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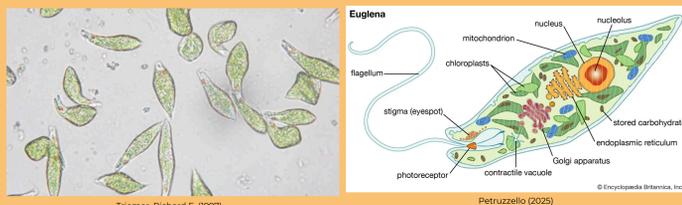
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## Abstract

*Euglena gracilis* has the ability to produce many interesting antioxidants such as beta-carotene, L-ascorbic acid, and  $\alpha$ -tocopherol. This research is intended to compare the efficiency of the extraction method. Between the microwave-assisted extraction method (MAE) and the ultrasound-assisted extraction (UAE) in the extraction with ethanol dissolver, study some biological effects of the extract obtained from both extraction methods. When studying biological properties by testing the antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-bis(3-ethylbenzothiazol-6-sulphonyl)propane (ABTS), potassium ferricyanide antioxidant power (RAPPF), and total phenolic content (TPC). It was found that the % yield of extract and paramylon obtained from both methods had similar amounts, which the extraction method found that the extract obtained from using microwave waves took less time than high-frequency sound wave 30 times. The extract from the microwave-assisted extraction method (MAE) was tested and exhibited better efficiency than the ultrasound-assisted extraction (UAE) and was found in scavenging activity of DPPH radical with in  $10.97 \pm 0.90$  mg GAE/g extract and high amounts of phenolic compounds in total phenolic content assay with in  $6.43 \pm 1.00$  mg GAE/g extract. The extract from the ultrasound-assisted extraction (UAE) was tested, by exhibited a higher efficiency in scavenging activity of the ABTS radical than the microwave-assisted extraction method (MAE) with in  $12.15 \pm 2.81$  mg TEAC/g extract. Moreover, the extract was found to include antibacterial activity bacteria (*Staphylococcus aureus* and *Escherichia coli*) that were determined by paper disc diffusion and the minimum bactericidal concentration (MBC). The result revealed that *E. gracilis* extract can act against only *Staphylococcus aureus*, the minimum bactericidal concentration (MBC) is at a concentration of 25 and 1.5625 mg/ml, respectively.

## Introductions



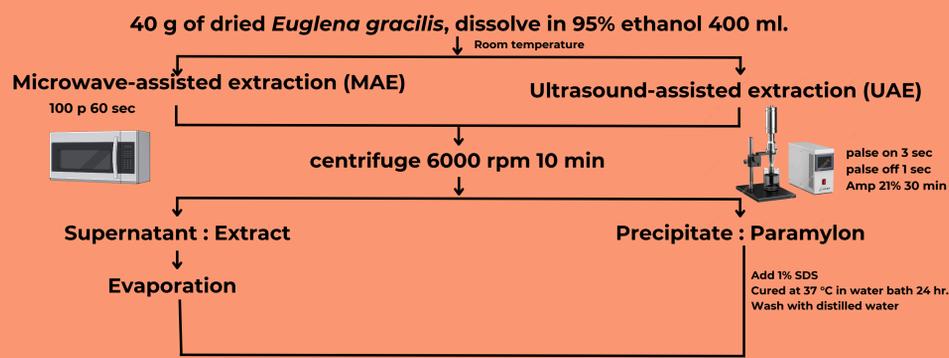
*Euglena* has a variety of nutritional growth patterns under different culture conditions. It contains many nutrients such as vitamins such as vitamins C and D, 9 types of minerals such as iron and calcium, amino acids such as lysine and alanine, unsaturated fatty acids such as DHA and EPA, and chlorophyll and paramylon ( $\beta$ -1,3-glucan). Therefore, the benefits of *Euglena* have been studied and methods of use have been developed in various aspects, including the application in cosmetics. Currently, the popularity of using extracts or natural products tends to increase every year. Therefore, the study of biological extracts from *Euglena* may lead to the discovery of new extracts that have the effect of inhibiting the work of free radicals and the ability to inhibit bacteria. (Gissibl et al., 2019)

## Objectives

- To study methods for extracting bioactive compounds from *Euglena* biomass
- To study the biological activities of *Euglena* extract.

## Methodology

### Extraction methods



### Activity assay

### Lyophilization

Extract is dissolved in 95% ethanol  
Paramylon is dissolved in 1 M NaOH

Extract is dissolved in 95% DMSO  
Paramylon is dissolved in 1 M NaOH

Mix by vortex mixer

Centrifuge 6000 rpm 10 min  
Dilute to a concentration of 1-200 mg/ml

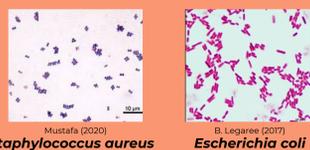
### Antioxidant activities

- DPPH assay (Bhadoriya et al., 2012)
- ABTS assay (Bunea et al., 2011)
- PFRAP assay (Benslama and Harare, 2016)

Total phenolic content  
(Khan et al., 2018 and Aryal et al., 2019))

### Antibacterial activities

- Paper disc diffusion method (Balouiri et al., 2016)
- Minimum inhibitory concentration (MIC) (CLSI, 2012)
- Minimum bacteria concentration (MBC) (CLSI, 1998)



## Results and discussion

### Productivity

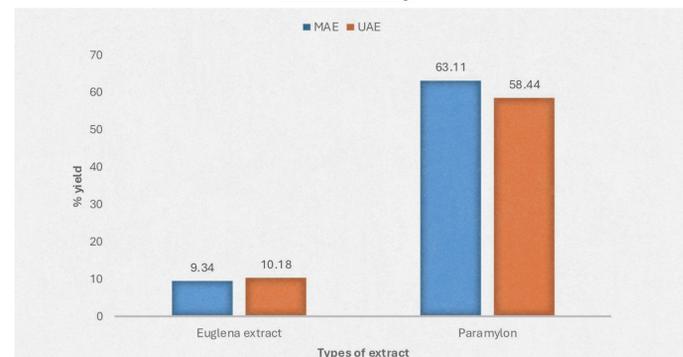


Figure 3. Productivity yield of extracts and paramylon from each methods.

### Antioxidant activities

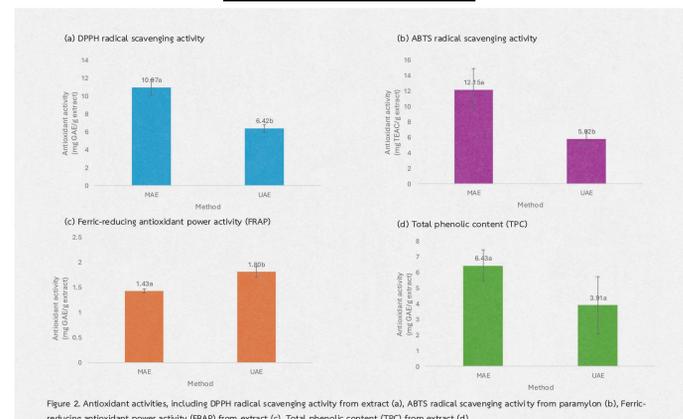


Figure 2. Antioxidant activities, including DPPH radical scavenging activity from extract (a), ABTS radical scavenging activity from paramylon (b), Ferric-reducing antioxidant power activity (FRAP) from extract (c), Total phenolic content (TPC) from extract (d)

### Antibacterial activities

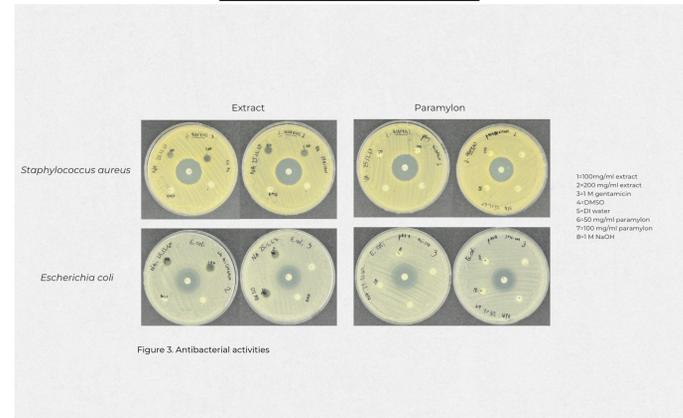


Figure 3. Antibacterial activities

From the experiment, it is found that the extraction method of MAE takes less time around 30 times and uses 10 times less electricity than the UAE method, but the yield is similar (figure 1). In addition, it is a method that helps preserve the important substances in extract better and energy is transferred directly to *Euglena gracilis* through molecular interaction, even though heat is generated in the system.

Antioxidant activity test of *E. gracilis* extract by DPPH, ABTS method found that *E. gracilis* extract by MAE method has the ability to inhibit DPPH and ABTS free radicals more than UAE method (figure 2). From the results of the total phenolic content MAE also have more quality than UAE. In the FRAP method, it was found that the extract from *E. gracilis* by the UAE method had a better ability to reduce agent or electron donor to free radicals than the MAE method, which the UAE method had a better ability to compete with Fe<sup>2+</sup> metal than the MAE method. From the research of Chaimuang (2019), comparing the antioxidant activity of *Spirogyra* spp. and *Cladophora* spp. extracts by the TD, MAE and UAE methods, it was found that the MAE method had the highest antioxidant value. Therefore, it can be said that the MAE method is the most effective in extracting antioxidants. When comparing the antioxidant values of *E. gracilis* extracts with *Eutrum* sp. AARL C001 from the research of Pongpanich (2019), *Spirogyra* spp. and *Cladophora* spp. algae from the research of Chaimuang (2019), it was found that the *E. gracilis* extract has less antioxidant activity.

In antibacterial activities, when testing the ability to inhibit *Staphylococcus aureus* and *Escherichia coli* by the paper disc diffusion method, it was found that the type of extract extracted from *E. gracilis* affects the inhibitory effect on bacteria, from MAE and UAE. The clear zone diameters of *S. aureus* is  $5.1 \pm 0.17$  mm and  $5.85 \pm 0.49$  mm, respectively, and the clear zone of *E. coli* is  $7.3 \pm 1.40$  mm and  $7.3 \pm 0.75$  mm, respectively. Paramylon has no inhibitory activity. It had the lowest MIC and MBC values of 25 mg/ml and 1.5625 mg/ml, respectively, only on *S. aureus*.

## References

- Gissibl et al., (2019). Bioproducts From *Euglena gracilis*: Synthesis and Applications. Retrieved February 26, 2025, from <https://www.frontiersin.org/journals/bioengineering-and-biotechnology/articles/10.3389/fbioe.2019.00108/pdf>.
- Gupta et al., (2021). *Euglena* Species: Bioactive Compounds and their Varied Applications. Retrieved February 26, 2025, from <https://www.eurekaselect.com/article/117333>.

## Acknowledgement

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## Conclusion

From the experiment it was found that the extract of *Euglena gracilis* from both methods had similar yield. However, the method using MAE used less time and energy for extraction than the method using ultrasound around 30 times. It has antioxidant activity, each method has different ability to inhibit free radicals and the extract from both methods has antibacterial activity against *Staphylococcus aureus*.