INTERNATIONAL JOURNAL OF BIOSCIENCES AND BIOTECHNOLOGY
eISSN: 2655-9994
pISSN: 2303-3371

Phytochemical Screening and Acute Toxicity Test of *Caulerpa lentillifera* J. Agardh Infusion Extract on Mice (*Mus musculus* L.)

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Received: 03-20-2023 **Revised:** 03-28-2024 **Accepted:** 04-25-2024

Citation: Adiyanti, S., Phabiola, T.A. & Sutari, N.W.S. (2024). Phytochemical Screening and Acute Toxicity Test of *Caulerpa lentillifera* J. Agardh Infusion Extract on Mice (*Mus musculus* L.). *International Journal of Biosciences and Biotechnology*, 11(2): 12-18. https://doi.org/10.24843/IJBB.2024.v11.i02.p03

Abstract: *Caulerpa lentillifera* possesses health-promoting components, including anti-cancer, antioxidant, anti-diabetic, and immunostimulatory properties. This study aims to analyze the phytochemical composition and the LD50 acute toxicity dose of *C. lentillifera* infusion extract on mice. The findings provide valuable information for the potential use of the extract as food or medicine. A qualitative method was used for the phytochemical screening. A total of twenty-five mice were used for the acute toxicity test and were divided into five treatment groups, *viz.* Control, 375 mg/kg BW, 750 mg/kg BW, 1.500 mg/kg BW, 3.000 mg/kg BW *C. lentillifera* oral infusion extract. Symptoms of toxicity and mortality were observed 24 hours after the treatment, and body weight was monitored for seven days. The phytochemical screening revealed that the presence of alkaloids, saponins, and tannins in the *C. lentillifera* infusion extract. The acute toxicity test showed no mortality at the tested dosages (up to 3000 mg/kg body weight), thus indicating that the *C. lentillifera* infusion extract was classified as non-toxic. These findings indicated that *C. lentillifera* was promising for the further investigation as a safe and potentially beneficial health-promoting ingredient.

Keywords: Acute toxicity, *Caulerpa lentillifera*, Infusion extract, Phytochemical screening

1. Introduction

Indonesia possesses seaweed as one of its marine resource commodities. According to the data from the Statistics Indonesia in 2019, the total seaweed production in Indonesia reached 9,746,946 tons. Seaweed has a variety of beneficial compounds and substances that might improve health, including fiber, omega-3 fatty acids, essential amino acids, also vitamins A, B, C, and E (Agung *et al*., 2019). Due to such diverse range of beneficial compounds, it is extensively used in food, health, cosmetics, and raw materials in various industrial sectors (Nurlaida *et al*, 2023).

One of the seaweeds found in Indonesia is *C. lentillifera*, commonly known as Bulung Anggur in Bali. It is a macroalgae species from the genus Caulerpa often found in coastal areas of Bali, including the West Tanjung Benoa Beach. The Balinese usually consume it as a salad or fruit mix. Previous research has reported that *C. lentillifera* contains anti-cancer properties (Maeda *et al*., 2012), acts as an antioxidant (Tian *et al*., 2021), has anti-diabetic benefits (Khairuddin *et al*., 2020), and stimulates the immune system (Sun *et al*., 2019). In addition, the polysaccharide content in *C. lentillifera* can prevent HeLa cells from the SARS-CoV-2 infection at a LC_{50} concentration of 48.48 µg/mL (You *et al*., 2022). Furthermore, *C. lentillifera* also exhibits potential as a highly promising functional food additive. The diverse combination of crude fiber, minerals, proteins, fats, and carbohydrates in the substance makes it a promising candidate for promoting dietary fiber intake and easily adaptable for food production (Tapotubun *et al*., 2020).

The potential use of a plant as a food or medicine ingredient is strongly influenced by the presence of phytochemical compounds and their toxicity levels. Phytochemical compounds in a plant are bioactive compounds naturally produced by a plant through primary or secondary metabolism (Chusniasih & Tutik, 2020). Phytochemical screening helps detect the presence of a phytochemical compound contained in a plant. The toxicity level of preparation can be determined by conducting toxicity tests on experimental animals to identify harmful effects on the biological system and obtain standard dose-response data from the test preparation (BPOM, 2022). This research aims to analyze the phytochemical composition and the LD_{50} acute toxicity dose of *C. lentillifera* infusion extract on mice so as to assess the safety of *C. lentillifera* for its potential use as food or medicine.

2. Methodology

The research was conducted from October to December 2022. Samples of *C. lentillifera* were collected from West Tanjung Benoa's beach, Kuta Selatan district, Badung Regency, Bali, Indonesia. Sample extraction, phytochemical screening, and toxicity testing were performed at the Laboratory of Genetic Resources and Molecular Biology, Universitas Udayana. Figure 1 shows the research flow chart. Figure 2 shows the map of the research area.

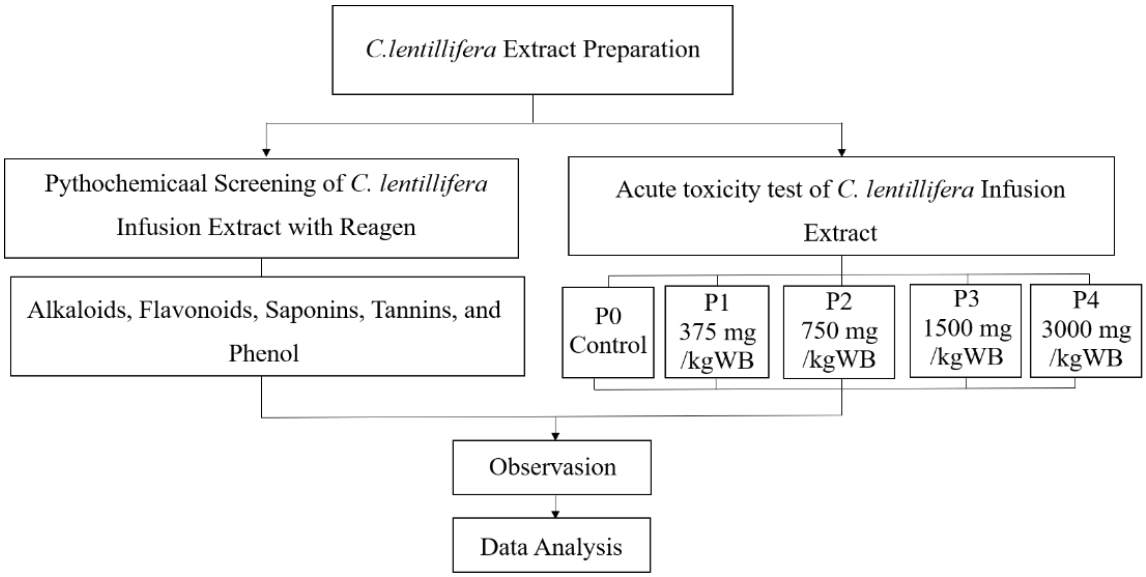

Figure 1. Research Flow Chart

Figure 2. Map of the Research Area

The materials used in this research were *C. lentillifera*, distilled water, DDY strain mice, standard mice feed, 70% alcohol, ketamine, 2N HCl, Mayer's reagent, Wagner's reagent, Dragendorff's reagent, magnesium (Mg), and 1% $FeCl_3$. The equipment used included aluminum foil, an oven, a blender, a digital scale, an analytical balance, a sieve, a graduated cylinder, a thermometer, an infusion pot, a water bath, a stirring rod, a filter cloth, a filter paper, vacuum filtration, test tubes, mouse cages, an oral probe, dropper pipettes, and disposable syringes.

2.1 Extract Preparation

The samples were washed with clean water to eliminate any dirt and epiphytes. Subsequently, they were subjected to a two-day natural air-drying in a shaded environment, followed by a seven-day drying in an oven set at a temperature of 45ºC. The dried *C. lentillifera* was ground into a fine powder using a sieve (Wirawan *et al*., 2022). The infusion extract was prepared using the infusion extraction method with concentrations of 2.25% w/v and 9% w/v. The powdered samples were mixed with distilled water in the infusion pot and thereafter heated in a water bath at a temperature of 90ºC for 15 minutes. Lastly, the extract underwent vacuum filtering with filter paper to yield the extract filtrate.

2.2 Phytochemical Screening

Alkaloids, flavonoids, saponins, tannins, and phenol were tested. The extract was added to a reaction tube and mixed with 2 mL of 2N HCl for alkaloids. One milliliter of the filtrate was transferred to three separate reaction tubes. Mayer's reagents were added, and the white precipitate indicated a positive result. Wagner's reagents were added, and the brown precipitate indicated a positive result. Simultaneously, orange or brown precipitate indicated a positive result on Dragendorff's reagents (Oktavia and Suyatno, 2021).

Two milliliters of the infusion were mixed with 2 mg of Mg powder and 2 mL of 2N HCl for the flavonoid test. A positive result was indicated by the color of the solution changing to orange, yellow, or red (Novindriani *et al*., 2013). Next, the saponin test was conducted by adding two milliliters of the infusion extract to a test tube and vigorously shaking it for 10 seconds. Upon the addition of a drop of 2N HCI, the foam production persisted, suggesting the presence of saponins in the sample (Martha and Atiqoh, 2018).

Two milliliters of the infusion were added into a reaction tube, followed by the addition of drops of 1% $FeCl_3$ solution for the tannin and phenol test. The change in color to bluish-black or greenish brown indicated the presence of tannins (Febriyanti et al., 2021). As for phenol, the color change to dark blue or black was considered as a favorable indication (Mukhlisa *et al*., 2021).

2.3 Acute Toxicity Test

The study employed a male DDY strain mouse model, comprising of 25 mice divided into 5 treatment groups, with each group consisting of 5 mice. The mice were selected based on their agility, age ranging from 8 to 12 weeks, and weight ranging from 28 to 31 grams. They were acclimatized for a week and provided ad libitum access to food and water while their cages were cleaned every 2 days. The mice fasted for 3-4 hours prior to the extract administration while still being provided with water. The oral administration of the *C. lentillifera* infusion extract was followed by a subsequent feeding of the mice 1-2 hours after the treatment.

The test mice were administered different doses of *C. lentillifera* infusion extract, *viz.* the control group with 1 mL/kg body weight of distilled water, 375 mg/kg body weight, 750 mg/kg body weight, 1.500 mg/kg body weight, and 3.000 mg/kg body weight. All were given as single doses over 24 hours. Observations were carried out at 30 minutes, 1 hour, and 24 hours after the extract administration, and once per day for 7 days. The detected factors encompassed the mortality rate of mice, their body weight, and clinical symptoms such as behavior, appetite, and thirst. The experimental animals were euthanized by administering a dose of ketamine (10 mg/kg) at a rate of 0.1 mL/kg body weight. Their necks were subsequently dislocated, and their carcasses were incinerated.

2.4 Data Analysis

The toxicity test results were analyzed descriptively, including the examination of observation data, the calculation of LD_{50} values using the Thomson and Weil method, the classification of the test substance's toxicity using the Hodge & Stener's classification (Stevani, 2016), and the statistical analysis of the mice's body weight using IBM SPSS Statistics 25 software.

3. Results and Disscussion

Table 1 reveals that the *C. lentillifera* infusion extract exhibited positive results for alkaloid, saponin, and tannin compounds during phytochemical screening. Alkaloids are chemical compounds containing at least one nitrogen atom in their heterocyclic ring structure and are recognized for their antioxidant properties. Tannins can also act as antioxidants and lower total cholesterol levels by blocking the oxidation of Low-Density Lipoprotein (Anggraito *et al*., 2018). Saponins possess expectorant, anti-inflammatory activities, and can inhibit the increase of blood glucose levels (Julianto, 2019).

Table 1. Phytochemical screening result of *C. lentillifera* infusion extract

Phytochemical Screening	Reagent	Result	Description
Alkaloid	HCl 2N + Mayer's reagents	(+)	White and had a white precipitate
	HCl 2N + Wagner's reagents	(+)	Brown and had precipitate
	HCl 2N + Dragendorff's reagents	(+)	Brown precipitate
Flavonoid	Mg + HCl 2N	(-)	Foamed and did not change into orange, yellow, or red
Saponin	HCl 2N	(+)	Stable foam
Tannin	$FeCl_3$ 1%	(+)	Brownish green
Phenol	$FeCl_3$ 1%	(-)	Brownish green and did not change into dark blue or black

Result: (+) showed positive reaction, (-) showed negative reaction

However, *C. lentillifera* infusion extract showed negative results in terms of flavonoid and phenol compound presence. Yap *et al*. (2019) reported that the flavonoids found in *C. lentillifera*, including isoflavones, flavanols, and flavones, have limited water solubility and exhibit a polarity similar to that of methanol. In addition, the *C. lentillifera* water extract had a total phenol concentration of 2.04 ± 0.36 mg GAE/g, which was much lower than that of the chloroform extract at 5.47 ± 0.75 mg GAE/g. Thus, it can be concluded that *C. lentillifera* predominantly contained semi-polar phenolic compounds.

The absence of mortality in all treatment groups receiving *C. lentillifera* infusion extract indicated a mortality rate of 0% in each treatment. However, the absence of death or mortality in mice made it unfeasible to determine the LD_{50} value using the Thomson and Weil method. LD_{50} calculation was not possible due to the unavailability of the f factor from the Thomson and Weil table. Due to the inability to determine the exact LD_{50} value in this

research and the absence of any fatalities at the highest dosage administered, the preparation can be classified as non-toxic. Nevertheless, administering doses exceeding 3,000 mg/kg BW may potentially cause toxicity and mortality rate of over 50% in the experimental animals.

No signs of toxicity were observed in the group receiving treatments up to a dose of 1,500 mg/kg BW. However, at a dose of 3,000 mg/kg BW, the motion activity in mice declined for one hour following the treatment. Their slower movements indicated a decrease in mice activity even upon tactile stimulation. The decrease in motor activity in these mice was suspected to be due to shock or stress caused by the administration of the test substance as well as the presence of saponin in the extract. Certain types of saponins possess toxicity and are commonly referred to as sapotoxins. According to Anggraito *et al*., 2018), saponins also have irritant properties to mucous membranes, indirectly suggesting that mice may experience discomfort.

The mice's body weight is another indicator observed in this toxicity test. Body weight might serve as an indicator of toxicity since poisoned individuals may develop digestive issues, which in turn can impact their ability to consume food and absorb nutrients (Fitria *et al*., 2020). The mean difference in body weight of male mice for 7 days following the administration of *C. lentillifera* infusion extract was not statistically significant ($p>0.05$) compared to the control group. This suggests that the administration of doses ranging from 375 mg/kgBW to 3,000 mg/kgBW had no side effects on the mice's body weight. Figure 3 displays the alterations in the mice's body weight. The graph illustrates the daily fluctuations in the mice's average weight in each treatment group, albeit not statistically significant. The observed decline in the mice's average weight on the second day was suspected to be due to stress experienced by experimental animals. It can stimulate heightened metabolic activity, leading to the breakdown of fat reserves for energy, ultimately resulting in a reduction in the mice's weight.

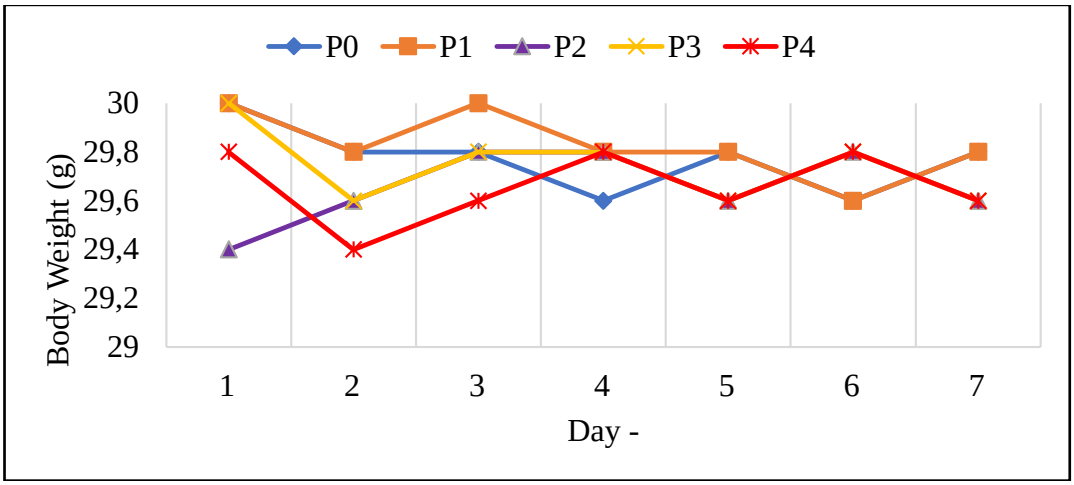

Figure 3. Graph of average daily weight of mice for 7 days

According to (Ubang *et al*.2022), the changes in the mice's weight can be affected by various factors, including the compounds present in the test substance and both internal and external factors of the experimental animals. Internal factors, e.g., genes, species, animal strains, gender, age, nutritional status, and hormonal regulation, can influence the response of experimental animals. Meanwhile, external factors, including the amount and length of exposure to the test substance, food intake, physical activity, temperature, and the surrounding environment, can also affect the mice's weight (Ubang *et al*., 2022; Yunedi, 2022).

4. Conclusions

The phytochemical screening showed the presence of alkaloids, saponins, and tannins in the *C. lentillifera* infusion extract, suggesting potentially beneficial properties. The acute toxicity test revealed no mortality at the administered dosages (up to 3000 mg/kg body weight) and had no side effects on the mice's body weight. In conclusion, the *C. lentillifera* infusion extract was classified as non-toxic. These findings indicated that *C. lentillifera* was promising for the further investigation as a safe and potentially beneficial health-promoting ingredient.

Author Contributions

Conceptualization, S.A and T.A.P.; methodology, **N.W.S.S**; validation, **S.A.**; formal analysis, **N.W.S.S**; investigation, **N.W.S.S**; data curation, N.W.S.S; writing—original draft preparation, T.A.P; writing—review and

editing, **S.A., T.A.P., N.W.S.S**; visualization, **T.A.P**. All authors have read and agreed to the published version of the manuscript.

Funding

Not applicable

Informed Consent Statement

Not applicable

Data Availability

Not applicable

Acknowledgements

Not applicable

Conflicts of Interest

The authors declare there no conflict of interest

References

Agung, I. G. A. A. A., Wiswasta, I. G. N. A., and Sudja, I. N. (2019). Bulung Sangu (*Gracilaria verrucosa*) Low Cost Balinese Seaweed Product, Nutrition, General and Orodental Health (A Review). *International Journal of Applied Science and Sustainable Development*. Vol. 1. Issue 2.

Anggraito, Y.U., R. Susanti, Retno, S.I., Ari, Y., Lisdiana, Nugrahaningsih, W.H., Noor, A.H., and Siti, H.B. (2018). *Metabolit Sekunder dari Tanaman: Aplikasi dan Produksi*. Semarang: Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Negeri Semarang.

Badan Pengawas Obat dan Makanan. (2022). Pedoman Uji Toksisitas Praklinik secara in vivo. *Peraturan Badan Pengawas Obat dan Makanan Nomor 10 Tahun 2022*

Chusniasih, D., and Tutik. (2020). Uji Toksisitas Dengan Metode *Brine Shrimp Lethality Test* (BSLT) dan Identifikasi Komponen Fitokimia Ekstrak Aseton Kulit Buah Kakao (T*heobroma cacao L.*). *Analit: Analytical and Environmental Chemistry*. Vol. 5(2). https://doi.org/10.23960/aec.v5.i2.2020.p192-201

Febriyanti, R., Muladi, P.M., and Rahmat, A. (2021). Skrining Fitokimia pada Ekstrak Hasil Proses Infundasi Akar Bajakah. *Laporan Penelitian*. Prodi DIII Farmasi Politeknik Harapan Bersama.Fitria, L., Tika, C. D., Kholidatus, S., Mulyati, Rahadian, Y. H., Slamet, W., dan Eko, A. S. (2022). Toksisitas Oral Akut *Arthrospira maxima* dan *Chlorella vulgaris* Isolat Glagah pada Tikus (*Rattus norvegicus* Berkenhout, 1769) Galur Wistar. *AL-KAUNIYAH: Jurnal Biologi*. Vol. 15(2): 209-219. https://doi.org/10.15408/kauniyah.v15i2.18716

Julianto, T.S. (2019). *Fitokimia Tinjauan Metabolit Sekunder dan Skrining Fitokimia.* Yogyakarta: Universitas Islam Indonesia.

Khairuddin, K., Sabri, S., Luqiang, H., and Zwe-Ling, K. (2020). *Caulerpa lentillifera* polysaccharides-rich extract reduces oxidative stress and proinflammatory cytokines levels associated with male reproductive functions in diabetic mice. *Applied Sciences (Switzerland)*, *10*(24), 1–14. https://doi.org/10.3390/APP10248768

Krisna, D., Patricius, K. A., and Ines, S. A. (2022). Efek Pemberian Sari Buah Berenuk (*Crescentia cujete* L.) Terhadap Berat Mencit Galur Swiss-Webster (*Mus musculus*). *Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati*, 108–120. https://doi.org/10.24002/biota.v7i2.5255

Maeda, R., Tomoaki, I., Hideshi, I., and Tatsuji, S. (2012). Induction of apoptosis in MCF-7 cells by β-1,3-xylooligosaccharides prepared from *Caulerpa lentillifera*. *Bioscience, Biotechnology and Biochemistry*, *76*(5), 1032–1034. https://doi.org/10.1271/BBB.120016

Martha, R.D. and Atiqoh, Z. (2018). Pengujian Fitokimia dan Penentuan Parameter Kinetik Enzim dengan Penambahan Ekstrak Infusa Air Seledri. *Jurnal Wiyata*, *5*(2), 106–111. http://dx.doi.org/10.56710/wiyata.v5i2.233

Mukhlisa, R., Liza, P., and Hadi, K. (2021). Uji Fitokimia Ekstrak Infusa Kulit Pisang (*Musa acuminata x Musa balbisiana*). *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN*. Vol. 5(1).

Novindriani, D., Bambang, W., and Mohammad, A. (2013). Uji Efek Sedatif Infusa Daun Kratom (*Mitragyna speciosa*) pada Mencit Jantan Galur BALB/c. *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN*. Vol. 3, No. 1.

Nurlaida., Husna and S., Aswar. (2023). Synthesis and Characterization of κ-Carrageenans from *Eucheuma cottonii* from Bantaeng Regency. *Jurnal Akta Kimia Indonesia.* Vol. 16, 13-16. http://dx.doi.org/10.20956/ica.v16i1.25487

Oktavia, F.D., and Suyatno, S. (2021). Skrining Fitokimia, Kandungan Flavonoid Total, dan Aktivitas Antioksidan Ekstrak Etanol Tumbuhan *Selaginella doederleinii*. *Jurnal Kimia Riset.* Vol. 6, No.2, 141–153. https://doi.org/10.20473/jkr.v6i2.30904

Stevani, H. (2016). *Praktikum Farmakologi*. Jakarta: Pusdik SDM Kesehatan.

Sun, Y., Liu, Y., Ai, C., Song, S., and Chen, X. (2019). Caulerpa lentillifera polysaccharides enhance the immunostimulatory activity in immunosuppressed mice in correlation with modulating gut microbiota. *Food Funct.*, *10*(7), 4315–4329. https://doi.org/10.1039/C9FO00713J

Tapotubun, M.A., Matrutty, E.A.A., Riry, J., Tapotubun, E.J., Fransina, E.G., Mailoa, M.N., Riry, W. A., Setha, B., and Rieuwpassa, F. (2020). Seaweed *Caulerpa* sp. position as functional food. *IOP Conf. Series: Earth and Environmental Science 517.* doi:10.1088/1755-1315/517/1/012021

Tian, H., Liu, H., Song, W., Zhu, L., and Yin, X. (2021). *Caulerpa lentillifera* polysaccharides enhance the immunostimulatory activity in immunosuppressed mice in correlation with modulating gut microbiota. *Natural Product Research*, *35*(20), 3417–3425. https://doi.org/10.1080/14786419.2019.1700507

Ubang, F., Siregar, V. O., and Herman, H. (2022). Efek Toksik Pemberian Ekstrak Etanol Daun Mekai (*Albertisia papuana* Becc.) Terhadap Mencit. *Proceeding of Mulawarman Pharmaceuticals Conferences*, *16*, 49–57. https://doi.org/10.25026/mpc.v16i1.672

Wirawan, I. G. P., Dewi, N. K. E. S., Sasadara, M. M. V., Sunyamurthi, I. G. N. A., Jawi, I. M., Wijaya, I. N., Darmawati, I. A. P., Suada, I. K., and Krisnandika, A. A. K. (2022). Phytochemical Analysis and Molecular Identification of Green Macroalgae *Caulerpa* spp. from Bali, Indonesia. *Molecules*, *27*(15). https://doi.org/10.3390/molecules27154879

Yap, W. F., Tay, V., Tan, S. H., Yow, Y. Y., and Chew, J. (2019). Decoding Antioxidant and Antibacterial Potentials of Malaysian Green Seaweeds: *Caulerpa racemosa* and *Caulerpa lentillifera*. *Antibiotics*, *8*(3). https://doi.org/10.3390/antibiotics8030152

You, Y., Song, H., Wang, L., Peng, H., Sun, Y., Ai, C., Wen, C., Zhu, B., and Song, S. (2022). Structural characterization and SARS-CoV-2 inhibitory activity of a sulfated polysaccharide from *Caulerpa lentillifera*. *Carbohydrate Polymers*, *280*, 119006. https://doi.org/10.1016/J.CARBPOL.2021.119006

Yunedi, A. 2022. Uji Toksisitas Akut Fraksi Daun Sungkai (*Peronema canescens* Jack.) serta Penentuan Nilai LD50 pada Mencit Putih Jantan. *Skripsi*. Fakultas Farmasi Universitas Andalas Padang.