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Phytochemical and Toxicity Analysis of *Sonneratia alba* Mangrove Leaf Extract using the Brine Shrimp Lethality Test (BSLT) Method

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Abstract

Mangroves are plants that contain various secondary metabolites with various potential pharmacological activities, one of which is cytotoxic activity. However, researchers have not extensively explored the evidence of secondary metabolite compounds and their toxicity effects in mangrove plants. This research aims to determine the content of secondary metabolite compounds and the toxicity of the leaf extract of the *Sonneratia alba* mangrove plant in the Ngurah Rai Grand Forest Park (TAHURA), Bali. In this experimental research, compound extraction was carried out using a maceration technique for 2x24 hours using two types of solvents, namely methanol and n-hexane. Phytochemical tests were carried out using qualitative methods and gas chromatography-mass spectrometry (GC-MS), while the toxicity test used was the brine shrimp lethality test (BSLT) method. Potassium dichromate and sea water without extract were used as positive and negative controls, respectively. The LC_{50} value was calculated using probit analysis in Microsoft Excel. Phytochemical test results showed that the methanol extract contains phenol, saponin, tannin, and steroid compounds. Meanwhile, n-hexane extract only contains steroid compounds. GC-MS analysis showed that the compounds with the largest area percentages in the methanol and n-hexane extracts were 1,2,3-benzenetriol (3.94%) and n-hexadecenoic acid (2.41%), respectively. Based on the LC_{50} value, the methanol extract of *S. alba* mangrove leaves was moderately toxic (178.17 ppm), while the n-hexane extract was weakly toxic (567.23 ppm). The results of this study provide information that the type of chemical solvent has a major influence on the level of toxicity of *S. alba* leaf extract, with polar methanol solvent providing the highest toxicity effect compared to nonpolar n-hexane solvent.

Keywords: BSLT; mangrove; phytochemicals; *Sonneratia alba*; toxicity.

INTRODUCTION

Cancer is a disease caused by the uncontrolled growth of abnormal cells in the body (Suryaningrum, 2021). In 2020, the number of deaths recorded in the world due to cancer reached almost 10 million (Pambudi, 2022). Commonly used methods of treating cancer, such as surgery, radiotherapy, and chemotherapy, are considered to be less effective because they do not selectively destroy cancer cells and are also toxic to normal cells (Latief et al., 2020). Furthermore, chemotherapy drugs also cause mild to severe side effects. Therefore, the discovery and development of safer and more selective anticancer compounds are still being conducted, especially in plants, considering that more than two-thirds of anticancer drugs on the market currently come from plants (Niksic et al., 2021). One of the natural resources that has the potential to be a new anticancer source is mangrove plants.

Several studies on the pharmacological activity of mangrove plants have shown that their compounds make them useful as traditional medicines (Kadir et al., 2019; Dotulong et al., 2020; Mairing & Ariantari, 2022). Various secondary metabolite compounds are reported to be contained in mangrove plants, such as flavonoids, tannins, alkaloids, saponins, steroids, and triterpenoids (Kadir et al., 2019). The content of these secondary metabolites makes mangrove plants have various pharmacological activities, namely antioxidants, anticancer, antibacterial, anti-inflammatory, antidiarrheal, antifungal, antinematode, antimalarial, and other pharmacological benefits (Rahmania et al., 2018). Among the mangrove plants, the *Sonneratia alba* species shows significant potential for development as a new source of chemotherapy agents.

Leaves of the *S. alba* mangrove plant contain secondary metabolites that have toxic effects, namely tannins, which cause damage to biological structures at the organ, tissue, cell, and biomolecular levels (Setiasih et al., 2016; Mairing and Ariantari, 2022). The toxic effect of a compound or extract can be determined from its lethal concentration 50 (LC_{50}) value. The LC_{50} value indicates the ability of an extract to kill test larvae by 50% (Fidyasari et al., 2020). An LC_{50} value of less than

1000 ppm is considered toxic and has the potential to be developed as an anticancer drug (Latief et al., 2020). The initial screening stage to map the potential toxicity before testing the anticancer activity of an extract is to carry out a toxicity test using the brine shrimp lethality test (BSLT) method (Latief et al., 2020).

This study explored the potential of *S. alba* from Ngurah Rai Mangrove Forest, Bali, Indonesia, as a source of anticancer agents. Two solvents, methanol, and n-hexane, were used to extract bioactive compounds from the leaves of *S. alba*, aiming to identify the most effective solvent for isolating compounds with cytotoxic properties. Methanol, a polar solvent, is expected to extract a broader range of hydrophilic compounds, while n-hexane, a non-polar solvent, is likely to target lipophilic compounds. By comparing the phytochemicals, toxicity profiles, and LC_{50} values obtained from the BSLT for both extracts, we aim to determine the most promising extract for further anticancer research. This investigation represents a significant step towards harnessing the therapeutic potential of *S. alba*, particularly in the development of novel, plant-derived anticancer agents.

MATERIALS AND METHODS

Sample preparation

A total of two kilograms of *S. alba* leaves were collected from five different plants at the Ngurah Rai Mangrove Forests. Taxonomy was identified at the Plant Conservation Research Center, Botanical Gardens and Forestry, Bedugul, Bali. Leave samples were cleaned using running water, cut into smaller sizes, and dried in an oven at 40°C. After drying, the sample was grounded using a blender until it became powder and then sieved (Akasia et al., 2021). To make extracts from two different solvents, a minimum of 200 grams of sample powder was prepared.

Extraction

The extraction stage was carried out using a maceration technique using two types of solvents with different polarities, namely methanol and n-hexane. 100 grams of simplicia were soaked in 500 ml of each solvent for 24 hours (v/v, 1:5) (Wijaya & Indraningrat, 2021). The filtrate from the first maceration was filtered using Whatmann No.1 filter paper and the resulting residue was macerated again using 500 ml of new solvent for 24 hours. The first and second maceration results are combined and then stored in the refrigerator until the next process. The extract evaporation process was carried out using a vacuum rotary evaporator at a temperature of 50°C, then the thick extract resulting from the evaporation process was weighed. The resulting extract was put into a tightly closed vial and stored at 4°C until the next research stage (Wijaya & Indraningrat, 2021).

Phytochemical testing

Testing for the content of phytochemical compounds in extracts was carried out using qualitative methods and Gas Chromatography-Mass Spectrometry (GC-MS) to identify the specific compounds in the extracts. The GC-MS method compares the mass spectrum and retention time of the sample with a standard standard (Suhaili et al., 2020). Briefly, 0.1 grams of the crude extract was prepared and sent to the Forensic Laboratory Polda Bali for further analysis. The extract was injected to the GC/MS instrument (Agilent Technologies 7890B/Agilent Technologies 5977B) based on the following setting**:** HP-5ms ultra inert column 30 m x250 µm x 0.25 µm, oven temperature (-60ºC to 325ºC), mode (splitless), pressure (25.523 psi), total flow (20.9 ml/min), average velocity (62.662 cm/sec), purge flow to split vent (15 mL/min at 0.75 min), and gas (He)**.** The chromatogram results were analyzed by matching the compound fragments from each chromatogram peak with literature to determine the type of content and function of the detected bioactive compounds.

Toxicity effect testing using the BSLT method

Tests were conducted using 10 newly hatched *Artemia salina* nauplii larvae (Fidyasari et al., 2020). The extract consisted of 7 concentrations, namely 2000; 1000; 500; 250; 100; 50; and 25 ppm. At each extract concentration, 10 larvae were placed in the extract and left for 24 hours. After 24 hours, surviving *A. salina* was counted and the lethal concentration was analyzed. The test was carried out with 3 repetitions and used potassium dichromate as a positive control and sea water without extract as a negative control. The toxicity effect was determined by calculating the percent death of *A. salina* in each vial after 24 hours. The calculation is done by counting the number of dead *A. salina* divided by the initial number and then multiplying by 100%. The LC_{50} value was calculated using probit analysis with Microsoft Excel software. An extract is declared to have a toxicity effect if it has an LC_{50} of less than 1000 ppm (Kurniawan & Ropiqa, 2021). The toxicity category of an extract is based on the LC_{50} value, namely the very toxic category with an LC_{50} of less than 30 ppm, the strongly toxic category with an LC_{50} of 30-100 ppm, the moderate toxic category with an LC_{50} range of 100-250 ppm, the weakly toxic category of 250-1000 ppm, and the non-toxic with LC_{50} more than 1000 ppm (Zulfiah et al., 2020).

Ethical clearance

The research has been approved by ethical committees of the Faculty of Medicine and Health Sciences, Warmadewa University, under Ethical Eligibility Number: 338/Unwar/FKIK/EC-KEPK/VII/2023.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical test showed that the methanol extract contained four compounds, namely tannin, phenol, steroid, and saponin. Meanwhile, only steroid was found in the n-hexane extract (Table 1). These results showed that steroid compounds were found in both *S. alba* mangrove leaf extracts.

Table 1. Phytochemical test results of methanol and n-hexane extracts.

Compounds	Reagent	*Sonneratia alba* Leaf Extracts	
		Methanol	N-Hexane
Tannins	FeCl3 1%	+	-
Flavonoids	Mg+HCl	-	-
Alkaloids	Dragendorff	-	-
Phenolics	FeCl3 1%	+	-
Saponins	Distilled water	+	-
Steroids	Liebermann Burchard	+	+
Triterpenoids	Liebermann Burchard	-	-

The GC-MS analysis showed that 251 compounds were found in the methanol extract. The five dominant compounds contained in the methanol extract can be seen in Table 2, with 1,2,3-Benzenetriol being the compound with the largest area (3.94%). On the other hand, the n-hexane extract consisted of 338 compounds. The five dominant compounds in the n-hexane extract can be observed in Table 2, with n-Hexadecanoic Acid being the compound with the largest area (2.41%).

Table 2. Phytochemical analysis of *S. alba* mangrove leaf extracts using GC-MS.

Peak	*Sonneratia alba* Leaf Extracts	Retention Time	Compounds	%Area
1	Methanol	16.291	1,2,3-Benzenetriol	3.94
2		27.832	Linoelaidic acid	3.28
3		25.471	n-Hexadecanoic acid	2.74
4		19.879	2,4-Hexadiene,3,4-dimethyl-,(E,Z)-	1.92
5		9.089	Benzyl alcohol	1.56
1	N-Hexane	25.499	n-Hexadecanoic acid	2.41
2		19.511	Dodecanoic acid	1.97
3		39.702	Oxirane, hexadecyl-	1.45
4		38.685	dl-.alpha.-Tocopherol	1.28
5		28.258	9,12,15-Octadecatrienoic acid, (ZZZ)-	1.21

Toxicity analysis

The toxicity screening of methanol extract showed that the highest toxicity was obtained at a concentration of 2000 ppm. From the linear regression formula, an LC_{50} value of 178.17 ppm was obtained, which is included in the moderate toxic category. Meanwhile, the toxicity test of n-hexane extract showed that the greatest toxicity effect with a death percentage of 70% was at a concentration of 2000 ppm. The probit analysis showed that the LC_{50} of n-hexane extract was 567.23 ppm, which is included in the weak toxic category.

Table 3. Cytotoxic activities of *S. alba* mangrove leaf extracts with potassium dichromate as positive control

Sonneratia alba Leaf Extracts	Concentration (ppm)	LC_{50} (ppm)	Toxicity Category
Methanol	(25-2000)	178.17	Moderate
N-Hexane	(25-2000)	567.23	Weak
Potassium dichromate	(25-2000)	58.59	Strong

Discussion

Based on the research results, it is known that methanol extract has a moderate toxic effect with an LC50 of 178.17 ppm. This result differs from a study by Eriani & Usman (2017) that reported that methanol extract of *S. alba* leaves from the East Kalimantan area yielded weak toxicity of 441.67 ppm (Eriani and Usman, 2017). Differences in toxicity effects can be influenced by

variations in sample ages, as well as differences in the living environment and habitat of the *S. alba* mangrove plant such as nutrient and climate differences (Supriatna et al., 2019). Qualitative phytochemical test results show that the methanol extract contains four phytochemical compounds, namely tannins, phenolics, saponins, and steroids. The research results differ from those of Muhaimin (2019), who found phytochemical content, namely alkaloids, flavonoids, terpenoids, phenolics, tannins, saponins, quinones, and glycosides. Differences in phytochemical content are caused by the age of the test samples, differences in habitat, and environmental conditions of mangrove plants, such as light intensity, season, and water temperature.(Supriatna et al., 2019).

The high tannin content in *S. alba* leaves can be correlated with observed toxicity in plant extracts due to the nature of tannins themselves. Tannins are a type of polyphenolic compound that can have astringent properties and can bind to proteins (Alfarabi, 2018). Phenolic is the largest class of natural compounds, including gallic acid, p-coumaric acid, ferulic acid, vanillic acid, and flavonoids, which have anticancer potential (Fatimah et al., 2022). Phenolic compounds are a diverse group of plant secondary metabolites that play essential roles in plant growth, development, reproduction, and defense against environmental stresses like herbivores, pathogens, and UV radiation (Lin *et al.*, 2016). While phenolic compounds generally benefit plants, they can also have toxic or inhibitory effects on other organisms. Steroid compounds cause death in larvae because they work as antifeedant (To'bungan et al., 2021). This antifeedants effect leads to feeding disorders in larvae by triggering toxic effects on digestion and starvation (Kurniawan & Ropiqa, 2021). Steroids also have anticancer and anti-inflammatory potential (To'bungan et al., 2021). Steroid compounds can also inhibit tumor cells in the human cervix and lungs (Putram et al., 2017).

Qualitative test results show that the methanol extract contains more phytochemical compounds than n-hexane extracts. This is in line with research by Gazali (2020) showing that the methanol extract of *S. alba* contains more phytochemical compounds compared to ethyl acetate and n-hexane extracts (Gazali et al., 2020). This can be caused by the difference in polarity between the three solvents. Based on the principle of like dissolves like, polar compounds can be extracted perfectly in polar compounds and nonpolar compounds can be perfectly extracted in zero-polar compounds, therefore compounds with different polarities cannot be extracted properly (Leksono et al., 2018). The methanol solvent is polar, while n-hexane is non-polar (Leksono et al., 2018). Consequently, methanol was able to extract the most active compounds, possibly because most of the compounds contained in *S. alba* leaves are polar compounds.

The results of quantitative phytochemical tests using the GC-MS method show five compounds with the largest percentages area in the methanol extract of *S. alba* including1,2,3-Benzenetriol, Linoelaidic acid, n-Hexadecanoic acid, 2,4-Hexadiene,3,4-dimethyl-,(E,Z)-, and Benzyl alcohol. Compound1,2,3-Benzenetriol is known to have anti-inflammatory, antioxidant, phytosterol, antifungal effects. However, there has been no research discussing the toxicity effects of this compound (Cahyani et al., 2021). The compound with the second largest percentage area is Linoelaidic acid, which is an unsaturated fatty acid and has anticancer activity by triggering the death of cancer cells and can increase necrosis in tumors (Dutta et al., 2023). The n-Hexadecanoic acid compound has been associated with cytotoxic effects on human colorectal carcinoma cells and has potential as an anticancer drug (Ravi & Krishnan, 2016). The Benzyl alcohol compound has been reported for antifungal and antibacterial activities, but there is no literature that mentions the toxicity activity of this compound (Sulaiman et al., 2020). On the other hand, the pharmacological effects of the compound 2,4-Hexadiene,3,4-dimethyl-,(E,Z)- remain unknown.

The n-hexane extract showed lower toxicity effects against brine shrimp larvae than the methanol extract. This may be because the n-hexane extract only contains steroids, compound known to have anti-inflammatory and anti-cancer activities (To'bungan et al., 2021). The GC-MS test results showed that the five compounds with the largest % area are n-Hexadecanoic acid, Dodecanoic acid, Oxirane, hexadecyl-, dl-alpha.-Tocopherol, and 9,12,15-Octadecatrienoic acid, (ZZZ)-. Of the five compounds, three compounds, namely n-hexadecanoic acid, 9,12,15-Octadecatrienoic acid, (ZZZ)-, and dodecanoic acid, are known to have cytotoxic and anticancer effects (Ravi & Krishnan, 2016). Apart from that, dodecanoic acid or also called lauric acid is a medium chain saturated fatty acid compound which is known to have antibacterial activity (Renugadevi et al., 2021). Another compound, namely dl-alpha-tocopherol or vitamin E, is a compound known to have antioxidant activity (Mubarak et al., 2017), but its toxicity effects are not yet known. Meanwhile, there has been no research regarding the pharmacological activity of the oxirane hexadecyl compound.

CONCLUSIONS

In conclusion, the toxicity of methanol and n-hexane extracts of *S. alba* mangrove leaves obtained in the Tahura Ngurah Rai Bali area was moderately toxic (178.17 ppm) and weakly toxic (567 ppm), respectively. The secondary metabolites contained in the methanol extract of the leaves of the *S. alba* mangrove plant are tannins, phenolics, saponins, and steroids, with 1,2,3-Benzenetriol as the compound with the largest % area in

the results of the GC-MS test analysis. The secondary metabolite in the n-hexane extract is steroids, with n-hexadecanoic acid being the compound with the largest % area in the GC-MS test analysis results.

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Competing Interests: The authors declare that there are no conflicts of interest.

REFERENCES

Akasia, A.I., Nurweda Putra, I.D.N., Giri Putra, I.N., 2021. Skrining Fitokimia Ekstrak Daun Mangrove Rhizophora mucronata dan Rhizophora apiculata yang Dikoleksi dari Kawasan Mangrove Desa Tuban, Bali. J. Mar. Res. Technol. 4, 16. https://doi.org/10.24843/jmrt.2021.v04.i01.p03

Alfarabi, M., 2018. Uji Toksisitas dan Identifikasi Fitokimia Ekstrak Ranting Leban (Vitex Pinnata) dari Putussibau, Kalimantan Barat. Maj. Kedokt. XXXIV, 69–73.

Cahyani, R.W., Wahyudi, A., 2021. Inventarisasi Jenis Tumbuhan Berpotensi sebagai Obat di Hutan Kerangas Kawasan Hutan dengan Tujuan Khusus (KHDTK) Kebun Raya Sampit, Kalimantan Tengah. Jurnal Penelitian Sosial dan Ekonomi Kehutanan. 7(1), 23-34. doi:10.20886/jped.2021.7.1.23-34.

Dotulong, A.R., Dotulong, V., Wonggo, D., Montolalu, L.A.D.., Harikedua, S.D., Mentang, F., Damongilala, L.J., 2020. Metabolit Sekunder Ekstrak Air Mendidih Daun Mangrove Sonneratia alba. Media Teknol. Has. Perikan. 8, 66. https://doi.org/10.35800/mthp.8.2.2020.28437

Dutta, A., Panchali, T., Khatun, A., Jarapala, S.R., Das, K., Ghosh, K., Chakrabarti, S., Pradhan, S., 2023. Anti-cancer potentiality of linoelaidic acid isolated from marine Tapra fish oil (Ophisthopterus tardoore) via ROS generation and caspase activation on MCF-7 cell line. Sci. Rep. 13, 1–16. https://doi.org/10.1038/s41598-023-34885-3

Eriani, I.R., Usman, 2017. Senyawa Metabolit Sekunder Ekstrak Metanol Daun Mangrove Sonneratia Alba Dan Sifat Toksisitasnya 129–132.

Fatimah, F., Diyan Martha, R., Danar Danar, dan, Karya Putra Bangsa Tulungagung Tulungagung-Blitar KM, S.J., Jawa Timur, S.-T., n.d. Analisis toksisitas dan potensi antikanker ekstrak metanol daun Majapahit (Crescentia cujete) dengan metode Brine Shrimp Lethality Test. https://doi.org/10.21831/jps.v1i1.43889

Fidyasari, A., Raharjo, S.J., Setyowati, M., 2020. Uji Toksisitas Akut Minuman Probiotik Sirsak Gunung (Annona montana Macf.) Dengan Metode BSLT (Brine Shrimp Lethality Test). J. Inov. Penelit. 1, 941–948. https://doi.org/10.47492/jip.v1i5.174

Gazali, M., Nurjanah, Ukhty, N., Nurdin, M., Zuriat, 2020. Skrining Senyawa Bioaktif Daun Perepat (Sonneratia alba J.E. Smith) sebagai Antioksidan asal Pesisir Kuala Bubon Aceh Barat. J. Pengolah. Has. Perikan. Indones. 23, 402–411. https://doi.org/10.17844/jphpi.v23i2.31684

Hardiansyah, M., Musa, Y., & Jaya, A. (2021). The Effectiveness of Giving Plant PGPR Rhizosphere Bamboo on Cocoa Seeds Germination at The Nursery Level. *Biology, Medicine, & Natural Product Chemistry, 10*(1), 1-5. doi:https://doi.org/10.14421/biomedich.2021.101.1-5

Kadir, M.A., Wibowo, E.S., Abubakar, S., Akbar, N., 2019. Manfaat Mangrove Bagi Peruntukan Sediaan Farmasitika Di Desa Mamuya Kecamatan Galela Timur Kabupaten Halmahera Timur (Tinjauan Etnofarmakologis). J. Enggano 4, 12–25. https://doi.org/10.31186/jenggano.4.1.12-25

Kurniawan, H., Ropiqa, M., 2021. Uji Toksisitas Ekstrak Etanol Daun Ekor Kucing (Acalypha hispida Burm.f.) Dengan Metode Brine Shrimp Lethality Test (BSLT). J. Syifa Sci. Clin. Res. 3, 52–62. https://doi.org/10.37311/jsscr.v3i2.11398

Latief, M., Nelson, N., Amanda, H., Tarigan, I.L., Aisyah, S., 2020. Potential Tracking of Cytotoxic Activities of Mangrove Perepate (Sonneratia alba) Root Extract as an Anticancer Candidate. Pharmacol. Clin. Pharm. Res. 5, 48–55. https://doi.org/10.15416/pcpr.v5i2.26790

Leksono, W.B., Pramesti, R., Santosa, G.W., Setyati, W.A., 2018. Jenis Pelarut Metanol Dan N-Heksana Terhadap Aktivitas Antioksidan Ekstrak Rumput Laut Gelidium sp. Dari Pantai Drini Gunungkidul – Yogyakarta. J. Kelaut. Trop. 21, 9. https://doi.org/10.14710/jkt.v21i1.2236

Lin, D. *et al.* (2016) 'An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes', *Molecules*, 21(10). Available at: https://doi.org/10.3390/molecules21101374.

Mairing, P.P., Ariantari, N.P., 2022. Review: Metabolit Sekunder dan Aktivitas Farmakologi Tanaman Mangrove Sonneratia alba. J. Farm. Udayana 11, 1. https://doi.org/10.24843/jfu.2022.v11.i01.p01

Mubarak, K., Natsir, H., Wahab, A.W., Satrimafitrah, P., 2017. Analisis Kadar α-Tokoferol (Vitamin E) dalam Daun Kelor (Moringa oleifera Lam) dari daerah Pesisir dan Pegunungan serta Potensinya sebagai Antioksidan [Analysis of α-Tokopherol (Vitamin E) Extracted from Moringa Leaves (Moringa oleifera Lam) Collected from Seashore and Highland Areas and Its Potencyl as Antioxidant]. Kovalen 3, 78–88.

Mariana, E., Cahyono, E., Rahayu, E.F., Nurcahyo, B., 2018. Indonesian Journal of Chemical Science Validasi Metode Penetapan Kuantitatif Metanol dalam Urin Menggunakan Gas Chromatography-Flame Ionization Detector, J. Chem. Sci.

Niksic, H., Becic, F., Koric, E., Gusic, I., Omeragic, E., Muratovic, S., Miladinovic, B., Duric, K., 2021. Cytotoxicity screening of Thymus vulgaris L. essential oil in brine shrimp nauplii and cancer cell lines. Sci. Rep. 11, 1–9. https://doi.org/10.1038/s41598-021-92679-x

Pambudi, D.B., 2022. Aktivitas Farmakologi Senyawa Bioaktif Tumbuhan Mangrove di Indonesia: Systematic Review.

Putram, N.M., Setyaningsih, I., Tarman, K., 2017. Anticancer Activity from Active Fraction of Sea Cucumber. J. Pengolah. Has. Perikan. Indones. 20, 53. https://doi.org/10.17844/jphpi.v20i1.16399

Rahmania, N., Herpandi, H., Rozirwan, R., 2018. Phytochemical Test of Mangrove Avicennia alba, Rhizopora apiculata and

Sonneratia alba From Musi River Estuary, South Sumatera. Biovalentia Biol. Res. J. 4, 8–15. https://doi.org/10.24233/biov.4.2.2018.116

Ranjith Kumar, S., Chozhan, K., Murugesh, K.A., Rajeswari, R., Kumaran, K., 2021. Gas chromatography-Mass spectrometry analysis of bioactive compounds in chloroform extract of Psoralea corylifolia L. J. Appl. Nat. Sci. 13, 1225–1230. https://doi.org/10.31018/jans.v13i4.2884

Ravi, L., Krishnan, K., 2016. Cytotoxic Potential of N-hexadecanoic Acid Extracted from Kigelia pinnata Leaves. Asian J. Cell Biol. 12, 20–27. https://doi.org/10.3923/ajcb.2017.20.27

Renugadevi, K., Valli Nachiyar, C., Zaveri, M., 2021. Bioactivity of dodecanoic acid extracted from geitlerinema sp. Trv57. Indian J. Pharm. Educ. Res. 55, 224–231. https://doi.org/10.5530/ijper.55.1.25

Setiasih, I.S., Hanidah, I.-I., Wira, D.W., Rialita, T., Sumanti, D.M., 2016. Uji Toksisitas Kubis Bunga Diolah Minimal (KBDM) Hasil Ozonasi. J. Penelit. Pangan (Indonesian J. Food Res. 1, 22–26. https://doi.org/10.24198/jp2.2016.vol1.1.04

Suhaili, R., Lucy Prima Ardi, Emil Salim, Mai Efdi, 2020. Analisis GC-MS ekstrak tanaman terfermentasi (ETT) dari kulit buah jengkol (Pithecellobium jiringa Prain). Chempublish J. 5, 36–45. https://doi.org/10.22437/chp.v5i1.7957

Sulaiman, M., Hassan, Y., Taskin Tok, T., Noundou, X.S., 2020. Synthesis, Antibacterial Activity and Docking Studies of Benzyl Alcohol D. J. Turkish Chem. Soc. Sect. A Chem. 7, 481–488. https://doi.org/10.18596/jotcsa.692113

Supriatna, D., Mulyani, Y., Rostini, I., Agung, M.U.K., 2019. Aktivitas Antioksidan, Kadar Total Flavonoid Dan Fenol Ekstrak Metanol Kulit Batang Mangrove Berdasarkan Stadia Pertumbuhannya. J. Perikan. dan Kelaut. 10, 35–42.

Suryaningrum, F.D., 2021. The Effect Of Mangrove Leaf Extract Dosage Sonneratia Alba On Hela Cell Viability. J. Stem Cell Res. Tissue Eng. 5, 30. https://doi.org/10.20473/jscrte.v5i1.29383

To'bungan, N., Jati, W.N., Zahida, F., 2021. Toksisitas Akut Ekstrak Etanol Batang Rumput Knop (Hyptis capitata Jacq.) dengan Metode Brine Shrimp Lethality Test (BSLT). Biota J. Ilm. Ilmu-Ilmu Hayati 52–57. https://doi.org/10.24002/biota.v6i1.3577

Wijaya, M.D., Indraningrat, A.A.G., 2021. Antibacterial Activity of Mangrove Root Extracts from Ngurah Rai Mangrove Forest, Denpasar-Bali. Biol. Med. Nat. Prod. Chem. 10, 117–121. https://doi.org/10.14421/biomedich.2021.102.117-121

Zulfiah, Z., Megawati, M., Herman, H., H. Ambo Lau, S., Hasyim, M.F., Murniati, M., Roosevelt, A., Kadang, Y.K., AR, N.I., Patandung, G., 2020. Uji Toksisitas Ekstrak Rimpang Temu Hitam (Curcuma aeruginosa Roxb.) Terhadap Larva Udang (Artemia salina Leach) dengan Metode Brine Shrimp Lethality Test (BSLT). J. Farm. Sandi Karsa 6, 44–49. https://doi.org/10.36060/jfs.v6i1.67