerous bioactive compounds in microalgae extracts, may support their use as functional foods and in the nutraceuticals industry in future.

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