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Inhibition of N^{ε} - (carboxymethyl) lysine (CML) and N^{ω} - (carboxymethyl) arginine (CMA) Formation by Using Microalgae Crude Extracts

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Abstract

Advanced glycation end-products (AGEs) are generated through glycation reactions, which take place between proteins, nucleic acids, or lipids and a reducing sugar. AGEs cause degeneration of cells, which contributes to aging-related diseases, metabolic syndromes, and hyperlipidemia, among others. Several studies found plant bioactive compounds such as phenolics (phlorofucofuroeckol-A and dieckol) and flavonoids (rutin and quercetin) could inhibit AGEs, but an alternative resource produced of those compounds and a new compound is still needed. This study was aimed to investigate the inhibitory effect of AGEs, especially N^{ε} - (carboxymethyl) lysine (CML) and N^{ω} - (carboxymethyl) arginine (CMA), formation by using three microalgae extracts produced from *Micractinium* sp. KLSc62 (G4), *Chlorella* sp. KLSc59 (KS), and *Chlorella* sp. KLSc61 (SR). Algal cells were extracted with 30% and 60% ethanol, and AGEs formation was determined by ELISA assay. The best result showed that 60% of ethanolic crude extract from *Micractinium* could inhibit CML formation up to 5%. This *Micractinium* extract was subjected to be fractionated through column chromatography (C18 resin) as six fractions, followed by ELISA detection of AGEs formation. We found that the 25% ethanolic fraction and 99.9% ethanolic fraction showed significantly most effectiveness in inhibiting CMA and CML formation, which were 23% and 11%, respectively. This preliminary finding suggested that 25% and 99.9% of *Micractinium* ethanolic fractions were likely to contain bioactive compounds capable of inhibiting CML and CMA formation. We propose to characterize these bioactive compounds using HPLC, preparative HPLC, and LC-MS/MS in further studies.

Keywords: Advanced glycation end-product, AGEs, CMA, CML, ELISA, Microalgae extract

1. Introduction

Nowadays, having foods such as hamburgers and buffet barbecues including high fructose syrup drinks and desserts are favorable because they are tasty, worth the price, and quick and easy to eat. Hamburgers and barbecues are processed foods which are cooked through high heat and this cooking method (grill) causes Maillard reaction, a non-enzymatic browning process occurs between amino acids and reducing sugars which finally generated AGEs product, as known as glycotoxins. Having high fructose corn syrup drinks and desserts also generates AGEs by Glycation reaction which takes place between amino groups of proteins, nucleic acids, or lipids and the carbonyl groups of reducing sugars. When people have those grilled foods and high sugar foods continuously with a large quantity, AGEs residues accumulated inside their body leads to tissue inflammation consistency. Finally, it can cause those people to have cancer, diabetes Type 2, coronary heart, stroke, and other chronic diseases (Tanaviyutpakdee, 2016; Uribarri et al., 2007; Uribarri et al., 2010). Furthermore, AGE residues can disrupt skin homeostasis and contribute to dermatological complications (Chen et al., 2022). Reducing consumption of those grilled and high sugar foods can reduce AGEs in the body and can help people remain healthy.

In several studies, AGEs products are found as N^{ε}-(carboxymethyl) lysine (CML), N^{ε}-(carboxyethyl) lysine (CEL), pentosidine, and pyrraline residues (Fotheringham et al., 2022; Tanaviyutpakdee, 2016; Uribarri et al., 2010). Among those AGE residues, CML is the most abundant and widely studied, making it a key target in inhibition of AGEs formation study (Li et al., 2012; Luevano-Contreras & Chapman-Novakofski, 2010). In medicine, aminoguanidine (AG), an AGE inhibitor, has been widely used as a drug to treat diabetes, but AG causes gastrointestinal disorders. In recent years, several studies have attempted to seek another strategy to inhibit AGEs. Several studies found that plant-derived substances could show inhibiting of AGE formation (Chen et al., 2019). For example, *Helichrysum melaleucum*, *Argyranthemum pinnatifidum*, and *Phagnalon lowei* were good candidates for development as alternative medicines to control of diabetes (Spínola & Castilho, 2017) because those plants consisted of a bioactive compound such as Thiamine effects on AGEs inhibition (Mrowicka et al., 2023). Although plant-based bioactive compounds were shown to be a promising medicine and plants were promising resources, they have some limitations such as cost, cultivation area, and use for human food. Seeking more alternative resources and a new bioactive compound is still needed in clinical tests. Like plants, microalgae are green and clean organisms, they use sun light, CO_2 to produce food, energy including synthesizing some bioactive compounds. In the past decade, microalgae became famous in research because people found some antioxidants and other substances that can treat people disease (Imai et al., 2022). In terms of AGEs inhibition, *Parachlorella beijerinckii*, a green microalga, showed a significant inhibition of CMA and CML formation as well as *Chlorella* sp. was reported as a promising species as an anti-glycation agent because they contain carotenoids and vitamins, having antioxidant and anti-inflammatory effects (Imai et al., 2022). In 2020, Manabe et al. (2020) reported that Siphonaxanthin, a ketocarotenoid found in some microalgae, can inhibit AGE-induced inflammation (Sugawara et al., 2014). In addition, Thiamine, Rutin, and Quercetin compounds in a carotenoids group strongly exhibited inhibiting CML formation. Indeed, Thiamine was typically found in microalgae, plants, and some fungi (Li et al., 2012; Mrowicka et al., 2023; Setyaningrum et al., 2023). Based on previous reports, we aimed to seek out other microalga candidates that has a property to inhibit AGEs residue, especially CML and/or CMA as a new resource.

In this study, we chose three microalgae strains from Cherdsak's laboratory collection to investigate the inhibitory effect of CML and CMA by using their crude extracts. The *Micractinium* sp. KLSc62 (Chae et al., 2021; Lim et al., 2023) and *Chlorella* spp. (*Chlorella* sp. KLSc59) and *Chlorella* sp. KLSc61) (Laokua et al., 2022; Preechaphonkul et al., 2024) were prepared as 30% and 60% ethanolic crude extracts and they were preliminary tested the inhibition of CML formation by ELISA assay. The best extract result would be selected to fractionate by using C18 Column chromatography, followed by ELISA assay to confirm anti-AGEs activity. The best fraction would be subjected to characterize a bioactive compound for inhibiting AGEs formation in the future experiment.

2. Materials and Methods

2.1 Algal standard growth curve and crude extract preparation

Three microalgae strains, *Micractinium* sp. KLSc62 (G4), *Chlorella* sp. KLSc59 (KS), and *Chlorella* sp. KLSc61 (SR), were obtained from Cherdsak's laboratory (Department of Biology, School of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand). Each microalga was grown and maintained on Tris-Acetate-Phosphate (TAP) agar (Gorman & Levine, 1965). To generate a standard growth curve of each microalga, a starter culture was performed by picking a single colony from a culture plate and transferring to 50 mL of TAP broth in 125 mL Erlenmeyer flask until cells reached the exponential phase at Day 3. Then, the starter culture was transferred to 100 mL of TAP broth in a new 250 mL Erlenmeyer flask with the initial cell number of $1x10^6$ cells/mL as an experimental flask. Algal cells were grown for 10 days until they reached the stationary phase. Microscopic cell count was performed every 24 h by using hemocytometer (Boeco, Germany) and the growth curve was generated thereafter by using Microsoft Excel (Islam et al., 2021).

To prepare all microalgae extracts, the starter culture was performed the same as the standard growth. After that, algal cells were transferred to 500 mL of TAP broth in a new 1-liter Erlenmeyer flask with the initial cell number of $1x10^6$ cells/mL and the cells were grown until they reached the early stationary phase on Day 5

(determined by the growth curve). Each cell sample was harvested in a new 50-mL conical tube by centrifugation at 5,000 x g for 5 min. The supernatant was discarded, and the cell pellet was used in the next step. The 5 g of the cell pellet of each microalga was disrupted by using Sonicator Probe (Sonics Materials VC-505 Ultrasonic Processor) (performed for 10 sec and stopped for 10 sec, a total 30 cycles for 10 min). After that, broken cells were soaked with 30% or 60% ethanol (1:10 w/v) and incubated at 4°C for 48 h. The supernatant (a microalgal extract) was collected by centrifuging at 5,000 x g for 5 min, transferred to a new 50 mL conical tube, and stored at 4°C until use.

All microalgae cultures were grown on a rotary shaker at 120 rpm with 7,400 lux of light intensity for 24 h of light exposure at 26°C in the culture room.

2.2 AGEs reaction and ELISA analysis

To perform AGE reaction and ELISA assay, Tominaga's protocol was used (Tominaga et al., 2020). Briefly describe, solution A was prepared by mixing of 25 mL of 60 mM Ribose in 200 mM K-PO_4 buffer with 25 mL of 0.4% Gelatin in 200 mM K-PO_4 buffer 25 mL (1:1 ratio of Ribose and Gelatin). After that, 495 µL of solution A was transferred to microcentrifuge tubes, and mixed as followed; with 5 µL of each microalgal ethanolic extract (30% and 60% ethanolic extract from the previous method in 2.1), with 5 µL of 1- and 10 mg/mL of Aminoguanidine (AG) as positive controls obtained from Nagai's laboratory (Department of Bioscience, School of Agriculture, Tokai University, Japan), with 5 µL of sterile distilled water as a negative control (two sets of negative controls were made). All AGEs reactions were incubated at 37°C for a week, except for one set of negative controls that was incubated at -20°C for a week.

After a week of all reaction incubation, AGEs formation (CMA and CML residues) was detected by ELISA assay which were performed by using anti-CMA and anti-CML as primary antibodies and immunoglobulin G-horseradish peroxidase (IgG-HRP) as secondary antibody, all primary and secondary antibodies were obtained from Nagai's laboratory (Department of Bioscience, School of Agriculture, Tokai University, Japan). The colorimetric reaction result as yellow color was developed by adding o-Phenylenediamine Dihydrochloride and detected by using microplate reader at absorbance 492 (A492) (Biochrom EZ Read 2000). The data was calculated as described in Tominaga's protocol.

2.3 C18 column chromatography

In this experiment, the best microalgal extract, the highest inhibition AGEs formation, from Materials and Methods section 2.2 would be chosen. Tominaga et al. (2020) was modified and used. Briefly described, C18 column chromatography was used to fractionate the microalgae crude extract with C18 resin packed in glass columns (1.8 x 20 cm) as stationary phase and distilled water, 25%, 50%, 75%, 99.9% ethanol and acetone as mobile phase. Each fraction was collected and the required volume for each fraction to be 3 CV (Column volumes) of eluent. Calculate the column volume based on the dimensions of the glass column and the packed resin inside, using the formula for the volume of a cylinder. Following the formula, as shown in Figure 1. All fractions were collected in new 50-mL conical tubes and concentrated by using a rotary evaporator, then AGEs (CMA and CML residue) product formation was detected by ELISA assay the same method as Materials and Methods section 2.2.

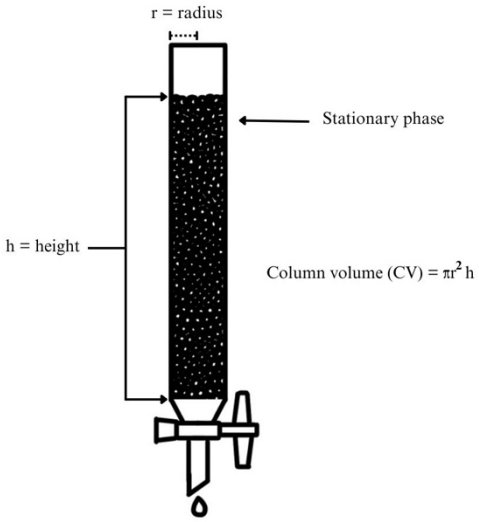

Figure 1. Column chromatography with C18 resin and the formula for calculating the column volume. Π, Pi is the irrational number, approximately equal to 3.14; r, radius; h, height.

2.4 Statistical analysis

Data were analyzed statistically using a Complete Randomized Design (CRD). The analysis of variance was performed using the one-way ANOVA method and the difference was measured using the Duncan method using IBM SPSS statistics version 29 (SPSS software, New York, USA) at a significant level of 0.05. Each experiment was conducted in triplicate.

3. Results and Discussion

3.1 Microalgal harvesting stage and primary screening inhibition of CML formation

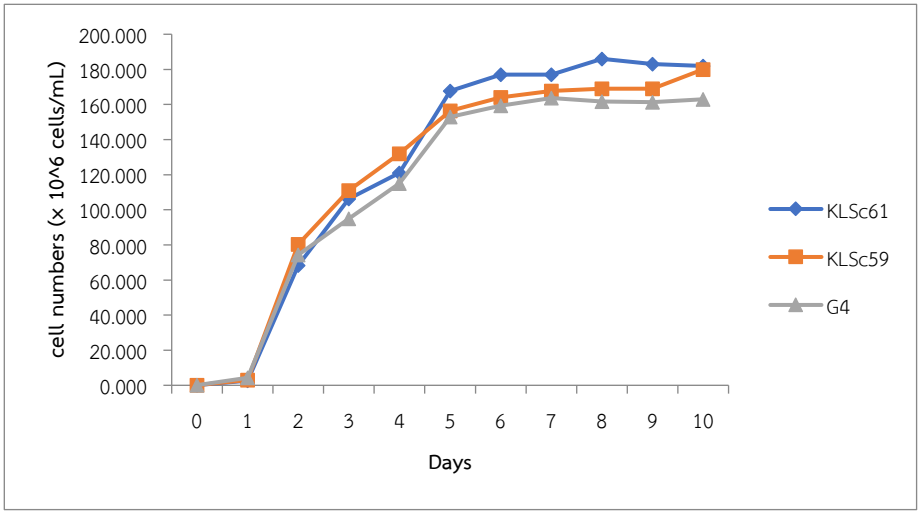

Figure 2. Growth phase of *Micractinium* sp. (G4), *Chlorella* sp. KLSc59 (KS) and *Chlorella* sp. KLSc61 (SR).

To determine the microalgae growth phase, each microalga was cultivated for 10 days, and cell counts were performed every 24 hours. The results showed that *Micractinium* sp. (G4), *Chlorella* sp. KLSc59 (KS), and *Chlorella* sp. KLSc61 (SR) showed significantly similar growth phases. They entered the log phase on Day 1, the mid-log phase on Day 3, and reached the early stationary phase on Day 5, as shown in Figure 2. Microalgal

biomass in the early stationary phase (Day 5) was harvested and used in the next experiment. This growth phase was chosen because algae cells had fully grown and stopped dividing, and their cells began accumulating secondary metabolites as bioactive compounds (Godbey, 2022; Kitrungloadjanaporn, 2017). We assumed that the microalgae extract from this stage would contain some bioactive compounds or substances that could inhibit CML and CMA formation.

To preliminarily screen for AGEs formation, only the CML residue was chosen for testing. Three microalgae strains, which were *Micractinium* sp. KLSc62 (G4), *Chlorella* sp. KLSc59 (KS), and *Chlorella* sp. KLSc61 (SR), were chosen from Cherdsak's laboratory collection, and they were prepared as 30% and 60% microalgal ethanolic extracts. AGE reactions were prepared, and the reactions were detected by ELISA assay. We found that all 30% ethanolic extracts of the three microalgal strains (SR 30%, KS 30%, G4 30%) did not show any significant difference in CML formation rate compared to controls (DW-20, DW37, AG1mg, and AG10mg), as shown in Figure 3. Interestingly, all 60% of microalgal ethanolic extracts exhibited the promising inhibition of CML formation. The highest CML formation was from *Micractinium* sp. KLSc62 extract (G4 60%), followed by *Chlorella* sp. KLSc59 extract (SR 60%) and *Chlorella* sp. KLSc61 extract (KS 60%), as 5%, 2%, and 1% of CML formation rate, respectively (Figure 3). This finding suggested that the 60% ethanolic extracts of all algal strains might contain interesting bioactive compounds, and the 60% ethanolic extract from *Micractinium* sp. KLSc62 (G4 60%) was chosen for the next experiment as the best extract inhibiting CML formation. Compared with another study, it had shown that using brown algae species to inhibit AGEs in the BSA and fructose model, a 10 mg/mL concentration of brown algae extract resulted in an inhibition rate of up to 52% (Park & Lee, 2021), but in our study, we used only 1 µg/mL of microalgal extract concentration in the AGEs reactions. A higher extract concentration was proposed and could improve the inhibition rate of CML formation for the future study.

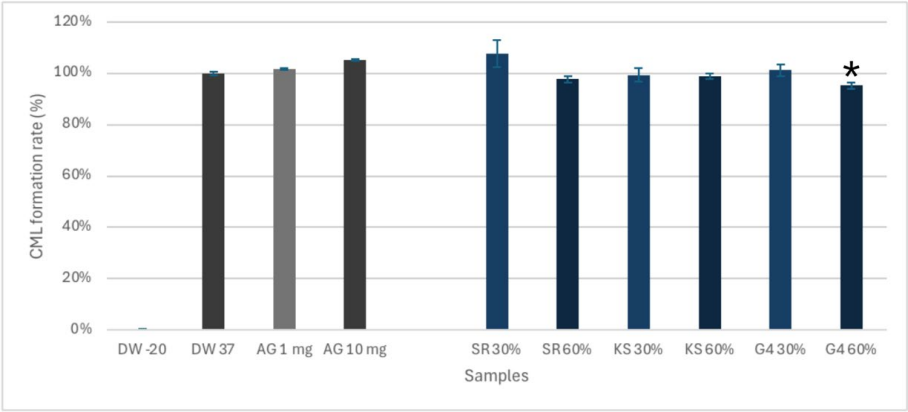

Figure 3. Inhibition of CML formation effects by using 30% and 60% ethanolic extract of *Micractinium* sp. KLSc62 (G4), *Chlorella* sp. KLSc59 (KS) and *Chlorella* sp. KLSc61 (SR) determined by ELISA (P value < 0.05).

3.2 Inhibition of CMA and CML formation from *Micractinium* sp. KLSc62 fractions

From the previous result in 3.1, 60% ethanolic extract from *Micractinium* sp. KLSc62 (G4 60%) showed the best inhibition of CML formation (Figure 3), it was chosen to perform in this experiment. The extract was fractionated by using C18 column chromatography and tested by ELISA with anti-CMA and anti-CML thereafter. The *Micractinium* sp. KLSc62 extract (G4 60%) was fractionated to six fractions with distilled water, 25%, 50%, 75%, 99.9% ethanol and acetone as a mobile phase. In Figure 4, after ELISA assay by using anti-CMA with those six fractions, the results showed that the best *Micractinium* sp. KLSc62 fraction with the highest inhibition of CMA formation rate was 25% ethanolic fraction (G4 25%). We found that CMA residue formation rate was 77%, which meant the inhibition rate was 23% compared with DW7 as a negative control. Whereas 75% ethanolic fraction (G4 75%) and 99.9% ethanolic fraction (G4 99.9%) could inhibit CMA residues at 6% and 1% of the inhibition rate, respectively when compared with DW7 as a negative control (Figure 4). Interestingly, G4 25%, G4 75% and G4 99.9% fractions exhibited the higher inhibition rate than AG 1 mg (Aminoguanidine 1 mg/mL, a

positive control), but not greater than AG 10 mg (Aminoguanidine 1 mg/mL, a positive control) because all fractions still were crude fractions, some compounds contained in these crude fractions still needed to be purified. The rest fractions, distilled water (G4 0%), 50% ethanolic (G4 50%) and acetone (G4 ACN) could not show any inhibition effect on CMA formation when compared with DW7, a negative control including AG 1 mg and AG 10 mg as positive controls (Figure 4). We concluded that G4 25% fraction of *Micractinium* sp. KLSc62 contained some bioactive compounds which affected on CMA residue formation, and it necessary to characterize those bioactive compounds in the future.

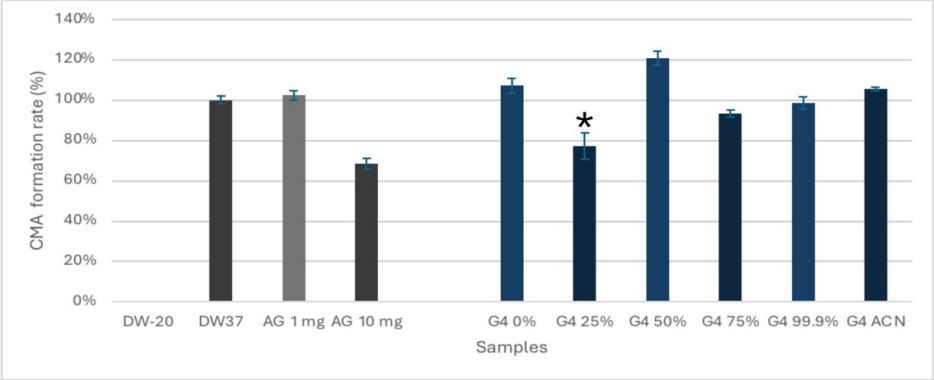

Figure 4. Inhibition of CMA formation effects of 60% *Micractinium* sp. ethanolic extract fractionated by C18 column chromatography (P value < 0.05).

On the other hand, all *Micractinium* sp. KLSc62 fractions showed slightly inhibitory effect of CML formation, which was less than 11%. To illustrate, 99.9% ethanolic fraction (G4 99%) presented the highest effect on inhibiting CML formation significantly up to 11% compared with DW37 as a negative control, CML residues could be generated 89% of formation rate (Figure 5). Whereas the 25% fraction had 7% of CML inhibition rate since CML residues had only generated 93%, and the rest of fractions (G4 75%, G4 0%, G4 50% and G4 ACN) could inhibit CML formation less than 7%, which were not significant different while comparing with all controls (Figure 5). This finding indicated that the 25% of *Micractinium* sp. KLSc62 ethanolic fraction could have some bioactive compounds which were able to inhibit CML formation. This fraction would be used to characterize a bioactive compound the same as in CMA experiment.

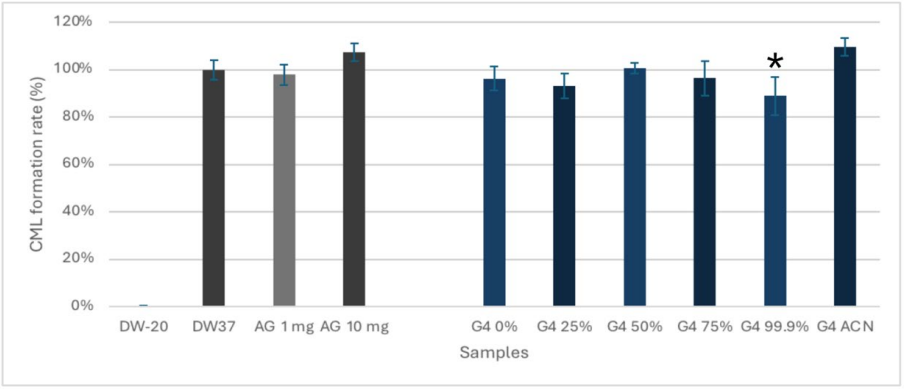

Figure 5. Inhibition of CML formation effects of 60% *Micractinium* sp. ethanolic extract fractionated by C18 column chromatography (P < 0.05).

From all the experimental results, using the ethanolic extract and fraction from *Micractinium* sp. KLSc62 (G4) showed the highest inhibitory effect of CMA and CML formation. *Micractinium* sp. is a green microalgae in Division Chlorophyta, Family Chlorellaceae (Trebouxiophyceae). *Micractinium* sp. is found closely related to

Chlorella species in both morphology and similarity determined by phylogenetic tree analysis (Krivina et al., 2023). Some *Micractinium* species have shown high biotechnological potential due to their ease of cultivation, high growth rate, and ability to accumulate valuable metabolites, especially polyunsaturated fatty acids (PUFA) (Adar et al., 2016; Krivina et al., 2023). A study has revealed that *Micractinium simplicissimum* extract consisted of some compounds such as Neophytadiene, a compound in the diterpenoid group found in many plants and marine algae (Selmy et al., 2023), and 9,12,15-Octadecatrienoic acid (α-linoleic acid) found in vegetable oils and in microalgae cells (Chen et al., 2021), both compounds has been investigated and showed anti-inflammatory activities (Chae et al., 2021; Lim et al., 2023). In conclusion, our findings could support the information that our *Micractinium* sp. KLSc62 might have some bioactive compounds, which was possibly a new compound or former ones that could help to reduce AGE production.

4. Conclusions

In this study, we investigated the inhibitory effect of AGEs, N^{ε}-(carboxymethyl) lysine (CML), and N^{ω}-(carboxymethyl) arginine (CMA) formation by using three microalgae strains. It was found that 25% of *Micractinium* sp. KLSc62 ethanolic fractions (G4 25%) presented the highest inhibition rate of CMA and CML formation, up to 23%, compared to the control (DW37). Several factors may influence the inhibition efficacy, such as the extraction method, algal culture conditions, or the extract and fraction concentration. In conclusion, our finding indicated that *Micractinium* sp. KLSc62 contained some bioactive compounds, potentially new or previously identified, that could help to reduce CMA and CML products. These results are in line with previous studies that have identified similar bioactive compounds in related species; they would give a better understanding and be useful for microalgae compounds in terms of pharmaceutical products and meditation applications for future studies.

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References

Adar, O., Kaplan-Levy, R. N., & Banet, G. (2016). High temperature Chlorellaceae (Chlorophyta) strains from the Syrian-African Rift Valley: The effect of salinity and temperature on growth, morphology and sporulation mode. *European Journal of Phycology*, *51*(4), 387–400. https://doi.org/10.1080/09670262.2016.1193772

Chae, H. J., Seo, J. B., Kim, S. H., Youn, E. J., Kim, S., & Suh, S. S. (2021). Antarctic freshwater microalga, *Micractinium simplicissimum*, suppresses inflammation. *Journal of Nanoscience and Nanotechnology*, *21*(7), 4098–4103(6). https://doi.org/10.1166/jnn.2021.19158

Chen, C.-Y., Zhang, J.-Q., Li, L., Guo, M.-M., He, Y.-F., Dong, Y.-M., Meng, H., & Yi, F. (2022). Advanced glycation end products in the skin: Molecular mechanisms, methods of measurement, and inhibitory pathways. *Frontiers in Medicine*, *9*, 837222. https://doi.org/10.3389/fmed.2022.837222

Chen, W., Wang, J., Ren, Y., Chen, H., He, C., & Wang, Q. (2021). Optimized production and enrichment of α-linolenic acid by *Scenedesmus* sp. HSJ296. *Algal Research*, *60*, 102505. https://doi.org/10.1016/j.algal.2021.102505

Chen, Y., Tang, S., Chen, Y., Zhang, R., Zhou, M., Wang, C., Feng, N., & Wu, Q. (2019). Structure-activity relationship of procyanidins on advanced glycation end products formation and corresponding mechanisms. *Food Chemistry*, *272*, 679–687. https://doi.org/10.1016/j.foodchem.2018.08.090

Fotheringham, A. K., Gallo, L. A., Borg, D. J., & Forbes, J. M. (2022). Advanced glycation end products (AGEs) and chronic kidney disease: Does the modern diet AGE the kidney?. *Nutrients*, *14*(13), 2675. https://doi.org/10.3390/nu14132675

Godbey, W. T. (2022). Cell growth. *Biotechnology and its applications* (2nd ed., pp. 117–150). Academic Press. https://doi.org/10.1016/B978-0-12-817726-6.00005-8

Gorman, D. S., & Levine, R. P. (1965). Cytochrome f and plastocyanin: Their sequence in the photosynthetic electron transport chain of *Chlamydomonas reinhardi*. *Proceedings of the National Academy of Sciences of the United States of America*, *54*(6), 1665–1669. https://doi.org/10.1073/pnas.54.6.1665

Imai, Y., Nakashima, Y., & Kanno, T. (2022). Inhibitory effects of *Parachlorella beijerinckii* extracts on the formation of advanced glycation end products and glycative stress-induced inflammation in an in vitro skin dermis-like model. *Evidence-Based Complementary and Alternative Medicine*, *2022*. https://doi.org/10.1155/2022/8789903

Islam, Z., Khatoon, H., Minhaz, T. M., Rahman, M. R., Hasan, S., Mahmud, Y., Hossain, M. S., & Sarker, J. (2021). Data on growth, productivity, pigments and proximate composition of indigenous marine microalgae isolated from Cox's Bazar Coast. *Data in Brief*, *35*, 106860. https://doi.org/10.1016/j.dib.2021.106860

Kitrungloadjanaporn, P. (2017). *Growth and lipid production of Chlorella vulgaris cultured in swine slaughterhouse wastewater* [Master's thesis]. Silpakorn University.

Krivina, E., Sinetova, M., Savchenko, T., Degtyaryov, E., Tebina, E., & Temraleeva, A. (2023). *Micractinium lacustre* and *M. thermotolerans* spp. nov. (Trebouxiophyceae, Chlorophyta): Taxonomy, temperature-dependent growth, photosynthetic characteristics and fatty acid composition. *Algal Research*, *71*, 103042. https://doi.org/10.1016/j.algal.2023.103042

Laokua, N., Rittiyan, N., Kornrawudaphikasama, Y., Klinsalee, R., Tonawut, Y., Preechaphonkul, N., Raksajit, W., Khetkorn, W., Dejtisakdi, W., & Maneeruttanarungroj, C. (2022). Optimal conditions for maximized H_2 yield from a new green algal strain *Chlorella* sp. KLSc61. *Journal of Applied Phycology*, *34*(4), 1909–1919. https://doi.org/10.1007/s10811-022-02779-y

Li, L., Han, L., Fu., Q., Li, Y., Liang, Z., Su, J. & Li, B. (2012). Formation and inhibition of N^{ε}-(carboxymethyl) lysine in saccharide-lysine model systems during microwave heating. *Molecules*, *17*, 12758–12770. https://doi.org/10.3390/molecules171112758

Lim, Y., Park, S.-H., Kim, E. J., Lim, H., Jang, J., Hong, I.-S., Kim, S., & Jung, Y. (2023). Polar microalgae extracts protect human HaCaT keratinocytes from damaging stimuli and ameliorate psoriatic skin inflammation in mice. *Biological Research*, *56*(1), 40. https://doi.org/10.1186/s40659-023-00454-1

Luevano-Contreras, C., & Chapman-Novakofski, K. (2010). Dietary advanced glycation end products and aging. *Nutrients*, *2*(12), 1247–1265. https://doi.org/10.3390/nu2121247

Manabe, Y., Takii, Y., & Sugawara, T. (2020). Siphonaxanthin, a carotenoid from green algae, suppresses advanced glycation end product-induced inflammatory responses. *Journal of Natural Medicines*, *74*, 127–134. https://doi.org/10.1007/s11418-019-01354-z

Mrowicka, M., Mrowicki, J., Dragan, G., & Majsterek, I. (2023). The importance of thiamine (vitamin B1) in humans. *Bioscience Reports*, *43*(10), BSR20230374. https://doi.org/10.1042/BSR20230374

Park, J. J., & Lee, W. Y. (2021) Anti-glycation effects of brown algae extracts and its phenolic compounds. *Food Bioscience*, *41*, 101042. https://doi.org/10.1016/j.fbio.2021.101042

Preechaphonkul, N., Sirikwanpong, S., & Maneeruttanarungroj, C. (2024). Freshwater green alga *Chlorella* sp. KLSc59 produced all forms of omega-3 oil: ALA, EPA, and DHA. *Algal Research*, *80*, 103532. https://doi.org/10.1016/j.algal.2024.103532

Selmy, A. H., Hegazy, M. M., El-Hela, A. A., Saleh, A. M., & El-Hamouly, M. M. (2023). In vitro and in silico studies of Neophytadiene; a diterpene isolated from *Aeschynomene elaphroxylon* (Guill. &Perr.) Taub. as apoptotic inducer. *Egyptian Journal of Chemistry*, *66*(10), 149–161. https://doi.org/10.21608/ejchem.2023.178261.7296

Setyaningrum, T. W., Budiman, A., & Suyono, E. A. (2023). Cobalamin and thiamine effect on microalgae biomass production in the Glagah consortium. *Journal of Tropical Biodiversity and Biotechnology*, *8*(3), 81949. https://doi.org/10.22146/jtbb.81949

Spínola, V., & Castilho, P. C. (2017). Evaluation of Asteraceae herbal extracts in the management of diabetes and obesity. Contribution of caffeoylquinic acids on the inhibition of digestive enzymes activity and formation of advanced glycation end-products (*in vitro*). *Phytochemistry*, *143*, 29–35. https://doi.org/10.1016/j.phytochem.2017.07.006

Sugawara, T., Ganesan, P., Li, Z., Manabe, Y., & Hirata, T. (2014). Siphonaxanthin, a green algal carotenoid, as a novel functional compound. *Marine Drugs*, *12*(6), 3660–3668. https://doi.org/10.3390/md12063660

Tanaviyutpakdee, P. (2016). Glycation and human diseases development. *Thai Journal of Toxicology*, *31*(2), 84–96.

Tominaga, Y., Sugawa, H., Hirabayashi, K., Ikeda, T., Hoshi, Y., & Nagai, R. (2020). *Drosera tokaiensis* extract containing multiple phenolic compounds inhibits the formation of advanced glycation end-products. *Archives of Biochemistry and Biophysics*, *693*, 108586. https://doi.org/10.1016/j.abb.2020.108586

Uribarri, J., Cai, W., Peppa, M., Goodman, S., Ferrucci, L., Striker, G., & Vlassara, H. (2007). Circulating glycotoxins and dietary advanced glycation endproducts: Two links to inflammatory response, oxidative stress, and aging. *The Journals of Gerontology: Series A*, *62*(4), 427–433. https://doi.org/10.1093/gerona/62.4.427

Uribarri, J., Woodruff, S., Goodman, S., Cai, W., Chen, X., Pyzik, R., Yong, A., Striker, G. E., & Vlassara, H. (2010). Advanced glycation end products in foods and a practical guide to their reduction in the diet. *Journal of the American Dietetic Association*, *110*(6), 911–916.e12. https://doi.org/10.1016/j.jada.2010.03.018