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Investigating the Anti-Inflammatory and Immunoregulatory Effects of *Euphorbia hirta* and Their Relation to Anti-Fatigue and Bioenergy Generation in Post-COVID Syndrome

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Introduction

Folklore medicines, such as Traditional Chinese Medicine, provide a variety of plants that are rich in phytochemicals. A great example is *Euphorbia hirta* L., a plant that thrives in tropical and subtropical regions. *E. hirta* or Tawa-Tawa has been discovered to have diverse pharmacological activities. Specifically, it has been valued as a prospective adjunctive anti-COVID-19 medicine. However, its effects on Post-COVID syndrome, as well as its anti-fatigue and bioenergy-generating characteristics have yet to be elucidated. This study investigated the anti-fatigue properties of Tawa-Tawa water extract *in vivo* and its anti-inflammatory and cytotoxic activities *in vitro*. The power density measurement of different concentrations of Tawa-Tawa was also deciphered by utilizing a Microbial Fuel Cell (MFC). As indicated, Tawa-Tawa increased the endurance of mice in a forced swimming test after administration. Anti-inflammatory and cytotoxicity assays resulted in a significant decrease in Interleukin-6 production of a primary chondrocyte cell line and a non-cytotoxic cell viability percentage, respectively. In addition, power density in MFC increased as the concentration of Tawa-Tawa increased. The findings suggest that Tawa-Tawa could exhibit anti-fatigue, anti-inflammatory, and bioenergy generation capabilities. Given its anti-inflammatory and non-cytotoxic nature, this study uncovers a new perception of the potential of Tawa-Tawa extract as a treatment for anti-fatigue. Furthermore, the bioenergy generation activity of Tawa-Tawa suggests that its treatment mechanism may be electron-transport chain-directed, involved in ATP production and that the plant exhibits antiviral activity, specifically anti-COVID-19. With this, Tawa-Tawa extract could possibly be a remedy for post-COVID-19 syndrome due to its capabilities of alleviating fatigue, inflammation, respiratory disorders, and viral infections.

Materials and methods

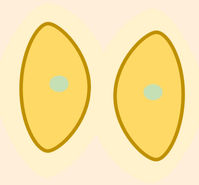
Animals and Treatment

In this study, 8 male ICR mice were used, obtained from BioLASCO Taiwan, following approval from the Institutional Animal Care and Uses Committee of Chang Jung Christian University (Ethical approval code: CJCU-109-001). The mice were 6–8 weeks old and weighed 18–22 g. They were housed in controlled conditions and provided standard food and water ad libitum. The mice were orally administered for 2 weeks before being used in subsequent experiments. All procedures followed the Principles of Institute Animal Care and Uses Committee and the guidelines of the Chang Jung Christian University Animal Research Committee.

Forced Swimming Test

The mice were subjected to a forced swimming test as the anti-fatigue assay. Thirty minutes after the final treatments, a forced swimming test was carried out by briefly placing the mice individually in a swimming pool filled with water (25 ± 1◦C) to a depth of 30 cm with a lead sheath (5% of the mouse's body weight) attached to the body of each mouse. The swimming time was immediately recorded when the physical strength of the mouse was exhausted, and it could not rise to the surface for more than 10 seconds.

Anti-Inflammatory Assay



Primary chondrocyte cells obtained from human ear cartilage were purchased from the American Type Culture Collection (Rockville, MD, USA). Cells were maintained and cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 µg/mL streptomycin (Gibco BRL, Grand Island, NY, USA) in a humidified incubator (5% CO2 at 37 ◦C). 200 mg/mL of the plant was administered to the cultured cells for anti-inflammatory testing. The interleukin-6 produced by the cells was then measured.

Power Density Measurements

A H-type double-chamber Microbial Fuel Cell (MFC), following Chen et al. (2020), utilized carbon rods as electrode material. The MFC had anodic and cathodic chambers separated by a proton exchange membrane (DuPontTM Nafion® NR-212) with a contact area of 0.000452 m2. Each chamber held 200 mL of solution. The cathodic chamber contained electrolyte composed of potassium ferricyanide and dipotassium hydrogen phosphate dissolved in deionized water. The anodic chamber held a culture broth with Aeromonas hydrophila (NIU01) electroactive dye-decolorizing bacteria, precultured in LB broth medium. Plant extract at various concentrations (250 ppm to 1500 ppm) was added to the cultured cell broth, with rinsing between concentrations.

Results

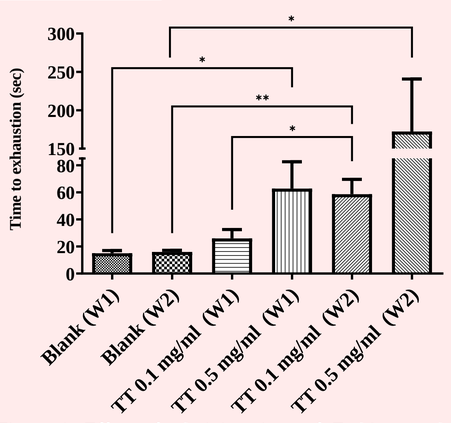


Figure 1. Effect of administrations of *E. hirta* crude water extract at 0.1 and 0.5 mg//mL dose on swimming exercise performance.

Fatigue described herein is a state of physical exhaustion that is an experience of low energy which is not usually taking place in normal life. Thus, it was suspected that ineffective biomass energy extracting and electron transfer-mediating capabilities would be the main cause of fatigue. The study utilized mice as an animal model for the anti-fatigue properties of the TT water extract at concentrations of 0.1 mg/mL and 0.5mg/mL using a forced swimming test. Figure 1 shows that the increase in the concentration of the plant extract increases the time to exhaustion for the experimental sets (W1 and W2). Clearly, 0.5 mg/mL extract demonstrated the highest exhaustion time in the two groups of mice. There is also a significant difference between the animal group that received a higher extract concentration than the control group. This result revealed that TT water extract could effectively increase endurance in mice models. Possibly, TT has promising electron-mediating characteristics that provided effective bioenergy extraction in mice.

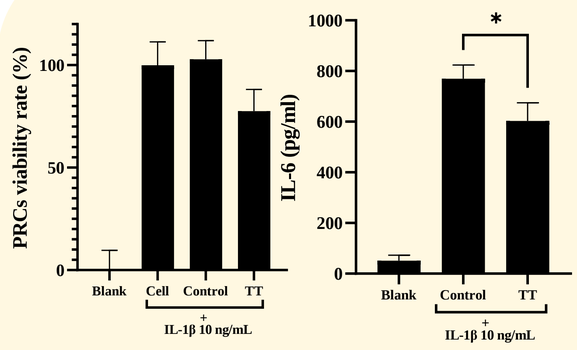


Figure 2. (A) Effect of *E. hirta* water extract on the production of IL-6 in primary chondrocyte cells; (B) Effect of *E. hirta* water extract on the cell viability of primary chondrocyte cells.

Under a dosage of 200 mg/mL of *E. hirta* crude water extract, an estimated 650.47 pg/mL of Interleukin-6 was produced. This result has been deemed non-toxic after performing cytotoxic assay. Figure 2A shows a statistically significant reduction in the amount of IL-6 in the primary chondrocyte cells administered with TT water extract compared to the control. On the other hand, Figure 2B depicted the effect of the extract on the cell viability of primary chondrocyte cells. The cell viability percentage of cells administered with TT was below 100% but above 50%, suggesting the non-cytotoxic nature of the plant extract. Thus, TT could be considered as biomaterials with low toxicity potency to chondrocyte cells.

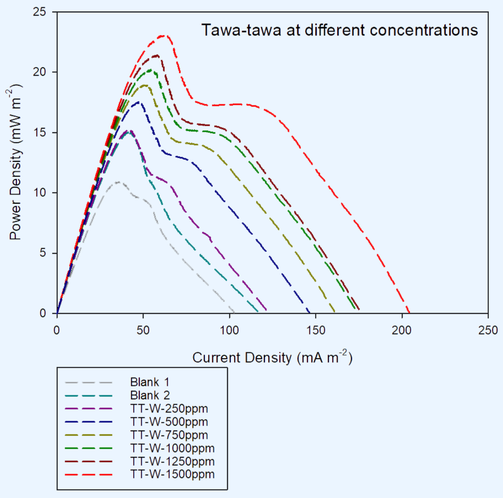


Figure 3. Power density profiles of water extract of Tawa-Tawa at different concentrations.

Table 1. Power density analysis and amplification factor of different Tawa-Tawa water extract concentrations. Amplification is with respect to blank 1.

Samples	Power Density	Amplification Factor
Blank 1	10.88 ± 0.03	-
Blank 2	14.70 ± 0.35	1.35 ± 0.16
TT-250ppm	14.94 ± 0.28	1.37 ± 0.12
TT-500ppm	17.16 ± 0.38	1.58 ± 0.17
TT-750ppm	18.45 ± 0.48	1.69 ± 0.22
TT-1000ppm	18.47 ± 1.75	1.70 ± 0.86
TT-1250ppm	20.97 ± 0.43	1.93 ± 0.20
TT-1500ppm	22.82 ± 0.27	2.10 ± 0.12

Since bioenergy-mediating and electron-shuttling capabilities would be strongly associated with the ON/OFF expression of physical fatigue, the bioenergy-expressing level could be exhibited as an indicator reporting on the physical conditions of the mice. The capability of the plant extract to generate bioelectricity was determined using a dual-chamber microbial fuel cell. As shown in Figure 3, an increased power density could be exhibited as TT concentration increased. This outcome is further proven by the amplification factor of power density calculated with respect to blank 1 (Table 1), implying the capability of TT water extract in electron mediating for enhanced bioelectricity generation. TT at ca. 1500 ppm concentration demonstrated the most favorable bioenergy-stimulating characteristics (i.e., amplification factor of 2.10 ± 0.12).

Conclusions

In this study, the water extract of *Euphorbia hirta* was used to test different biological properties *in vivo* and *in vitro*. This study aimed to understand the anti-fatigue, bioenergy generation, anti-inflammatory, and immunoregulatory characteristics of Tawa-Tawa. These properties provide insight on the effects of Tawa-Tawa against Post-COVID-19 Syndrome. The results indicated that the extract exhibited anti-fatigue and anti-inflammatory properties and no cytotoxicity potency. Furthermore, bioenergy generation of the extract shows a potential source of electroactive species. It can be further explored for possible novel mechanisms in demonstrating its biological properties and the interaction of the secondary metabolites with infectious agents such as bacteria, viruses, or fungi. The findings of the study suggest that the aerial parts of *E. hirta* could potentially be a treatment for anti-fatigue, which may be electron-transport chain driven based on its bioenergy generation capabilities. Most importantly, the findings suggest the potential of Tawa-Tawa extract against COVID-19 and post-COVID-19 syndrome given its diverse pharmacological activities.