

Effects of Marine Algae, *Caulerpa lentillifera*, on Lowering Lipid Accumulation and Oxidative Stress in *Caenorhabditis elegans* Model

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Abstract

Obesity is a condition characterized by excessive accumulation of body fat and its prevalence continues to increase worldwide with projections of further growth in the future. Prevention and management strategies for obesity include lifestyle modification, physical activity, pharmacological treatment, and bariatric surgery; however, some approaches may have adverse effects. Therefore, maintaining a balanced diet plays an important role in controlling obesity and reducing the risk of related diseases. Sea grape, *Caulerpa lentillifera*, is a marine alga with potential benefits in preventing and improving metabolic syndrome. This study aimed to investigate the effects of *C. lentillifera* on lipid accumulation, reactive oxygen species (ROS) levels, and lifespan by using *Caenorhabditis elegans* as a model organism. *C. elegans* were induced to become obese worms by exposure to 50 mM glucose, followed by treatment with *C. lentillifera* extract to evaluate its effects compared with a control group. The results showed that *C. lentillifera* extract at concentrations of 10 and 50 µg/mL decreased lipid accumulation, lipid droplets sizes and ROS levels compared to the glucose-induced control group, but it did not effect on lifespan. These results suggested that *C. lentillifera* extract has strong potential to reduce fat accumulation and oxidative stress, leading to the potential application to further develop to combat obesity in the future.

Keywords: Obesity, sea grapes, *Caulerpa lentillifera*, *Caenorhabditis elegans*

ฤทธิ์การลดไขมันสะสมและอนุพลอิสระของสารสกัดจากสาหร่ายพวงองุ่น *Caulerpa lentillifera* ในสัตว์ทดลองต้นแบบ *Caenorhabditis elegans*

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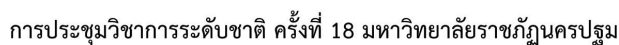
บทคัดย่อ

โรคอ้วนเป็นภาวะที่มีการสะสมไขมันในร่างกายมากเกินไป โดยปัจจุบันมีแนวโน้มเพิ่มสูงขึ้นอย่างต่อเนื่องและคาดว่าจะเพิ่มขึ้นในอนาคต การป้องกันและจัดการโรคอ้วนสามารถทำได้โดยการปรับพฤติกรรมบริโภค การออกกำลังกาย การใช้ยา และการผ่าตัด อย่างไรก็ตาม บางวิธีอาจมีผลข้างเคียง ดังนั้น การรับประทานอาหารที่สมดุลจึงมีบทบาทสำคัญในการควบคุมโรคอ้วนและลดความเสี่ยงของโรคที่เกี่ยวข้อง โดยสาหร่ายพวงองุ่น *Caulerpa lentillifera* เป็นสาหร่ายที่มีศักยภาพในการป้องกันและบรรเทาภาวะกลุ่มอาการเมตาบอลิก งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาฤทธิ์ของสาหร่ายพวงองุ่นต่อการสะสมไขมัน ระดับอนุพลอิสระ และอายุขัย ในหนอน *Caenorhabditis elegans* ซึ่งใช้เป็นสัตว์ทดลองต้นแบบในการศึกษา โดยทำการเหนี่ยวนำให้หนอนเกิดภาวะอ้วนด้วยน้ำตาลกลูโคสที่ความเข้มข้น 50 mM และให้สารสกัดจากสาหร่ายพวงองุ่นเพื่อประเมินผลต่อการสะสมไขมันเมื่อเปรียบเทียบกับกลุ่มควบคุม ผลการทดลองพบว่ากลุ่มหนอนที่ได้รับสารสกัดจากสาหร่ายพวงองุ่นที่มีความเข้มข้น 10 และ 50 µg/mL สามารถลดการสะสมไขมัน ลดขนาด lipid droplets และลดระดับอนุพลอิสระได้ดีกว่ากลุ่มควบคุมที่ได้รับน้ำตาลเพียงอย่างเดียว แต่ไม่มีผลในการยืดอายุขัยของหนอน งานวิจัยแสดงให้เห็นว่าสารสกัดจากสาหร่ายพวงองุ่นมีศักยภาพในการลดไขมันสะสมและอนุพลอิสระ ซึ่งจะนำไปสู่การพัฒนาเพื่อต้านภาวะโรคอ้วนในอนาคต

คำสำคัญ: โรคอ้วน สาหร่ายพวงองุ่น *Caulerpa lentillifera*, *Caenorhabditis elegans*

1. Introduction

Obesity is a major global public health problem characterized by excessive accumulation of body fat that may negatively affect health. The primary cause of obesity is an imbalance between energy intake and energy expenditure and is commonly associated with physical inactivity, sedentary behavior, and excessive consumption of high-calorie foods. In addition, obesity is a major risk factor for several noncommunicable diseases (NCDs), including cardiovascular diseases, type 2 diabetes, and hypertension [1]. Various strategies for the prevention and management of obesity, including physical activity, lifestyle modification, pharmacological treatment, and surgical intervention, may help reduce excessive body fat accumulation and lower obesity-related health risks [2]. However, drug therapy and surgical interventions



Marine macroalgae, commonly known as seaweeds, are rich sources of nutrients and bioactive compounds with potential pharmaceutical properties. *Caulerpa lentillifera*, also known as sea grapes, is an edible green macroalgae with high nutritional value and potential benefits in metabolic health [4]. Previous studies in Wistar rats fed a high-carbohydrate and high-fat diet showed that supplementation with *C. lentillifera* reduced body weight, systolic blood pressure, and plasma total cholesterol levels [5]. In addition, a recent study on screening of *C. lentillifera* extracts for lipid-lowering effect showed that the ethyl acetate extract was the most effective in reducing lipid accumulation and ROS levels in glucose-fed *Caenorhabditis elegans* model of obesity [4]. Therefore, this study aims to determine the potential of bioactive fraction isolated from *C. lentillifera* ethyl acetate extract in reducing lipid accumulation and oxidative stress using a glucose-fed *C. elegans* model.

2. Material and methods

C. elegans strains, including Bristol N2 (wild-type) and LIU-1 (*dhs-3p::dhs-3::GFP + unc-76(+)*), were obtained from *Caenorhabditis* Genetics Center (CGC) at the University of Minnesota, USA. They were maintained on solid nematode growth medium (NGM) plates seeded with the *Escherichia coli* OP50 at 20 °C.

The *C. lentillifera* ethyl acetate (CLEA) extract was fractionated. The obtained fraction 1, namely CLEA-F1, in various concentrations (1, 5, 10 and 50 µg/ml) were dissolved in 0.5% DMSO for used in next experiments.

Oil Red O (ORO) is a fat-soluble dye that stains neutral lipids that is commonly used to detect and quantify lipid accumulation in *C. elegans* [8]. The synchronized wild-type L1 larvae were transferred onto NGM plates containing *E. coli* OP50 supplemented with 50 mM glucose and different concentrations of CLEA-F1 extract until they reach the L4 stage, and were transferred to NGM plates containing 5-fluoro-2'-deoxyuridine (FUDR) (for preventing progeny production). The worms were collected at adult stage, washed with 1X PBS, and fixed in 2x MRWB buffer containing 2% paraformaldehyde. Then, the worms were

incubated with 60% isopropanol followed by staining with 60% Oil Red O solution overnight [9]. The stained worms were washed with 1XPBS, and the supernatant were removed before adding 1X PBS containing 0.01% triton X-100. The worms were subsequently mounted on 2% agarose slides and approximately 105 worms were photographed under the bright-field microscope (Nikon Eclipse upright microscope Eclipse Ci, Tokyo, Japan). The staining intensity was quantified using ImageJ software (National Institute of Health, Bethesda, MD, USA), and statistical analysis was conducted using GraphPad Prism. The experiment was performed in triplicate.

2.4 Lipid droplet quantification

The LIU-1 (*dhs-3p::dhs-3::GFP + unc-76(+)*) strain was used for visualization and quantification of lipid droplets in *C. elegans*. The worms were exposed to same condition as previous assay. Approximately 105 adult worms were washed with M9 and anesthetized with 1% sodiumazide (NaN_3) then mounted on 2% agarose slides [7]. The intensity was photographed under a fluorescence microscope (Nikon Eclipse upright microscope Eclipse Ci, Tokyo, Japan). Size of lipid droplets of 30 worms were photographed by FLUOVIEW™ FV4000 confocal laser scanning microscope (Olympus, Tokyo, Japan). The images were analyzed by ImageJ. All experiments were performed in triplicate.

2.5 Analysis of reactive oxygen species (ROS) levels

The synchronized wild-type L1 larvae were transferred onto NGM plates containing *E. coli* OP50 supplemented with 50 mM glucose and different concentrations of CLEA-F1 extract until they reach the L4 stage, and transferred to NGM plates containing FUDR. N2 (wild-type) in adult stages were washed from plate with M9 for three times. The worms were stained with 50 μM $\text{H}_2\text{DCF-DA}$ and incubated in dark at room temperature for 1 hour [10]. The fluorescence intensity about 105 worms were measured using a fluorescence microscope. The fluorescence intensity was quantified using ImageJ software. The experiment was repeated three times.

2.6 Lifespan assay

After synchronized wild-type L1 larvae, the worms were transferred onto NGM plates containing *E. coli* OP50 supplemented with 50 mM glucose and different concentrations of CLEA-F1 extract until they reach the L4 stage, and transferred to NGM plates containing FUDR. Approximately 40-50 L4-stage N2 worms were picked into 35 mm NGM plates containing FUDR. The worms were incubated at 22 °C [4]. The numbers of live and dead worms were counted daily until the end of the experiment. All experiments were performed in triplicate. The data were analyzed and presented as survival curves. The assay was conducted three times.

2.7 Statistical analysis

The statistical analysis was performed using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA, USA). All results of the treated groups and their control groups were presented as the mean $\pm$ SEM and analyzed by one way-ANOVA with Dunnett's test. The lifespans were analyzed using Kaplan-

Meier survival curves and log-rank tests. Statistical significance was set at P values < 0.05, 0.01, 0.001 and 0.0001.

3. Result and Discussion

3.1 Effect of *C. lentillifera* extract, CLEA-F1, on lipid accumulation

3.1.1 Oil Red O staining

Glucose-induced worm showed increased Oil Red O staining intensity, indicating enhanced lipid accumulation compared with the non-glucose-treated control group, with staining intensity levels of 126.12% and 126.24%. Treatment with CLEA-F1 at concentrations of 1, 5, 10, and 50 $\mu\text{g/mL}$ decreased lipid storage in *C. elegans*, as assessed by Oil Red O staining (Fig. 1). The corresponding staining intensities were 114.9%, 114.0%, and 108.4% at 5, 10, and 50 $\mu\text{g/mL}$, respectively, which were significantly lower than that of the glucose-induced control group (50 mM glucose with 0.5% DMSO), representing reductions of 11.19%, 12.16%, and 17.74%, respectively. In contrast, no significant differences between worms treated with 1 $\mu\text{g/mL}$ CLEA-F1 compared the glucose-induced control group (50 mM glucose with 0.5% DMSO). These findings suggested that CLEA-F1 extract exerted a lipid-lowering effect in *C. elegans* N2, with the strongest effect at 50 $\mu\text{g/mL}$.

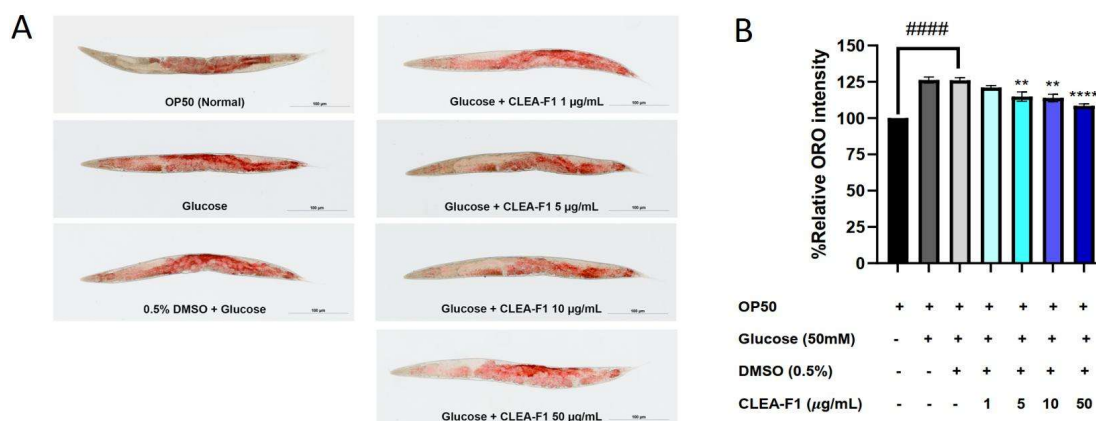


Figure 1. Lipid lowering effects of *C. lentillifera* extract (CLEA-F1) on glucose-induced lipid accumulation in *C. elegans* N2 wild-type worms. Representative images (A) and graph (B) showed Oil Red O staining in worms treated with glucose and *C. lentillifera* extract (CLEA-F1). The asterisks ** and **** indicated P value < 0.01 and 0.0001, respectively, compared with the glucose mixed with 0.5% DMSO control group, while #### indicated P value < 0.0001 compared with the normal control group fed with *E. coli* OP50.

3.1.2 Lipid droplet quantification

Lipid droplets (LDs) play important roles in lipid storage and metabolic regulation, and DHS-3::GFP has been widely used as a lipid droplet marker in the intestinal cells of *C. elegans* [11]. The fluorescence intensity of lipid droplets was evaluated by detecting expression of green fluorescent protein (GFP) under the control of the *dhs-3* promoter as a marker for lipid droplets. The result showed that the

A

OP50 (Normal)

Glucose + CLEA-F1 10 $\mu\text{g/mL}$

Glucose

Glucose + CLEA-F1 50 $\mu\text{g/mL}$

0.5% DMSO + Glucose

B

% Relative fluorescence intensity

OP50

Glucose (50mM)

DMSO (0.5%)

CLEA-F1 ($\mu\text{g/mL}$)

Condition	% Relative fluorescence intensity	Significance
OP50	100	
Glucose (50mM)	~115	##
DMSO (0.5%)	~110	##
CLEA-F1 (10 $\mu\text{g/mL}$)	~90	****
CLEA-F1 (50 $\mu\text{g/mL}$)	~85	****

Previous studies have demonstrated that the ethyl acetate extract of *C. lentillifera* exhibits anti-obesity effects by downregulating lipogenesis-related genes and upregulating lipolysis-related genes [4]. Therefore, it is possible that CLEA-F1 may exert its lipid-lowering effect through similar molecular pathways. However, the underlying molecular mechanisms responsible for the lipid-lowering effects of CLEA-F1 will be further elucidated.

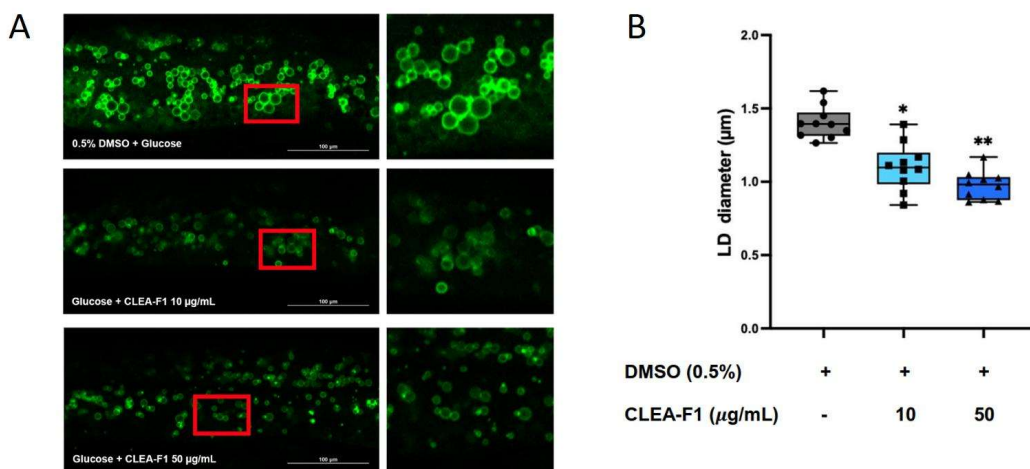


Figure 3. Effect of *C. lentillifera* extract (CLEA-F1) on reducing the sizes of lipid droplets in glucose-induced *C. elegans* model. Representative images (A) and graph (B) showed sizes of lipid droplets in worms treated with glucose and *C. lentillifera* extract (CLEA-F1). The asterisks * and ** indicated P value < 0.05 and 0.01 , respectively, compared to the glucose with 0.5% DMSO control group.

3.2 Effect of *C. lentillifera* extract, CLEA-F1, on ROS levels

High glucose exposure in *C. elegans* induced oxidative stress by increasing the production of ROS, which is associated with obesity-related effects in this model [12]. ROS levels was investigated in obese N2 worm treated with 50 mM glucose, with and without 0.5% DMSO. Both groups showed significantly increased intensity levels of 133.20% and 134.60%, respectively. However, treatment with CLEA-F1 extract at 10 and 50 µg/mL was able to decrease fluorescence intensity level of 105.30% and 93.03%, representing decreases of 29.27% and 41.58%, respectively (Fig. 4).

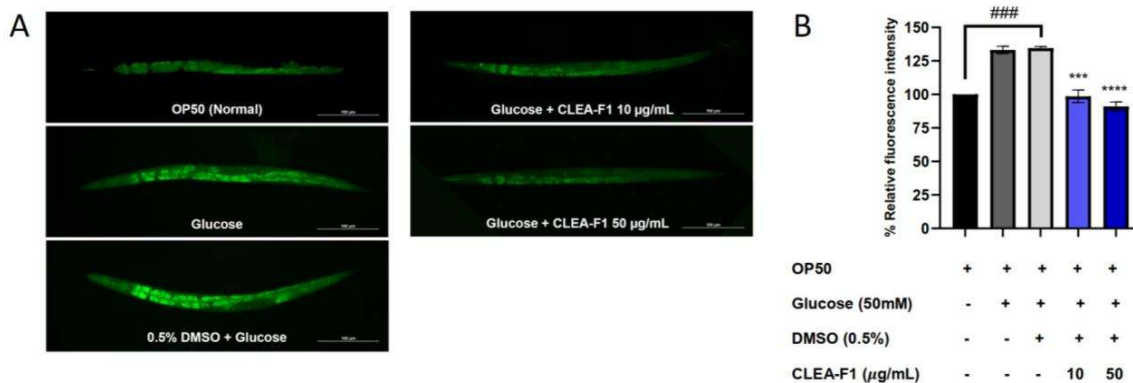


Figure 4. Effect of *C. lentillifera* extract (CLEA-F1) on reducing ROS levels in obese model *C. elegans*. Representative images (A) and graph (B) showed fluorescence intensity in worms treated with glucose and *C. lentillifera* extract (CLEA-F1). The asterisks ** and *** indicated P value < 0.01 and 0.001 , compared to the glucose with 0.5% DMSO control group, while ### indicated P value < 0.001 compared to the with the normal control group fed with *E. coli* OP50.

3.3 Effect of *C. lentillifera* extract, CLEA-F1, on lifespan

The result of lifespan revealed that there were no significant differences on lifespan in obese worms treated with the CLEA-F1 extract compared to the glucose with 0.5% DMSO control (Fig 5). This result suggested that the CLEA-F1 extract did not affect longevity-related pathways in glucose-fed *C. elegans*.

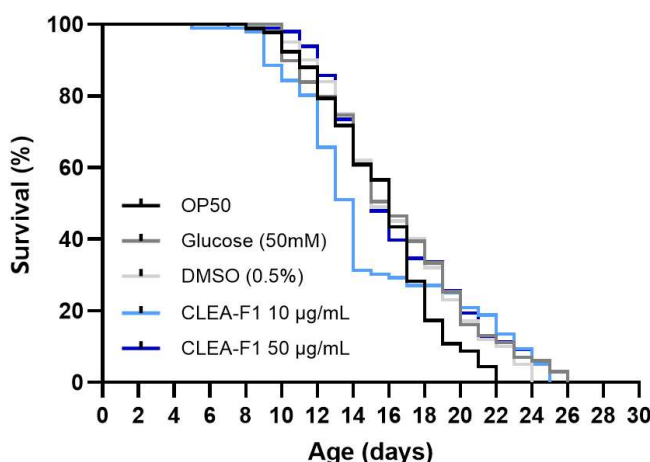


Figure 5. Effect of *C. lentillifera* extract (CLEA-F1) on lifespan in glucose-induced *C. elegans* model.

Table 1. Mean lifespan, percentage increase of lifespan, and significance (*P* value) of *C. elegans* under various treatment conditions compared to the control group.

Compound	Mean Lifespan (Days $\pm$ SD)	% Increase Lifespan	Significance (<i>P</i> value)
OP50	15.59 $\pm$ 3.31	-	ns
Glucose (50 mM)	16.40 $\pm$ 4.36	5.20 ^A	ns
Glucose + 0.5% DMSO	16.36 $\pm$ 3.96	4.94 ^A	ns
Glucose + CLEA-F1 10 μ g /mL	14.91 $\pm$ 5.00	-8.86 ^B	ns
Glucose + CLEA-F1 50 μ g /mL	16.43 $\pm$ 4.01	0.43 ^B	ns

Note : ^A indicated comparison with OP50, ^B indicated comparison with glucose (50 mM) + 0.5% DMSO and, ns indicated a non-significance.

4. Conclusion

The present study showed that CLEA-F1 obtained from *C. lentillifera* ethyl acetate extract decreased lipid accumulation and sizes of lipid droplets in glucose-induced *C. elegans* model of obesity. Interestingly, the CLEA-F1 extract also reduced the oxidative stress; however, it did not extend lifespan. Therefore, CLEA-F1 extract has the potential to reduce fat deposits and may be further developed as a therapeutic agent for the prevention and treatment of obesity.

5. References

- [1] World Heart Federation. (n.d.). *Obesity*. <https://world-heart-federation.org/what-we-do/obesity/>.
- [2] American Diabetes Association Professional Practice Committee for Diabetes*. (2026). 8. *Obesity and Weight Management for the Prevention and Treatment of Diabetes: Standards of Care in Diabetes—2026. Diabetes Care*. American Diabetes Association.
- [3] Magwaza, S. N., & Islam, M. S. (2023). *Roles of Marine Macroalgae or Seaweeds and Their Bioactive Compounds in Combating Overweight, Obesity and Diabetes: A Comprehensive Review*. *Marine drugs*, 21(4), 258. <https://doi.org/10.3390/md21040258>
- [4] Chumphoochai, K., Manohong, P., Niamnont, N., Tamtin, M., Sobhon, P., & Meemon, K. (2023). *Anti-Obesity Effects of Marine Macroalgae Extract Caulerpa lentillifera in a Caenorhabditis elegans Model*. *Marine drugs*, 21(11), 577. <https://doi.org/10.3390/md21110577>.
- [5] Manoppo, J. I. C., Nurkolis, F., Pramono, A., Ardiaria, M., Murbawani, E. A., Yusuf, M., Qhabibi, F. R., Yusuf, V. M., Amar, N., Karim, M. R. A., Subali, A. D., Natanael, H., Rompies, R., Halim, R. F., Bolang, A. S. L., Joey, G., Novianto, C. A. and Permatasari, H. K. (2022) *Amelioration of obesity-related metabolic disorders via supplementation of Caulerpa lentillifera in rats fed with a high-fat and high-cholesterol diet*. *Front. Nutr.* 9:1010867. doi: 10.3389/fnut.2022.1010867
- [6] Dyczkowska, A. A., & Chabowska-Kita, A. (2021). *Caenorhabditis elegans as a model organism in obesity research*. *Biotechnologia*, 102(3), 337–362. <https://doi.org/10.5114/bta.2021.109094>
- [7] Bouyanfif, A., Jayarathne, S., Koboziev, I. and Moustaid-Moussa, N. (2019). *The Nematode Caenorhabditis elegans as a Model Organism to Study Metabolic Effects of ω -3 Polyunsaturated Fatty Acids in Obesity*. *Advances in Nutrition*, 10(1), 165-178. <https://doi.org/10.1093/advances/nmy059>
- [8] Wang, F. Y., & Ching, T. T. (2021). *Oil Red O Staining for Lipid Content in Caenorhabditis elegans*. *Bio-protocol*, 11(16), e4124. <https://doi.org/10.21769/BioProtoc.4124>.
- [9] Chumphoochai, K., Promtang, S., Chalorak, P., Manohong, P., Niamnont, N., Tamtin, M., Sobhon, P., Tuck, S. and Meemon, K. (2025), *Halymenia durvillei Extracts Exert Antiobesity Effects by Targeting hosl-1-Mediated Lipolysis in a Glucose-Induced Caenorhabditis elegans Model*. *eFood*, 6(3): e70065. <https://doi.org/10.1002/efd2.70065>
- [10] Thongdechsrī, S., Kamonnat, C., Sanguanphun, T., Kraokaew, P., Jattujan, P., Niamnont, N., Smith, S. J., Cummins, S. F., Jongkamonwiwat, N., Sobhon, P., & Meemon, K. (2026). *Triterpenoid saponin-containing Holothuria leucospilota extract mitigates amyloid- β proteotoxicity in transgenic C. elegans through activations of protein clearance and stress resistance pathways*. *Food Bioscience*, Volume 77, 108427. <https://doi.org/10.1016/j.fbio.2026.108427>.
- [11] Xie, K., Zhang, P., Na, H., Liu, Y., Zhang, H., & Liu, P. (2019). *MDT-28/PLIN-1 mediates lipid droplet-microtubule interaction via DLC-1 in Caenorhabditis elegans*. *Scientific reports*, 9(1), 14902. <https://doi.org/10.1038/s41598-019-51399-z>.
- [12] Núñez, S., Millán-Laleona, A., Cano-Lou, J., Corella, A., Moliner, C., Cásedas, G., Maggi, F., López, V., & Gómez-Rincón, C. (2025). *Black mulberry (Morus nigra L.) prevents deleterious effects of excess*



glucose in obese C. elegans decreasing lipofuscin accumulation and ROS production. Heliyon, 11(2), e41898. <https://doi.org/10.1016/j.heliyon.2025.e41898>.