

Acute Toxicity LD₅₀ Fraction Ethyl Acetate *Aquilaria malaccensis*, *Ficus benjamina*, *Mikania micrantha*, and Fraction Water *Cinnamomum burmanii* in *Mus Musculus*

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Abstract

Research on the acute toxicity of LD₅₀ had been carried out on the ethyl acetate fraction of Gaharu leaves (*Aquilaria malaccensis*), beringin leaves (*Ficus benjamina*), sembung rambat leaves (*Mikania micrantha*), and kayu manis cortex (*Cinnamomum burmanii*) water fraction the base of use as traditional treatment by the Suku Anak Dalam (SAD) empirically obtained from ancestors. This research method was experimental and it used male and female mice. The control group was given a 1% NaCMC doses of 625 mg/kg body weight, 1,250 mg/kg body weight, 2,500 mg/kg body weight, and 5,000 mg/kg body weight. The test preparation was administered orally once per day. The Thomson-Weil method was used for the measurement of LD₅₀ values. The weight ratio of organs was applied for heart, liver, lungs, kidneys, and stomach. The results showed that the LD₅₀ value for male mice was 2454mg/kg body weight; 2454mg/kg body weight; 1546 mg/kg body weight and 2065mg/kg body weight respectfully. The LD₅₀ value for female mice was a pseudo LD₅₀. The value of the organ weight ratio in each sample showed P<0.05 values that were obtained in gaharu leaves in the liver and kidney organs (male); heart in females. Beringin leaves value P<0.05 lung organs (male). Sembung rambat leaves P<0.05 values of the heart, liver, and stomach organs (male); heart and stomach (female). Cinnamomum cortex value P<0.05 liver and stomach organs (male). The conclusions showed that the LD₅₀ value of the ethyl acetate fraction of gaharu leaf, sembung rambat leaf, beringin leaf, and kayu manis cortex in male mice was slightly toxic, while in female mice it could not be counted.

Keywords: Beringin leaf (*Ficus benjamina* L.); Gaharu leaf (*Aquilaria malaccensis*); Kayu manis cortex (*Cinnamomum burmanii*); Sembung rambat leaf (*Mikania micrantha* Kunth); Toxicity acute LD₅₀.

INTRODUCTION

Suku Anak Dalam (SAD) is part of a minority group on the island of Sumatra, precisely in the interior area of Jambi province, Indonesia (Setiyadi et al., 2020). At the end of the 18th century, the jungle people (Suku Anak Dalam) encountered foreigners who brought in the infectious disease, smallpox, and reached epidemic and severe levels. This encouraged people to seek healing from plants in the forest and in addition to the knowledge of traditional medicine obtained from ancestors (Ayuningtyas et al., 2020; Mustika & Dastina, 2020; Najib, 2020). Some plants that can be used in traditional medicine are gaharu leaf, beringin leaf, sembung rambat leaf, and kayu manis cortex. Gaharu leaves are used to overcome insomnia, beringin leaves are usually used as influenza medicine, sembung rambat leaves are used as wound medicine and kayu manis

cortex can be used as a solution for diabetes, this is used empirically.

Several studies have been reported related to the plant that gaharu leaf contains secondary metabolites, namely flavonoids, steroids, tannins, triterpenoids, saponins, alkaloids, and sesquiterpene metabolites (δ -cadinene, gurjunene) (Fitriani & Erlyn, 2019; Wong et al., 2015). Beringin leaves contain kaempferol, chlorogenic acid, alkaloids, flavonoids, saponins, steroids, and polyphenols (Aslamiah & Haryadi, 2013; Corrêa et al., 2012). Sembung rambat leaf contains lignin (Lim et al., 2020), Isoledene, δ -Cadinene, Debromofiliformin, Trans-Caryophyllene, β -Bisabolene, Germacrene-D, Zingiberene (Saikia et al., 2020), protein, phenolics, and proline (Jali et al., 2021). The kayu manis cortex contains polyphenols (Rozi et al., 2022) alkaloids, tannins, phenolics, flavonoids, quinones, and triterpenoids (Hananti et al., 2012).

The acute toxicity test is a pre-clinical trial aimed at measuring the degree of the toxic effect of a compound within 24 hours after a single dose. The purpose of the acute toxicity test is to determine the potential for acute toxicity (LD₅₀) (Khumaidi et al., 2018; OECD, 2022; Ugwah-Oguejiofor et al., 2019). Based on literature studies that have been carried out, no research has been found using the ethyl acetate fraction of gaharu leaf, beringin leaf, sembung rambat leaf, and kayu manis cortex water fractions to test for acute toxicity of LD₅₀. We interested to know the acute toxicity test of LD₅₀ in mice as a research experimental animal so that the use of active doses is more guaranteed.

MATERIALS AND METHODS

The study area

Quantitative experimental studies have been conducted to trace the acute toxicity value of LD₅₀ in four types of medicinal plants in Jambi province. The results of a fraction of ethyl acetate *Aquilaria malaccensis*, *Ficus benjamina*, *Mikania micrantha*, and fraction water *Cinnamomum burmanii* were used as samples on albino mice (*Mus musculus*). Furthermore, tracing the impact of sampling on the liver, kidney, heart, lung and stomach organs was carried out.

Tools and Materials

The tools used in this study were a set of vacuum rotary evaporators, analytical scales, balance, lumps and stampers, glass bottles, stirring rods, filter paper, funnels, measuring cups, aluminum foil, Erlenmeyer, measuring flask, separator funnels, spatulas, vaporizing cups, drip pipettes, masks, hand scoops, tissue, napkins, syringe, needles, surgical tools mice, styrofoam, and squeak basins.

The ingredients used were mice (25 males and 25 females), gaharu leaf extract, beringin leaf extract, sembung rambat leaf extract, kayu manis cortex extract, 70% ethanol, n-hexane, ethyl acetate, distilled water, HgCl₂, KI, I₂, Subnitric bismuth, glacial acetic acid (CH₃COOH), HCL, ammonia (NH₃), chloroform (CHCl₃), magnesium (Mg), water, iron (III) Chloride solution (FeCl₃), Ether, Sulfuric acid (H₂SO₄), and acetic acid anhydride (C₄H₆O₃).

Procedure

Plant Determination

Determination of plants is was carried out to avoid mistakes in the identification of plants. Determination of gaharu leaf plants, beringin leaf, sembung rambat leaf, and kayu manis cortex at the Biota Identification and Determination Laboratory, Padjadjaran University (UNPAD) Indonesia.

Ethical Clearance

Ethical Clearance is a written statement given by the Research Ethics Commission for research involving living things and which states that a research proposal is feasible to be carried out according to 7 WHO standards. Ethical Clearance was carried out at the Ethics Committee of the Health Service Poltekkes of the Ministry of Health, Jambi. Ethically feasible information that has been carried out consecutively for acute toxicity tests LD₅₀ Ethyl Acetate Fraction of Gaharu leaf, Beringin leaf, Sembung rambat leaf and Kayu Manis cortex Water Fractions in White Mice No. LB. 02.06/2/104/2021; No. LB.02.06/2/090/2021; No. LB. 02.06/2/122/2021.

Sample and Material Processed

Samples of gaharu leaf were used as much as 5 kg, beringin leaf as much as 1 kg, sembung rambat leaves as much as 5 kg, and kayu manis cortex as much as 1 kg. The sample was washed thoroughly using running water first to separate the dirt, the next step was drying it by aeration. Samples that had been dried then chopped for maceration (Hermes et al., 2021).

Extraction

In the extraction process of samples that had been cleaned, dried, and pureed, the next step was to extract by the maceration method using 70% ethanol (1: 10 b/v) in a closed dark bottle allowed to stand for 24 hours. Further filtered and squeezed, the pulp is added again 70% ethanol until submerged, soaked, and filtration is carried out for 3 days with 3 changes of solvent or carried out until the color of the solvent was clear. After all, the resulting filtrates were then mixed, then the next step was concentration using a rotary evaporator at a temperature of 50°C until finally the results of the viscous extract were obtained (Dechayont et al., 2021; Khan & Islam, 2012). The working principle of a rotary evaporator was to evaporate the extraction solvent and leave only the distraction resulting compound called extract.

Fractionation

Extracts of gaharu leaf, beringin leaf, spliced leaves of propagation, and kayu manis cortex are concentrated and then fractionated by liquid-liquid extraction (ECC) using a solvent of 1: 10 based on the degree of polarity. The first thing to do was that the sample extract was stirred well-used water (polar), after mixed was put in the separator funnel then n-hexane (non-polar) is added in a ratio of 1: 1, close the separator funnel put under the hand then shake several times interspersed with opened the faucet of the separator funnel to remove the gas, so as not to cause too strong pressure to occur. Then after the sound of the gas does not come out again, it was allowed to stand for a few minutes so that there was a separation of 2 phases, namely the n-hexane phase and

the water phase, separate the n-hexane phase to the limit of solubility to the container. Furthermore, the ethyl acetate (semi-polar) fraction with the same workmanship was used, after all the fractionation of the n-hexane solvent, ethyl acetate and the remained water obtained, were separated first based on the solvent. Then re-concentrated using a rotary evaporator until it gets a viscous fraction result (Liu et al., 2013; Rachmawaty et al., 2019; Shah & Gilani, 2012).

Selection and setup of Test Animals

The test animals used were healthy male and female mice, aged 2-3 months with a body weight of mice of 20-30 grams. Prepared 50 heads of mice. Mice are divided into 5 groups and each group consists of 5 male mice and 5 female mice. Mice are acclimatized for 7 days and before treatment were fasted for 18 hours. The doses used were the control group given Na CMC 1%, the treatment group was given a dose of 625 mg/kgBB, 1,250 mg/kgBB, 2,500 mg/kgBB and 5,000 mg/kgBB (OECD, 2022; Vogel, 2002).

Organ Weight Ratio

All groups of test animals performed surgery and observations on the organs of the liver, heart, lungs, stomach, and heart. The organ was then weighed and compared with body weight to obtain the organ index (Rajeh et al., 2012).

Data Analysis

Used the SPSS (Statistical Product and Service Solutions) program to see the difference in the ratio of squeak organ weights between sexes and dose groups after administration of the test preparation, if the data was not distributed normally, the Kruskal-Wallis test is continued. The significance of the obtained results was judged at the 5% level

RESULTS AND DISCUSSION

Results

Plant Determination

Plants were determined at the Biota Identification and Determination Laboratory, Padjadjaran University (UNPAD). The determination results obtained that the sample used was *Aqularia malaccensis* Lam, *Ficus benjamina* L., *Mikania micrantha* Kunth, and *Cinnamomum burmanii*.

Extraction Results

The extraction method used was the maceration method. The sample was soaked with 70% ethanol solvent and let stand in a sealed dark vial for 24 hours. Soaked and filtration are carried out for 3 days with 3 solvent changes. After all, filtrates were obtained and then evaporated using a rotary evaporator (Dillasamola et al., 2021; Halim et al., 2020). A total of 1,400 g, 1,000 g, 1,000 g, and 1,000 g of Gaharu leaf powder extracted, beringin leaf extracted, sembung rambat leaf extracted, and kayu manis cortex extracted respectfully were successively obtained condensed extracts of 85.41 g, 77.72 g, 70.29 g, 81.6 g with a yield of 6.10%, 6.4%, 7.02%, 8.16%.

Fractionation Results

Fractionation aims to separate the components of the active compound from the extract that has been produced. The results of the viscous extract of the sample are further fractionated by separation based on their polarity. Fractionation in this study used non-polar solvents, namely n-hexane, and semi-polar solvents, namely ethyl acetate (Liu et al., 2013; Rachmawaty et al., 2019; Shah & Gilani, 2012). The results of the yield of the fractionation of gaharu leaf, beringin leaf, sembung rambat leaf, and kayu manis cortex successively accorded to the polarity of the solvent obtained an n-hexane fraction of 13.43%; 10,8%; 12,73%; 0,06%. The ethyl acetate fraction was obtained 7.62%; 6,09%; 11,50%; 2.16% and the residual fraction of water obtained 23.00%; 22%; 35,86%; 30,00%.

Table 1. Total Number of Male Mice Deaths on Gaharu leaf, Beringin leaf, Sembung Rambat leaf, and Kayu Manis cortex.

| | Normal | | 625mg/kgBB | | 1250mg/kgBB | | 2500mg/kgBB | | 5000mg/kgBB | | Total | |
|---------------------|---------|------|------------|------|-------------|------|-------------|------|-------------|------|---------|------|
| | survive | dead | survive | dead | survive | dead | survive | dead | survive | dead | survive | dead |
| Gaharu leaf | 5 | 0 | 4 | 1 | 5 | 0 | 2 | 3 | 2 | 3 | 18 | 7 |
| Beringin leaf | 5 | 0 | 3 | 2 | 4 | 1 | 2 | 3 | 3 | 2 | 17 | 8 |
| Sembung Rambat leaf | 5 | 0 | 5 | 0 | 3 | 2 | 2 | 3 | 2 | 3 | 17 | 8 |
| Kayu Manis cortex | 5 | 0 | 5 | 0 | 4 | 1 | 3 | 2 | 1 | 4 | 18 | 7 |

Table 2. Total Number of Female Mice Deaths on Gaharu leaf, Beringin leaf, Sembung Rambat leaf, and Kayu Manis cortex.

| | Na-CMC 1% | | 625mg/kgBB | | 1250mg/kgBB | | 2500mg/kgBB | | 5000mg/kgBB | | Total | |
|---------------------|-----------|------|------------|------|-------------|------|-------------|------|-------------|------|---------|------|
| | survive | dead | survive | dead | survive | dead | survive | dead | survive | dead | survive | dead |
| Gaharu leaf | 5 | 0 | 5 | 0 | 4 | 1 | 3 | 2 | 3 | 2 | 20 | 5 |
| Beringin leaf | 5 | 0 | 4 | 1 | 5 | 0 | 5 | 0 | 2 | 3 | 21 | 4 |
| Sembung Rambat leaf | 5 | 0 | 5 | 0 | 4 | 1 | 4 | 1 | 3 | 2 | 21 | 4 |
| Kayu Manis cortex | 5 | 0 | 5 | 0 | 4 | 1 | 3 | 2 | 3 | 2 | 20 | 5 |

Table 3. The data value of the influence of the ratio of organ weights on each sample to the organ of mice ($P>0.05$).

| | Heart | | Liver | | Lungs | | Kidney | | Stomach | |
|---------------------|-------|--------|-------|--------|-------|--------|--------|--------|---------|--------|
| | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| Gaharu leaf | No | Yes | Yes | Yes | No | No | Yes | Yes | No | No |
| Beringin leaf | No | No | No | No | Yes | Yes | No | No | No | No |
| Sembung Rambat leaf | Yes | Yes | Yes | Yes | No | No | No | No | Yes | Yes |
| Kayu Manis cortex | No | No | Yes | Yes | No | No | No | No | Yes | No |

Discussion

LD₅₀

In this study, the fifty mice used were divided into 5 groups, each group consists of 5 male mice and 5 female mice. Before treatment, all mice were acclimatized for 7 days with the aim that the test animals could adapt to the laboratory atmosphere (tested environment). Before being given the treatment of mice, they were fasted for 18 hours but were still given a drink. Mice are satisfied so that when given the sample treatment were expected to directly interact with the digestive system and not be disturbed by the presence of food contained in the digestion of mice. Mice were administered sample extracts orally (Vogel, 2002).

In male mice with Gaharu leaf samples, the results of this study showed that the value of LD₅₀ ethyl acetate fraction of gaharu leaves obtained a result of 2,454 mg/kg body weight are included in the category of slightly toxic. The toxicity ethyl acetate fraction of beringin leaves obtained a result of 2,454 mg/kg body weight belongs to the category of slightly toxic. The toxicity ethyl acetate fraction of sembung rambat leaf obtained a result of 1546 mg/kgBB, belonging to the category of slightly toxic. The toxicity water fraction of the cinnamomum cortex obtained a result of 2065 mg/kg body weight belongs to the category of slightly toxic.

In female mice, no meaningful value of LD₅₀ founded after being given extracts of gaharu leaf, beringin leaf, sembung rambat leaf, and kayu manis cortex. It can be said that this is included in the pseudo LD₅₀. This is possible due to the influence of hormonal cycles that can cause estrogen hormone levels to increase. If the level of the hormone estrogen increases then the immune system also increases or was stronger than the immune system of the male mice, so that the male mice can experience death. In female mice, LD₅₀

calculations could not be carried out because they did not meet the mortality criteria of the Thompson-Weil method.

Organ Weight Ratio

In the statistical test of the organ weight ratio after administration of the ethyl acetate fraction of Gaharu leaf, there was a significant difference in female mice in the heart organs. While in male mice there are significant differences in the liver organs and kidney organs. In the ethyl acetate fraction of the leaves of the splice of the sembung rambat leaf, there were significant differences in female mice in the organs of the heart and stomach. While in male mice there were significant differences in the organs of the liver, heart, and stomach. This was likely due to the presence of content of secondary metabolite compounds. Secondary metabolites are chemical compounds that were formed in plants. Secondary metabolites in addition to having physiological effects that can nourish or cure diseases can also cause undesirable side effects on the human body or certain animals. In plants, each compound works together to influence the other to increase its activity or effectiveness. As for compounds that can damage liver cells, including alkaloids and saponins, both compounds can damage cells and tissues and even cause death in experimental animals.

For data on the ratio of organ weights after administration of the ethyl acetate extract fraction of beringin leaf to the heart, liver, kidney, and stomach organs of male and female mice, data were obtained with $p>0.05$ meant that there was no noticeable difference in the weight of the heart, liver, kidney and stomach organs at various doses, while data on the weight ratio of male lung organs were obtained data marked $p<0.05$ which means that there was a difference

in the weight of the male lung organs in various doses. In statistical tests of kayu manis cortex fraction on the weight of mice organs, there were significant differences in the lung organs of male mice and the gastric organs of male and female mice.

The ethyl acetate fraction of beringin leaf and the water fraction of kayu manis cortex may contain flavonoid compounds, which is known if flavonoids at excess levels in the cells can cause a halt in active transport which causes an uncontrolled intake of ions in the cells so that it can cause cell death (necrosis) in the lung organs (Tanduwinata et al., 2015). In the lungs, there is an alveolus that functions as a place for gas exchange, where each wall is coated by cells. The alveolus is declared damaged when dilation of the alveolus is founded and this can trigger the onset of inflammatory infiltrates.

There are significant differences in the gastric organs of male and female mice, it can be concluded that there is gastric damage in male white mice and female white mice. Kayu Manis cortex may contain flavonoid compounds that are thought to have an inhibitory effect against lipoxygenase and cyclooxygenase. Where one of the product pathways of cyclooxygenase, namely prostaglandins, is thought to have a protective effect on the stomach. A deficiency of prostaglandins is suspected to lead to a decrease in the production of mucus, phospholipids, HCO₃⁻ secretions, the proliferation of mucosal cells, and microvascular flow of the stomach that is suspected to cause discontinuities in the epithelium of the gastric mucosa known as peptic ulcers. The mechanism is suspected to be the cause of swelling of the gastric organs in male white mice and female white mice after administration of the Kayu Manis cortex fraction (Astri et al., 2012).

CONCLUSIONS

Based on the studies that have been carried out, it can be concluded that the LD₅₀ values of gaharu leaf, geringin leaf, sembung rambat leaf, and kayu manis cortex in male mice are categorized as slightly toxic and in female mice cannot be calculated or show pseudo-LD₅₀ values. The value of the organ weight ratio in each sample shows P<0.05 values were obtained in gaharu leaves in the liver and kidney organs (male); heart in females. Beringin leaves value P<0.05 lung organs (male). sembung rambat leaves P<0.05 values of the heart, liver, and stomach organs (male); heart and stomach (female). Cinnamomum cortex value P<0.05 liver and stomach organs (male).

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carried out the laboratory work for Gaharu leaves (*Aquilaria malaccensis*). Rizky Yulion, Lili Andriani and Lesra Indriani carried out the laboratory work for Beringin leaves (*Ficus benjamina*). Rizky Yulion, Santi Perawati and Suci Ramadani carried out the laboratory work for Sembung rambat leaves (*Mikania micrantha*). Rizky Yulion, Barmi Hartesi and Nadia Monika carried out the laboratory work for Kayu manis cortex (*Cinnamomum burmanii*). Rizky Yulion analyzed the data. Rizky Yulion wrote the manuscript. All authors read and approved the final version of the manuscript

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REFERENCES

- Aslamiah, S., & Haryadi, H. (2013). Identifikasi Kandungan Kimia Daun Pohon Beringin (*Ficus Benyamina* L.) sebagai Obat Tradisional. *Jurnal Anterior*, 13(1). <https://doi.org/10.33084/anterior.v13i1.287>
- Astri, Y., Sitorus, T., Sigit, J. I., & Sujatno, M. (2012). Toksisitas Akut per Oral Ekstrak Etanol Daun Dewa (*Gynura pseudochina* (Lour.) DC) terhadap Kondisi Lambung Tikus Jantan dan Betina Galur Wistar. *Majalah Kedokteran Bandung*, 44(1), 38–43. <https://doi.org/10.15395/mkb.v44n1.71>
- Ayuningtyas, D., Utami, R. R. M., & Maulidya, A. N. (2020). The readiness of Batang Hari district as a district of human rights care: case study of the Anak Dalam tribe. *International Journal of Human Rights in Healthcare*, 13(3), 209–217. <https://doi.org/10.1108/IJHRH-04-2019-0027>
- Corrêa, R., Agertt, V., & Boligon, A. A. (2012). Natural Product Research: Formerly Natural Product Letters In vitro antimycobacterial activity and HPLC – DAD screening of phenolics from *Ficus benjamina* L. and *Ficus luschnathiana* (Miq.) Miq. leaves. *Natural Product Research*, 26(23).
- Dechayont, B., Phuaklee, P., Chunthong-Orn, J., Juckmeta, T., Prajuabjinda, O., & Jiratsatit, K. (2021). Antibacterial, anti-inflammatory and antioxidant activities of Mahanintangtong and its constituent herbs, a formula used in Thai traditional medicine for treating pharyngitis. *BMC Complementary Medicine and Therapies*, 21(1), 1–12. <https://doi.org/10.1186/s12906-021-03274-6>
- Dillasamola, D., Aldi, Y., Wahyuni, F. S., Rita, R. S., Dachriyanus, Umar, S., & Rivai, H. (2021). Study of Sungkai (*Peronema canescens*, Jack) leaf extract activity as an immunostimulators with in vivo and in vitro methods. *Pharmacognosy Journal*, 13(6), 1397–1407. <https://doi.org/10.5530/PJ.2021.13.177>
- Fitriani, N., & Erlyn, P. (2019). Aktivitas Antidiabetik Kombinasi Ekstrak Etanol Daun Ciplukan (*Physalis angulata*) dan Daun Gaharu (*Aquilaria malaccensis*) pada Tikus Diabetes. *Syifa' MEDIKA: Jurnal Kedokteran Dan Kesehatan*, 9(2), 70. <https://doi.org/10.32502/sm.v9i2.1660>
- Halim, K. F. K., Jalani, K. J., Mohsin, H. F., & Wahab, I. A. (2020). Phytochemical Screening of *Peronema Canescens*

- Jack. *International Journal of Pharmaceuticals*, 1(March), 7–15.
- Hananti, R. S., Hidayat, S., & Yanti, L. (2012). Uji Aktivitas Antidiabetes Ekstrak Etanol Kulit Kayu Manis (*Cinnamomum burmanii* Nees ex.Bl.) Dibandingkan dengan Glibenklamid pada Mencit Jantan Galur Swiss Webster dengan Metode Toleransi Glukosa. *Indonesian Journal of Pharmaceutical Science and Technology*, 1(1), 13–21.
- Hermes, L., Römermann, J., Cramer, B., & Esselen, M. (2021). Quantitative Analysis of β -Asarone Derivatives in *Acorus calamus* and Herbal Food Products by HPLC-MS/MS. *Journal of Agricultural and Food Chemistry*, 69(2), 776–782. <https://doi.org/10.1021/acs.jafc.0c05513>
- Jali, P., Samal, I. P., Jena, S., & Mahalik, G. (2021). Morphological and biochemical responses of *Macrotyloma uniflorum* (Lam.) Verdc. to allelopathic effects of *Mikania micrantha* Kunth extracts. *Heliyon*, 7(8), e07822. <https://doi.org/10.1016/j.heliyon.2021.e07822>
- Khan, M. A. A., & Islam, M. T. (2012). Analgesic and cytotoxic activity of *Acorus calamus* L., *Kigelia pinnata* L., *Mangifera indica* L. and *Tabernaemontana divaricata* L. *Journal of Pharmacy and Bioallied Sciences*, 4(2), 149–154. <https://doi.org/10.4103/0975-7406.94820>
- Khumaidi, A., Ihwan, & Yusuf, A. (2018). Uji Toksisitas Akut Dan Letal Dose (LD50) Ekstrak Etanol Daun Pepolo (*Bischofia javanica* Blume) Pada Mencit Putih (*Mus musculus*) Acute Toxicity Test And Lethal Dose (LD50) of Pepolo Leaf Ethanol Extract (*Bischofia javanica* Blume) on White Mice (*Mus mus*. *Journal of Science and Technology*, 7(1), 110–116.
- Lim, C. J., Arumugam, M., Lim, C. K., & Ee, G. C. L. (2020). Mercerizing Extraction and Physicochemical Characterizations of Lignocellulosic Fiber from the Leaf Waste of *Mikania micrantha* Kunth ex H.B.K. *Journal of Natural Fibers*, 17(5), 726–737. <https://doi.org/10.1080/15440478.2018.1527742>
- Liu, X. C., Zhou, L. G., Liu, Z. L., & Du, S. S. (2013). Identification of insecticidal constituents of the essential oil of *Acorus calamus* rhizomes against *Liposcelis bostrychophila* badonnel. *Molecules*, 18(5), 5684–5696. <https://doi.org/10.3390/molecules18055684>
- Mustika, D., & Dastina, W. (2020). Religious, Economic, and Political Values Shifting in Inheritance System of Suku Anak Dalam. *Ahkam: Jurnal Ilmu Syariah*, 20(1), 49–78. <https://doi.org/10.15408/ajis.v20i1.13568>
- Najib, K. (2020). Government Ecology and the Indigenous Religion of the Suku Anak Dalam: Intersubjective Relations in Forest Conservation in Jambi, Indonesia. *Jurnal Manajemen Hutan Tropika*, 26(3), 303–315. <https://doi.org/10.7226/JTFM.26.3.303>
- OECD. (2022). *Test Guideline No . 425 Acute Oral Toxicity : Up-and-Down Procedure*.
- Rachmawaty, F. J., Andianty, E., Tamhid, H. A., & Julianto, T. S. (2019). Antibacterial activity of fractions (chloroform, ethyl acetate and methanol) red betel leaf ethanol extract (*Piper crocatum*) against *Streptococcus pyogenes* bacteria. *International Journal of Infectious Diseases*, 79. <https://doi.org/10.1016/j.ijid.2018.11.110>
- Rajeh, M. A. B., Kwan, Y. P., Zakaria, Z., Latha, L. Y., Jothy, S. L., & Sasidharan, S. (2012). Acute toxicity impacts of *Euphorbia hirta* L extract on behavior, organs body weight index and histopathology of organs of the mice and *Artemia salina*. *Pharmacognosy Research*, 4(3), 170–177. <https://doi.org/10.4103/0974-8490.99085>
- Rozi, F., Silvy, D., & Syukri, D. (2022). Characterization of Herbal Tea Bags Made From Starfruit Leaves (*Averrhoa bilimbi*), Gotu Kola Leaves (*Centella Asiatica*) and Cinnamon Powder (*Cinnamomun Burmanii*) As Functional Drinks. *Andalasian International Journal of Agriculture and Natural Sciences (AIJANS)*, 3(01), 47–54. <https://doi.org/10.25077/aijans.v3.i01.47-54.2022>
- Saikia, S., Tamuli, K. J., Narzary, B., Banik, D., & Bordoloi, M. (2020). Chemical characterization, antimicrobial activity, and cytotoxic activity of *Mikania micrantha* Kunth flower essential oil from North East India. *Chemical Papers*, 74(8), 2515–2528. <https://doi.org/10.1007/s11696-020-01077-6>
- Setiyadi, B., Ranati, A., & Atani, M. H. (2020). Isolasi Masyarakat Terasing: Kajian Kegiatan Pksmt Pada Suku Anak Dalam. *Sosial Horizon: Jurnal Pendidikan Sosial*, 7(1), 92–103. <https://doi.org/10.31571/sosial.v7i1.1737>
- Shah, A. J., & Gilani, A. H. (2012). Aqueous-methanolic extract of sweet flag (*Acorus calamus*) possesses cardiac depressant and endothelial-derived hyperpolarizing factor-mediated coronary vasodilator effects. *Journal of Natural Medicines*, 66(1), 119–126. <https://doi.org/10.1007/s11418-011-0561-7>
- Tanduwina, A., Istiqomah, H. A., Jamilah, Caesaria, N. L. ., Saputra, R. ., & Aulanni'am. (2015). Bioactive potency of red algae (*Gracillaria verrucosa*) extract towards malondialdehyde level and lung histology of white rat (*Rattus novergicus*) after formalin induction. *Jurnal Ilmu Kimia Universitas Brawijaya*, 10(2), 86–87.
- Ugwah-Oguejiofor, C. J., Okoli, C. O., Ugwah, M. O., Umaru, M. L., Ogbulie, C. S., Mshelia, H. E., Umar, M., & Njan, A. A. (2019). Acute and sub-acute toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* N. E. Brown in mice and rats. *Heliyon*, 5(1), e01179. <https://doi.org/10.1016/j.heliyon.2019.e01179>
- Vogel, H. G. (2002). Drug Discovery and Evaluation Pharmacological Assays. In *Human & Experimental Toxicology* (Ed II, Vol. 17, Issue 10).
- Wong, Y. F., Chin, S. T., Perlmutter, P., & Marriott, P. J. (2015). Evaluation of comprehensive two-dimensional gas chromatography with accurate mass time-of-flight mass spectrometry for the metabolic profiling of plant-fungus interaction in *Aquilaria malaccensis*. *Journal of Chromatography A*, 1387, 104–115. <https://doi.org/10.1016/j.chroma.2015.01.096>