Malaysian Applied Biology (2022) 51(5): 153-158
https://doi.org/10.55230/mabjournal.v51i5.2405

Research Article

Chlorella sp. (UKM8), A Local Microalgae Isolate with Anti-Human Herpes Virus and Antioxidant Properties

Abdul Fattah Shaima, Nazlina Haiza Mohd Yasin* and Nazlina Ibrahim

Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600, UKM Bangi, Selangor, Malaysia
*Corresponding author: nazlinayasin@ukm.edu.my

ABSTRACT

Microalgae are an invaluable source of new and safe therapeutics with potential antiviral and free-radical scavenging compounds. This study aimed to investigate the antiviral and antioxidant properties of local microalgae, *Chlorella* sp. (UKM8). The UKM8 methanol extract (UKM8-ME) was tested for antiviral activity using plaque reduction assay against Human Herpes Virus type 1 (HHV-1). The antioxidant activity of UKM8-ME was evaluated for the radical scavenging activity (RSA) according to the elimination of 1,1-diphenyl-2-pikrilhydrazil (DPPH) radicals and total phenolic content (TPC) by the Folin-Ciocalteu reactions. UKM8-ME effective concentration that inhibits 50% (EC_{50}) of plaque formation was 222.33 ± 24.54 µg/mL. The calculated selective index is 19 indicating potential antiviral activity. In the DPPH assay, the IC_{50} value of positive control and UKM8-ME were 122.9 ± 29.1 and 198.78 ± 14.35 µg/mL, respectively. The TPC of positive control and UKM8-ME were 263.414 ± 9.6 and 254.793 ± 3.31 mg GAE/g, respectively. Evaluation in RSA and TPC concludes that UKM8-ME has high antioxidant activity. In conclusion, UKM8-ME has two unique properties in anti-HHV-1 and antioxidant activities that can be further evaluated for potential in pharmaceutics and food ingredients.

Key words: Anti-HHV-1, antioxidant, biomass, *Chlorella*, methanol extract

Article History
Accepted: 28 November 2022
First version online: 26 December 2022

Cite This Article:
Shaima, A.F., Mohd Yasin, N.H. & Ibrahim, N. 2022. *Chlorella* sp. (UKM8), a local microalgae isolate with anti-human herpes virus and antioxidant properties. Malaysian Applied Biology, 51(5): 153-158. https://doi.org/10.55230/mabjournal.v51i5.2405

INTRODUCTION

Microalgae can live in diverse habitats owing to their adaptive survival strategy (Phang *et al*., 2015). *Chlorella* spp. has been shown previously to be an excellent source of valuable bioactive compounds with antimicrobial activities (Jafari *et al*., 2018; Zielinski *et al*., 2020). A local isolate, *Chlorella* sp. (UKM8) has been shown to have potential as an antibacterial agent with various compounds including phenols that may contribute to the antiviral and antioxidant activity (Shaima *et al*., 2022). The non-cytotoxicity of Vero cells has also been demonstrated hence the potential for antiviral properties can be further evaluated.

Human Herpes Virus (HHV) is one of the most common opportunistic viral infections globally. HHV can cause a variety of diseases in humans, from small irritations to life-threatening acute infections. New effective compounds are needed due to the development of virus resistance towards current antiviral agents such as acyclovir (Adamson, 2020). Natural products gained attention as alternative sources for antiviral activity due to their availability, efficiency, safety, and cost-effectiveness. Several compounds with such activity have been isolated from microalgae (Besednova *et al*., 2021; El-Sheekh *et al*., 2022).

During microbial infection, reactive oxygen species (ROS) such as hydroxyl (OH), peroxide (ROO), and nitric oxide (NO) are produced, damaging the membrane cells through chain reactions (Martelli & Giacomini, 2018). In such events, it would be favorable to find agents that can combine antioxidant and antimicrobial properties. In this regard, microalgae as a natural reservoir of chemical diversity can

be implemented. In this study, we evaluated the antiviral activity of the methanol extract (ME) of *Chlorella* sp. UKM8 (UKM8-ME) against Human Herpes Virus (HHV-1) and the antioxidant activity.

MATERIALS AND METHODS

Microalgae cultivation and extraction

The cultivation and extraction with methanol for *Chlorella.* sp. UKM8 (NCBI accession number: KT452082) follows Shaima *et al*. (2022). Cultivation was done in 1 L of Bold Basal Medium (BBM) in a Duran bottle and biomass was harvested in approximately ten days. Biomass (1 g) was collected and immersed overnight at 25 °C in 100 mL absolute methanol. The extract was then filtered onto filter paper (Whatman No. 1), concentrated, and dried using a rotary evaporator (Buchi, Germany) at 40 °C. The dried extract of UKM8-ME was stored at 4 °C for subsequent use.

Antiviral activity evaluation

A plaque reduction assay (PRA) was performed to evaluate the antiviral activity against a clinical strain of HHV-1 following the method described by Ismaeel *et al* (2018). Confluent Vero-cells were overlaid with 200 µL of DMEM containing 50 pfu HHV-1 for an hr at 37 °C in a humidified 5% (v/v) CO_2 atmosphere before being discarded. Mock treated and various concentrations of UKM8-ME ranging from 15.63 to 1000 µg/mL were added to 1% (w/v) methyl cellulose in DMEM supplemented with 5% (v/v) Fetal Bovine Serum (FBS). Plaques were allowed to develop for at least 48 h at 37 °C in a humidified 5% (v/v) CO_2 atmosphere. The number of plaques was stained using crystal violet and calculated. The percentage of plaque reduction indicative of viral inhibition for UKM8-ME at various concentrations was computed using the following equation:

$$\% \text{ of plaque reduction} = \frac{PC-PT}{PC} \times 100$$

Where PC indicates the number of plaques derived from virus-infected cells with no treatment. PT represents the number of plaques derived from virus-infected cells with treatment.

The EC_{50} value i.e. extracts concentration required to inhibit 50% of the virus plaque reduction compared to the control was computed using Graph Pad Prism 8 by plotting the plaque reduction percentage to UKM8-ME concentration. The selective index (SI) value was calculated as in the following equation:

$$\text{Selective index (SI)} = CC50 / EC50$$

The concentration of UKM8-ME that kills 50% of the cell population data or CC_{50} value was taken from Shaima *et al*. (2022).

DPPH radical scavenging activity assay

The assay was carried out according to Choochote *et al*. (2014). UKM8-ME was diluted with methanol to various concentrations (0.031, 0.063, 0.125, 0.25, 1.5, & 1 g/L). A volume of 0.5 mL at each concentration was added to 1 mL of 0.5 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma Aldrich, USA) in absolute methanol. The mixture was shaken vigorously and then kept to stand at 25 °C for 30 min in the dark. The absorbance was measured using a Mini-1240 UV spectrophotometer (Shimadzu, Japan) at 517 nm. UKM8-ME only without DPPH was used as blank. Gallic acid (GA) was used as the reference standard at concentrations from 31.25 to 500 µg/mL. The radical scavenging activity (in percentage) was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = ((A_0-(A-A_b))/A_0) \times 100$$

where A0 is the absorbance of the control (DPPH solution only), A is the absorbance of the tested extract with DPPH solution, and Ab is the absorbance of the blank (a sample only). The IC_{50} value is the concentration of a sample or a standard antioxidant in (g/L) that give 50% of the radical scavenging activity and was determined using Graph Pad Prism 8.

Determination of total phenolic content (TPC)

The TPC of the microalgae extract was determined by the Folin–Ciocalteu method according to Manivannan *et al*. (2012). UKM8-ME (0.2 mL) at 1 g/L in methanol was mixed with 1 mL of 1:10 Folin–Ciocalteu reagent (FCR) in a test tube. After four min, 0.8 mL of 7.5 % (w/v) sodium carbonate in distilled water was added. The sample was incubated for 120 min at room temperature in the dark. The absorbance of the reaction mixture was measured at 765 nm using a mini-1240 UV spectrophotometer (Shimadzu, Japan). Gallic acid (GA) was used to produce a standard curve graph, and TPC was determined from the regression equation (R^2=0.9988). The TPC is estimated to be equal to the extracts' equivalent of gallic acid (GAE). The TPC of sample extracts was expressed in milligram gallic acid equivalent (GAE)/g dry weight of sample using the following equation:

$$TPC = (c \times v)/M$$

TPC is the total phenolic content (mg/g) of the sample in GAE; c is the GA concentration obtained from the standard curve (mg/mL); v is the sample volume (mL); M is the weight of the sample (g). The analyses were performed in triplicates. Data were analyzed statistically using One Way Anova by Graph Pad Prism 8.

RESULTS AND DISCUSSION

Plaque reduction assay of UKM8-ME against HHV-1 strain

The concentration used to determine the antiviral activity of UKM8-ME was based on a non-toxic concentration previously determined in our previous study (Shaima *et al*., 2022). The percentage of plaque reduction of UKM8-ME at different concentrations is presented in Figure 1. The antiviral inhibition activity of UKM8-ME can be considered dose-dependent with an EC_{50} value of 222.33 ± 24.55 µg/mL. The CC_{50} value determined was 4.21 mg/mL (Shaima *et al*., 2022), and the calculated selective index (SI) for UKM8-ME is 19. SI values less than one are considered weak antiviral, while values above one have moderate activity and those above ten have great potential (Chattopadhyay *et al*., 2009). Therefore, UKM8-ME can be considered as having potential as an antiviral agent but a more in-depth study may be needed.

Reports on the antiviral studies from *Chlorella* sp. against HHV-1 are limited. However, extracts from *Chlorella* spp. are effective against rotavirus (Cantú-Bernal *et al*., 2020), hemorrhagic septicemia virus (VHSV) and African swine fever virus (ASFV) (Fabregas *et al*.,1999), vesicular stomatitis virus (VSV) (Fukada *et al*., 1968), influenza virus (El-feky *et al*., 2020), betanodavirus causing viral encephalopathy and retinopathy (VER) (Katharios *et al*., 2005). Viral inhibition ranged from 5 to 99% in these studies is relatively comparable to plaque inhibition of up to 78.68% (Figure 1).

Chemical profiling showed that UKM8-ME was rich in phenol (Shaima *et al*., 2022). Several researchers have reported that phenols exhibit a wide range of biological activities such as antimicrobial, antioxidant, antiviral, and anti-cancer activities (Rico *et al*., 2017; Sawant & Mane, 2018). Phenol from *Chlorella* sp. has been reported by Santoyo *et al*. (2010) to inhibit HHV-1. Therefore, it is suggested that phenol in UKM8-ME may contribute to the anti-HHV-1 properties.

Antioxidant activity

The antioxidant activity of UKM8-ME was determined by RSA and TPC. UKM8-ME showed dose-dependent RSA with the highest RSA of UKM8-ME and GA was observed with % DPPH radical inhibition of 63.31 and 69.66 (Table 1). The IC_{50} value is a concentration that prevents 50% of the radical material from showing the extract's ability as an antioxidant agent. UKM8-ME showed an IC_{50} value of 198.78±14.35 µg/mL. The positive control GA showed an IC_{50} value at 122.897 ± 29.102 µg/mL. According to the RSA% and IC_{50} of the UKM8-ME, the extract showed remarkable antioxidant capacity.

TPC is the most frequent and easy to use for measuring the phenolic amounts in plants or food with gallic acid equivalent (GAE) was used for expressing the total phenolic concentration. The standard curve for gallic acid is used to calculate the total phenolic content. UKM8-ME showed a TPC of 254.793 ± 3.31mg GAE/g, almost comparable to the positive control gallic acid of 263.414 ± 9.6 at the concentration of 250 µg/mL with no significant differences (p<0.05).

The high TPC in UKM8-ME is attributed to the phenol content confirmed by Shaima *et al*. (2022). Phenols are hydrophilic compounds that are more soluble in highly polar solvents (Ningsiha *et al*., 2016). In this study, methanol was used as the extractant. Barchan *et al*. (2014) found that methanol extract showed higher TPC than other solvent extracts with lower polarity. TPC of *Chlorella* sp. extracts has been widely studied. It was observed for *Chlorella* sp. E53 (35.5 mg GAE/g) and *Chlorella* sp. ED53 (29 mg GAE/g) (Choochote *et al*., 2014), *C. vulgaris* (45 mg GAE/g) (Pradhan *et al*., 2021), *C. sorokiniana* (73.7 mg GAE/g) and *C. zofingiensis* (40.8 mg GAE/g) (Azaman *et al*., 2017). In general, every algae species has a different amount of phenolic compounds, therefore, the variation in the TPC of algae samples is noted (Rajasekaran & Kalaimagal, 2011; Vello *et al*., 2018). Moreover, various factors can influence the phenolic content of algae including, extraction procedure, particle size, as well as the sample's time and storage condition. The presence of chemicals in the algae that can interfere with the extraction of phenolic substances such as pigments, waxes, and fats can also impact the amount of the extracted phenolic compounds (Arguelles *et al*., 2022).

Chlorella sp. is known for its rapid adaptation to extreme conditions (Japar *et al*., 2021), thus can offer a great reservoir of antioxidant compounds and one of the most known antioxidants formers (Andrade *et al*., 2018). Other studies also confirm the antioxidant ability of *Chlorella* sp., while a wide range of antioxidant compounds was identified, including hexadecanoic acid, 2-hexadecanoic-1-Ol, cyclodecasiloxane, 4-Eicosaenoic acid from *C. vulgaris* (El-fayoumy *et al*., 2020), lutein from *C. zofingensis* and peptide from *C. ellipsoidea* (Choochote *et al*., 2014). Antioxidant activity of UKM8-ME has been confirmed in this study with compounds that have antioxidant properties including 7,10-Hexadecadienoic acid, 9,12-Octadecadienoic acid, Cis-8-methyl-exo-tricyclo, [5.2.1.0(2.6)]decane, Eicosane, Cetene and Z-12-Pentacosene (Shaima *et al*. 2022). More comprehensive studies are required to identify these compounds and their mechanism of activity to unleash their potential as pharmaceuticals.

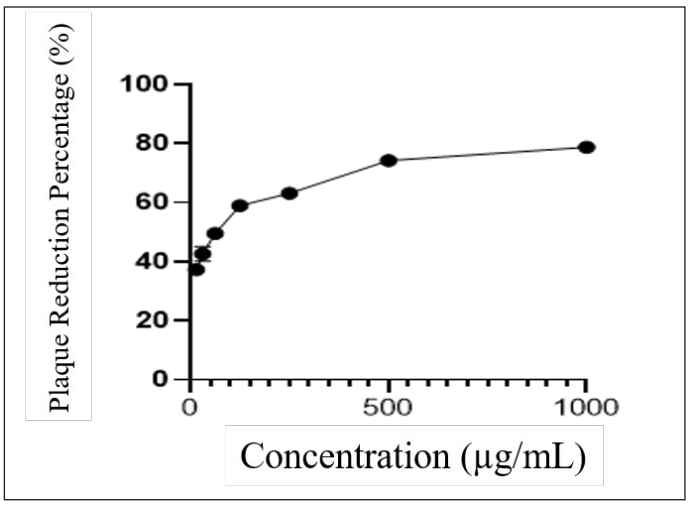

Fig. 1. Plaque reduction percentage against different concentrations (µg/mL) of UKM8-ME.

Table 1. Radical scavenging activity RSA % of UKM8-ME and GA

Sample concentration (µg/mL)	UKM8-ME RSA (%)	GA RSA (%)
31.25	34.66 ± 0.82	45.05 ± 1.64
62.5	37.33 ± 0.49	42.61 ± 2.83
125	38.06 ± 0.89	51.55 ± 1.36
250	58.53 ± 1.03	60.33 ± 1.56
500	65.26 ± 1.03	69.66 ± 1.36
1000	63.13 ± 1.83	nd*

*nd, not determined

CONCLUSION

Local microalgae, *Chlorella* sp. UKM8-ME has good potential against HHV-1 and can be associated with free radicals scavenging activity. The antioxidant ability of UKM8-ME suggests that the extract can be exploited further as food supplements or additives.

ACKNOWLEDGEMENTS

This research is funded by the Malaysian Ministry of Higher Education (FRGS/1/2018/TK02/UKM/01/1). The Organization for Women in Science for the Developing World (OWSD) and Swedish International Development Cooperation Agency (SIDA) are acknowledged for the fellowship to Shaima Abdul Fattah.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Adamson, C.S. 2020. Antiviral agents: discovery to resistance. Viruses, 12(4): 406. https://doi.org/10.3390/v12040406

Andrade, L.M., Andrade, C.J., Dias, M. & Nascimento, C.A.O. 2018. *Chlorella* and *Spirulina* microalgae as sources of functional foods, nutraceuticals, and food supplements; an overview. Food Science and Technology, 6: 45–58. https://doi.org/10.15406/mojfpt.2018.06.00144

Azaman, S.N.A., Nagao, N., Yusoff, F.M., Tan, S.W. & Yeap, S.K. 2017. A comparison of the morphological and biochemical characteristics of *Chlorella sorokiniana* and *Chlorella zofingiensis* cultured under photoautotrophic and mixotrophic conditions. Peer Journal, 5: 3473. https://doi.org/10.7717/peerj.3473

Arguelles, E.D.L.R. 2020. Biochemical composition and bioactive properties of *Chlorella minutissima* (Chm1) as a potential source of chemical compounds for nutritional feed supplement and disease control in aquaculture. Current Applied Science and Technology, 21(1): 65-77. https://DOI10.14456/cast.2021.9

Barchan, A., Bakkali, M., Arakrak, A., Pagán, R. & Laglaoui, A. 2014. The effects of solvents polarity on the phenolic contents and antioxidant activity of three Mentha species extracts. International Journal of Current Microbiology and Applied Sciences, 3(11): 399-412.

Besednova, N.N., Andryukov, B.G., Zaporozhets, T.S., Kryzhanovsky, S.P., Fedyanina, L.N., Kuznetsova, T.A., Zvyagintseva, T.N. & Shchelkanov, M.Y. 2021. Antiviral effects of polyphenols from marine algae. Biomedicines, 9: 200. https:// doi.org/10.3390/biomedicines9020200

Cantú-Bernal, S., Domínguez-Gámez, M., Medina-Peraza, I., Aros-Uzarraga, E., Ontiveros, N., Flores-Mendoza, L., Gomez-Flores, R., Tamez-Guerra, P. & González-Ochoa, G. 2020. Enhanced viability and anti-rotavirus effect of *Bifidobacterium longum* and *Lactobacillus plantarum* in combination with *Chlorella sorokiniana* in a dairy product. Frontiers in Microbiology, 11: 1–9. https://doi.org/10.3389/fmicb.2020.00875

Chattopadhyay, D., Sarkar, M.C., Chatterjee, T., Dey, R.S., Bag, P., Chakraborti, S., Tareq, M. & Khan, H. 2009. Recent advancements for the evaluation of anti-viral activities of natural products. New Biotechnology, 25: 347–368. https://doi.org/10.1016/j.nbt.2009.03.007

Choochote, W., Suklampoo, L. & Ochaikul, D. 2014. Evaluation of antioxidant capacities of green microalgae. Journal of Applied Phycology, 26: 43–48. https://doi.org/10.1007/s10811-013-0084-6

El-fayoumy, E.A., Shanab, S.M.M. & Shalaby, E.A. 2020. Metabolomics and biological activities of *Chlorella vulgaris* grown under modified growth medium (BG 11) composition. Chiang Mai University Journal of Natural Sciences, 19: 93. https://doi.org/10.12982/CMUJNS.2020.0007

El-feky, A.M., Elbatanony, M.M., Naser, A.F.A., Kutkat, O.M., El, A.E.B. & Hamed, M.A. 2020. Phytoconstituents and in vitro anti-oxidant, anti-viral, anti-hyperlipidemic and anticancer effects of *Chlorella vulgaris* microalga in normal and stress conditions. Der Pharma Chemica, 12: 9–20.

El-Sheekh, M.M., Shabaan, M.T., Hassan, L. & Morsi, H.H. 2022. Antiviral activity of algae biosynthesized silver and gold nanoparticles against Herpes Simplex (HSV-1) virus in vitro using cell-line culture technique. International Journal of Environmental Health Research, 32(3): 616-627. https://doi:10.1080/09603123.2020.1789946

Fabregas, J., García, D., Fernandez-Alonso, M., Rocha, A.I., Gómez-Puertas, P., Escribano, J.M., Otero, A. & Coll, J.M. 1999. In vitro inhibition of the replication of haemorrhagic septicaemia virus (VHSV) and African swine fever virus (ASFV) by extracts from marine microalgae. Antiviral Research, 44: 67–73. https://doi.org/10.1016/S0166-3542(99)00049-2

Fukada, T., Hoshino, M., Endo, H., Mutai, M. & Shirota, M. 1968. Photodynamic antiviral substance extracted from *Chlorella* cells. Applied Microbiology, 16: 1809–1810. https://doi.org/10.1128/am.16.11.1809-1810.1968

Ismaeel, M., Dyari, H.R.E., Nor, N.S.M., Yaacob, W.A. & Ibrahim, N. 2018. Anti-human herpesvirus type-1 activity of *Phaleria macrocarpa* fruits methanol extract and fractions. Malaysian Applied Biology, 47(5): 31-40.

Jafari, S., Mobasher, M.A., Najafipour, S., Ghasemi, Y., Mohkam, M., Ebrahimi, M.A. & Mobasher, N. 2018. Antibacterial potential of *Chlorella vulgaris* and *Dunaliella salina* extracts against *Streptococcus mutans*. Jundishapur Journal of Natural Pharmaceutical Products, 13(2): e13226. https://doi.org/10.5812/jjnpp.13226

Japar, A.S., Takriff, M.S. and Yasin, N.H.M. 2021. Microalgae acclimatization in industrial wastewater and its effect on growth and primary metabolite composition. Algal Research, 53:102163. https://doi.org/10.1016/j.algal.2020.102163

Katharios, P., Papadakis, I.E., Prapas, A., Dermon, C.R., Ampatzis, K. & Divanach, P. 2005. Mortality control of viral encephalopathy and retinopathy in 0+ grouper *Epinephelus marginatus* after prolonged bath in dense *Chlorella minutissima* culture. Bulletin of the European Association of Fish Pathologists, 25(1): 28-31.

Manivannan, K., Anantharaman, P. & Balasubramanian, T. 2012. Evaluation of antioxidant properties of marine microalga *Chlorella marina* (Butcher, 1952). Asian Pacific Journal of Tropical Biomedicine, 2(1): 342-346. https://doi.org/10.1016/S2221-1691(12)60185-3

Martelli, G. & Giacomini, D. 2018. Antibacterial and antioxidant activities for natural and synthetic dual-active compounds. European Journal of Medicinal Chemistry, 158: 91-105. https://doi.org/10.1016/j.ejmech.2018.09.009

Ningsiha, I.Y., Zulaikhaha, S., Hidayata, A. & Kuswandib, B. 2016. Antioxidant activity of various kenitu (*Chrysophyllum cainito* L.) leaves extracts from Jember, Indonesia. Agriculture and Agriculture Science Procedia, 9: 378-85. https://doi.org/10.1016/j.aaspro.2016.02.153

Phang, S., Mustafa, E.M., Ambati, R.R., Meriam, N., Sulaiman, N., Lim, P., Majid, N.A. & Dommange, X. 2015. Checklist of microalgae collected from different habitats in Peninsular Malaysia for selection of algal biofuel feed-stocks. Malaysian Journal of Science, 34(2): 141-167. https://doi.org/10.22452/mjs.vol34no2.2

Pradhan, B., Patra, S., Dash, S.R., Nayak, R., Behera, C. & Jena, M. 2021. Evaluation of the anti-bacterial activity of methanolic extract of *Chlorella vulgaris* with special reference to antioxidant modulation. Future Journal of Pharmaceutical Sciences, 7(1): 1-11. https://doi.org/10.1186/s43094-020-00172-5

Rajasekaran, M. & Kalaimagal, C. 2011. In vitro antioxidant activity of ethanolic extract of a medicinal mushroom, *Ganoderma lucidum*. Journal of Pharmaceutical Sciences and Research, 3(9): 1427.

Rico, M., González, A.G., Santana-Casiano, M., González-Dávila, M., Pérez-Almeida, N. & Tangil, M.S.D. 2017. Production of primary and secondary metabolites using algae. In: Prospects and Challenges in Algal Biotechnology. B.N. Tripathi & D. Kumar (Eds.). Springer, Singapore. pp. 311-326. https://doi.org/10.1007/978-981-10-1950-0

Santoyo, S., Plaza, M., Jaime, L., Ibañez, E., Reglero, G. & Señorans, F.J. 2010. Pressurized liquid extraction as an alternative process to obtain antiviral agents from the edible microalga *Chlorella vulgaris*. Journal of Agricultural and Food Chemistry, 58(15): 8522-8527. https://doi.org/10.1021/jf100369h

Sawant, S.S. & Mane, V.K. 2018. Nutritional profile, antioxidant, antimicrobial potential, and bioactives profile of *Chlorella emersonii* kj725233. Asian Journal of Pharmaceutical and Clinical Research, 11(3): 220-225. https://doi.org/10.22159/ajpcr.2018.v11i3.21990

Shaima, A.F., Yasin, N.H.M., Ibrahim, N., Takriff, M.S., Gunasekaran, D. & Ismaeel, M.Y. 2022. Unveiling antimicrobial activity of microalgae *Chlorella sorokiniana* (UKM2), *Chlorella* sp. (UKM8) and *Scenedesmus* sp. (UKM9). Saudi Journal of Biological Sciences, 29(2): 1043-1052. https://doi.org/10.1016/j.sjbs.2021.09.069

Vello, V., Umashankar, S., Phang, S. & Chu, W. 2018. Metabolomic profiles of tropical *Chlorella* and *Parachlorella* species in response to physiological changes during exponential and stationary growth phase. Algal Research, 35: 61–75. https://doi.org/10.1016/j.algal.2018.08.014

Zielinski, D., Fraczyk, J., Debowski, M., Zielinski, M., Kaminski, Z.J., Kregiel, D., Jacob, C. & Kolesinska, B. 2020. Biological activity of hydrophilic extract of *Chlorella vulgaris* grown on post-fermentation leachate from a biogas plant supplied with stillage and maize silage. Molecules, 25(8): 1790. https://doi.org/10.3390/molecules25081790