Al-Kimia, 11-2-2023, 41-51
Available online at: http://journal.uin-alauddin.ac.id/index.php/al-kimia

Toxicity Test of Elo (*Ficus racemosa* L.) Leaf Extracts and Fractions Using Brine Shrimp Lethality Test Methods

Novia Suryani*, Serli Gustiana

Tadris Chemistry Study Program, Faculty of Tarbiyah and Teacher Training, Mataram State Islamic University, Jl. Pendidikan No.35 Dasan Agung Baru, Selaparang, West Nusa Tenggara, Indonesia

*Corresponding Author: noviasuryani@uinmataram.ac.id
Received: August, 22, 2023 /Accepted: December, 1, 2023
doi: 10.24252/al-kimiav11i2.40821

Abstract: *This study aims to determine the toxicity of the Ficus racemosa L. plant, commonly known as the elo plant, which has been widely used as an alternative medicine for natural products. Using natural ingredients as drug candidates is a viable option to enhance the value of natural resources and contribute to Indonesia's biodiversity. The research employed the maceration method to obtain leaf extract. The extract was then partitioned using n-hexane, ethyl acetate, and chloroform solvents to obtain various leaf fractions. Qualitative tests were conducted to identify secondary metabolite compounds. The toxicity of the elo plant extract and its fractions was evaluated using the Brine Shrimp Lethality Test (BSLT) with Artemia salina Leach as an initial toxicity screening. The results revealed that the extract and fractions of the leaves contained secondary metabolites such as flavonoids, alkaloids, and saponins. The toxicity test yielded LC50 values of 91.76 ppm for the leaf extract, 220.47 ppm for the n-hexane fraction, 249.13 ppm for the ethyl acetate fraction, and 338.16 ppm for the chloroform fraction. Based on the LC50 values, the leaf extract and fractions of the elo plant exhibit toxic potential.*

Keywords: *BSLT, Extraction, Ficus racemose L., Toxicity.*

INTRODUCTION

As an archipelago, Indonesia benefits from favourable geographical conditions, flanked by two oceans and continents. This unique positioning contributes to its status as a megadiverse country. The plant diversity within Indonesia provides substantial opportunities for the exploration and utilization of plants in the food and health sectors (Kusmana & Hikmat, 2015). As a nation rich in biodiversity, Indonesia offers numerous plant samples with potential secondary metabolite compounds. Exploring plants derived from natural materials is crucial in harnessing the potential of existing biodiversity (K & AN, 2018). Plants produce secondary metabolite compounds (Twaij & Hasan, 2022) with diverse and unique biological activities (Uswatun & Wijayanti, 2020). These compounds, resulting from secondary metabolic processes (Mohiuddin, 2020), include active chemical components such as flavonoids (Abusufyan et al., 2018), alkaloids, terpenoids, steroids, saponins, and tannins. These compounds have been extensively studied for their potential as active compounds in cancer treatment. Efforts to develop cancer treatments continue to evolve, ranging from traditional remedies using natural materials to chemical treatments, including drugs and cancer therapies (Muthukrishnan et al., 2017; Antonyan et al., 2016). The development of treatment methods utilizing natural materials as alternatives to traditional medicine remains an area of significant research interest (Mohiuddin, 2020). The diverse natural materials have proven valuable lead compounds for developing active compound medicine candidates (Kumar Dubey et al., 2018) with potential as active substances in cancer drugs.

Ficus racemosa L., known as the elo plant in East Java and the Ara plant in West Java and West Nusa Tenggara, has been traditionally utilized as an alternative medicine for various ailments (Kumar Dubey et al., 2018). Its uses include treatments for diarrhoea (Shiksharthi & Mittal, 2011) in both adults and children, as well as anticoccidial (Khan et al., 2023), antimicrobial (Suryawanshi et al., 2020), anti-filarial (Ahmed & Urooj, 2010), chemotherapeutic (Singh et al., 2013), antipyretic (Jain et al., 2013), and anti-inflammatory applications (Kumar Dubey et al., 2018). The plant's various parts contain significant bioactive compounds, including its fruit and root bark. The root bark is particularly noted for its high flavonoid composition, which has demonstrated potential as a natural antioxidant, with an IC50 value of 1.66 ppm. This flavonoid was identified as quercetin. Additionally, racemosic acid, isolated from the elo plant, shows promise for treating inflammatory diseases, exhibiting IC50 activity in inhibiting COX-1 and 5-LO in vitro at 90 and 18 µM, respectively. The fruit of the elo plant, known for its bitter taste, has potential anticancer properties against HepG-2 cells through in vitro methods (Sivakumar et al., 2019). Furthermore, the roots and leaves of the elo plant are being explored for their potential as antidiabetic drugs (Sharma et al., 2023). These findings highlight the elo plant's significant potential in developing natural treatments for various diseases, reinforcing its traditional uses and paving the way for further scientific exploration and validation of its medicinal properties.

Methods for analyzing the bioactivity of natural materials include in vitro, in silico, and in vivo techniques. One of the simple in vitro methods for determining the toxic properties of a natural product is the Brine Shrimp Lethality Test (BSLT) using **Artemia salina** Leach (Zamzami et al., 2021). This method effectively monitors the pharmacological activity of natural extracts (Chan et al., 2021). It is advantageous due to its cost-effectiveness, repeatability, minimal sample requirement, and simplicity (Ntungwe et al., 2020), making it a functional preliminary bioactivity test before further testing (Golla et al., 2011). The BSLT method determines the LC50 value, representing the extract concentration required to kill 50% of the *Artemia salina* larvae (Clemen-Pascual et al., 2022). Artemia salina larvae are commonly used in this assay because of their high sensitivity to toxic properties, making them ideal for identifying toxic compounds in natural material samples (Albarano et al., 2022). Given the potential of the active secondary metabolites produced by the elo plant, it is essential to explore its toxicity activity. This can be done using the BSLT method as an initial toxicity screening, employing *Artemia salina* Leach shrimp larvae as test organisms (Musyarrifah et al., 2014). This approach can help identify the toxic potential of elo plant extracts, paving the way for their use as candidates for anticancer drugs derived from natural ingredients.

RESEARCH METHODS

Materials and Tools

The material used in this research is elo leaves which form the Krembangan Village, Jombang, East Java. Ethanol 96% (Merck), n-hexane (Merck), chloroform (Merck), ethyl acetate (Merck), dimethyl sulfoxide (DMSO) (Merck), Wagner reagent (Sigma Aldrich), Dragendorff reagent (Sigma Aldrich), H_2SO_4 p.a (Merck), HCl p.a (Merck), magnesium (Mg) (Merck), $FeCl_3$ (Merck), NaCl (Merck), dan aquades (Chemika Karya). The tools used are beaker glass (pyrex), volumetric flask (pyrex), test tube (pyrex), hotplate (thermos scientific), measuring pipette (pyrex glass funnel (pyrex), separatory funnel (pyrex), drop pipette, spatula, analytical balance (sonic), rotary evaporator (B-ONE), oven (Memmert), water bath (Memmert), aquarium, filter paper, aluminum foil,

macerator, sieve 60 mesh, blender (Panasonic), vaporizer cup, drop plate, magnetic stirrer, vials, erlenmeyer Buchner (pyrex), and vacuum desiccator (Duran).

Procedures

Extraction of Elo Leaves

The elo leaves of about 150 were put into a beaker glass containing 750 mL of 96% ethanol solvent and soaked completely for 1x24 hours. The soak results were filtered every 1x24 hours and separated between the results of the macerate and dregs. The sample dregs will be soaked again using a new solvent using the same maceration procedure as before, up to 3 repetitions. The total macerate results were combined and concentrated using a rotary evaporator at 89ºC Solvent evaporation is done until there is no more solvent dripping. The sample extract was stored in a desiccator before being used for further analysis.

% Moisture Content (Suryani et al., 2022)

The samples were washed thoroughly using clean running water and then dried at room temperature for ± 1 x 24 hours. The samples were put into the oven and dried at 105 ºC for 3 hours. The oven process was repeated until a constant mass-weighing result was obtained. The drying process was stopped after the measurement of moisture content had met the SNI standard, which did not exceed 10%.

Partition of leaf extract (Suryani et al., 2022)

A total of 1 g of extract that has been obtained is partitioned using various solvents such as n-hexane, ethyl acetate, and chloroform, each as much as 10 mL. The partition process uses a 50 mL separating funnel. Partition results that are further analyzed are solutions in the organic phase.

Phytochemical Qualitative Test

Flavonoid Test (Suryani et al., 2022)

A total of 1 mL of sample was added as much as 0.01 g of Mg powder and concentrated HCl as much as 3-5 drops. The formation of color changes into orange, brick red, pink, and dark red colors indicated the presence of flavonoids in the sample.

Alkaloid Test (Marinho et al., 2022)

A total of 1 mL was put into 2 different test tubes and marked. Each tube is added to the reagent Dragendroff and Wagner as much as ± 5-10 drops. A comparison of alkaloids present in the sample is characterized by the formation of a red-brown precipitate if added Dragendorff reagent was and a brick-red brown precipitate formed if added Wagner reagent.

Saponin Test (Ojah et al., 2021)

Saponin testing on the sample is done by dissolving 1 mL of sample into 4 mL of hot distilled water then cooled and filtered. The filtrate obtained was shaken very vigorously for 15 to 20 seconds. Next, concentrated HCl has added as much as 1-3 drops to the foam formed. If the foam remains after the addition of concentrated HCl, the sample is indicated to contain saponins.

Salkowski Test (Stamenković et al., 2021)

A total of 2 ml of sample was added with 2 ml of chloroform. Furthermore, a few drops of concentrated H2SO4 were added to the mixture and shaken gently to observe the changes that occurred. The presence of steroids/terpenoids is indicated by the formation of

2 layers, namely organic with a red color change and water layer with a yellow to green color change.

Tannin Test (Carabelly & Aspriyanto, 2020)

A total of 1 mL of sample was added with 10 mL of aqudes and then brought to a boil in a water bath. The cooled filtrate added as much as 5 mL of $FeCl_3$ 1% (b/v). The formation of color changes to dark blue, blue-black, brown-black, or green-black indicates the sample contains tannins.

Toxicity Assay (Marliza and Oktaviani, 2021), (Sukandar, et al., 2009), (Suryawanshi et al., 2020), (Rahamouz-Haghighi et al., 2022)

The primer solution of each extract fraction that has been obtained is prepared. 100 mg of extract fractions were dissolved in DMSO (unique, complex samples). The solution was transferred to a 10 mL vial tube with a concentration variation of 50 ppm, 100 ppm, 250 ppm, 500 ppm, and 1000 ppm. Each vial contains a solution of 48-hour-old *Artemia salina*Leach shrimp larvae actively moving as many as ten tails. Dilute the solution using seawater solution until the volume reaches 10 mL. The same work was done on the control solution and replicated three times. The % mortality value of shrimp larvae was observed after incubation for 1 x 24 hours. Based on the % mortality data, the data were processed based on the regression data obtained to determine the LC50 value. The LC50 value is obtained based on the calculation of the probit value by converting the % mortality value with the Probit table. The results of plotting the data between the probit value and log concentration will produce a regression line equation that will be used to determine the LC50 value of each elo plant sample.

RESULTS AND DISCUSSION

The samples to be used need to be measured for moisture content to determine if the dry samples (dry simplicial) have met the SNI rules; namely, the maximum percentage of moisture content in natural material samples is 10%. This standard indicates that the sample still contains water but with a minimum tolerance level, so disturbing microorganisms do not live and multiply in the sample during storage (Vera Zambrano et al., 2019). A minimal measure of water content will be beneficial during the maceration process because the sample can interact maximally with the solvent during the maceration process. Based on the calculation results, the percentage value of leaf sample water content is 1.039%.

Elo Leaf Extract

Prepared samples will be used to make extracts using the maceration method. The maceration method has a working principle of interaction between the sample and the solvent used so that the secondary metabolite compounds present in the sample can dissolve in the solvent. This method has the advantage that the process can be done and does not need to use heat for a certain period, which can create a risk of damage to the components of active compounds that are sensitive to temperature or heat in the natural ingredients used. However, this method has disadvantages, namely the use of many solvents and the fact that it could be more time efficient (long time needed) (Farahani, 2021). The choice of solvent in the maceration process plays a vital role in determining the type of secondary metabolite compounds that will dissolve. This is to the principle of like dissolve like; namely, secondary metabolite compounds that have the same properties as the solvent will

attract the solvent, while secondary metabolite compounds that have different properties from the solvent cannot dissolve in the solvent used (Permana & Andhikawati, 2021).

A suitable solvent can be selected based on the order of the solvent most often used during the maceration process. The degree of solubility of the solvent is also a factor that should be considered in the selection of solvents. Specifically, solvent selection can be based on the nature of solvent polarity, solvent selectivity properties, and volatile solvent properties so that it is easily separated from the active compounds to be taken (Al-Shabibi et al., 2022), solvents that have stable chemical properties, and solvents with low prices and are readily available. If viewed based on the polarity of the solvent, it can be sorted from water, ethanol, methanol, chloroform, ether, and acetone.

This study used a solvent and sample ratio of 1:5 (b/v) and maceration for 1 x 24 with repetition of maceration up to three repetitions. Every 1 x 24 hours after maceration, the maceration results were filtered to separate the residue and filtrate. The residue that has been obtained will be used for the following maceration process using a new solvent with the same ratio. Each filtrate obtained will be combined into one in the same container. Repetition of maceration up to three times aims to maximize the process of extracting secondary metabolite compounds in natural materials. Therefore, the solvent used is a new solvent to avoid solvent saturation, which results in the solvent no longer being able to attract secondary metabolite compounds in the sample.

The Fraction of Elo Leaf

Partitioning is done using a separating funnel tool with liquid-solid principles. The thick sample obtained will be dissolved first with distilled water and added with the three partition solvents. Furthermore, it is shaken using a separating funnel so that separation occurs according to the principle of like dissolve like. The shaking results will form two phases: the distilled water phase and the organic phase (the phase containing the organic solvent used). The separation of these two phases can occur due to differences in the distribution of secondary metabolite compounds based on the similarity of their properties with the solvent used. At the same time, the phase position can be determined based on the weight density of each phase. If the density of a phase is greater, the position of the phase is at the bottom. The partition results will be used as a fraction sample according to the solvent used. The solvent will be evaporated again to obtain a thick fraction extract that will be used for further analysis. Especially for n-hexane, ethyl acetate, and chloroform solvents, solvent evaporation is carried out using a water bath tool because the solvent is very volatile compared to ethanol solvents.

Phytochemical Qualitative Test

Secondary metabolites are compounds in plants or natural materials formed from the results of side reactions or secondary reactions of primary metabolism (Parbuntari et al., 2018). The existence of secondary metabolite compounds in plants has a function as a substance that can defend itself or maintain its existence in the environment. Although they are the result of side reactions and have a smaller amount when compared to primary metabolite compounds, secondary metabolite compounds have many benefits and potential (Fecker et al., 2020).

Table 1. The results of secondary metabolites of elo leaf

Compound	Color if the result is positive	Information & Samples Color Observed			
		Leaf	F. hexane	F.EA	F.$CHCl_3$
Flavonoid	Yellow or orange or red	(+) Orange	(+) Orange	(+) Yellow	(-) Light green
Alkaloid	Red-brown or brick red-brown	(+) Red-brown	(+) Red-brown	(+) Red-brown	(+) Red-brown
Saponin	Foam stable	(+) Foam stable	(+) Foam stable	(-) No foam	(-) No foam
Steroid	Red to yellow	(-) Green	(-) Green	(-) Green	(-) Green
Terpenoid	Red to yellow	(-) Green	(-) Green	(-) Green	(-) Green
Tanin	Blackish green	(-) Light green	(-) Light green	(+) Blackish green	(-)

Note:
F. hexane = fraction of n-hexane extract
F. EA = fraction of ethyl acetate
F. $CHCl_3$ = fraction of chloroform
(+) = indicated contain secondary metabolite compound
(-) = indicated not contain secondary metabolite compound

Based on Table 1, show that the secondary metabolite compounds contained in leaf extract samples and n-hexane fractions are flavonoids, alkaloids, and saponins. While the ethyl acetate and chloroform fractions only contain alkaloid compounds. The qualitative test results obtained provide information regarding the possibility of what secondary metabolite groups are contained in the sample but cannot provide information regarding the structure of the metabolite compounds in question. But, based on research before informed that leaf *Ficus racemosa* L., contains flavonoids, alkaloids, tannins, sterols, triterpenoids, and triterpenes (Pahari et al., 2022), (Kumar Dubey et al., 2018)

Toxicity Assay

Based on the calculation of the probit value and log concentration of each sample of extracts and fractions of elo plant leaves, the LC_{50} value has been obtained respectively (Clemen-Pascual et al., 2022). The ability of each sample that can cause mortality (death) to 50% of the test sample in the form of *Artemia salina* Leach larvae is thought to contain secondary metabolite compounds that have the ability of toxic properties (Suryawanshi et al., 2020). While the category of toxicity properties can be seen in Table 2:

Table 2. The category of toxicity based on LC_{50} value

Category	LC_{50} (µg/mL)
Very toxic	≤ 30
Toxic	31 – 1000
Not toxic	> 1000

The BSLT test method is a simple method that is commonly used to determine the toxic potential (Ntungwe N et al., 2020) of an active compound derived from natural plants or synthetic materials based on its ability to cause death in *Artemia salina* Leach larvae (as bioindicators) (Muthukrishnan et al., 2017). The results of the BSLT test of elo plants in the form of extract samples and leaf fractions against *Artemia salina* Leach shrimp are presented in the following Table 3:

Table 3. LC_{50} leaf extract

Log Concentration	% Mortality	Probit	LC_{50} (ppm)
1,699	26,7	4,375	
2,000	56,7	5,169	
2,398	83,3	5,966	**91,76**
2,699	90,0	6,282	
3,000	96,7	6,808	

Based on Table 3, the LC_{50} value obtained was 91.76 ppm. This shows that to be able to cause death as much as 50% of the total shrimp larvae required a sample concentration of 91.76 ppm. Refers to Table 2, the LC_{50} value that has been obtained is included in the toxic category. As an initial screening information on the toxicity properties of leaf extract samples can be recommended as a candidate containing anticancer drug compounds.

Table 4. LC_{50} fraction of n-hexane

Log Concentration	% Mortality	Probit	LC_{50} (ppm)
1,699	6,7	3,5015	
2,000	33,3	4,5684	
2,398	60,0	5,2533	**220,47**
2,699	63,3	5,3398	
3,000	93,3	6,4985	

Table 5. LC_{50} fraction of ethyl acetate

Log Concentration	% Mortality	Probit	LC_{50} (ppm)
1,699	0,0	0,0000	
2,000	13,3	3,8877	
2,398	50,0	5,0000	**249,13**
2,699	90,0	6,2816	
3,000	100,0	8,7190	

Table 6. LC_{50} fraction of chloroform

Log Concentration	% Mortality	Probit	LC_{50} (ppm)
1,699	0,0	0,0000	
2,000	0,0	0,0000	
2,398	33,3	4,5684	**338,16**
2,699	76,7	5,7200	
3,000	100,0	8,7190	

Based on Tables 4, 5, and 6, the LC50 values of h-hexane, ethyl acetate, and chloroform fractions were 220.47 ppm, 249.13 ppm, and 338.16 ppm, respectively. This indicates that these concentrations are required to cause the death of 50% of the total shrimp larvae. When referring to Table 2, the LC_{50} value that has been obtained is included in the toxic category as initial screening information of the toxicity properties of leaf fraction samples in n-hexane, ethyl acetate, and chloroform solvents can be recommended as candidates containing anticancer drug compounds. Tests on cancer cell lines as a follow-up test to the cytotoxic test results that have been obtained still need to be done. This is the basis for determining the potential of elo plants as candidates for anticancer drug

compounds. Based on qualitative phytochemical tests that have been carried out, it is suspected that the toxic properties given can be influenced by the presence of certain organic compound functional groups, such as hydroxy (OH) functional groups and nitrogenous bases on amine (NH2) functional groups. The active secondary metabolite compounds in the sample can cause the death of shrimp larvae. These active compounds can be toxic to larvae, thus disrupting their digestive system (Yani et al., 2023).

CONCLUSIONS

Based on the results of the research that has been done, it can be concluded that the content of secondary metabolite compounds contained in extracts and fractions of elo plant leaves is in the form of flavonoid compounds, saponins, and alkaloids (Table 1). The LC_{50} value of elo plant leaf extract obtained was 91.76 ppm; leaf n-hexane fraction was 220.47 ppm; leaf ethyl acetate fraction was 249.13 ppm; and leaf chloroform fraction was 338.16 ppm. Based on the LC_{50} value, it shows that the leaf extract and fraction of the elo plant have toxic potential.

ACKNOWLEDGMENTS

Thanks to the Center for Research and Scientific Publications (LP2M) Mataram State Islamic University which has provided funds for the completion of this research and to all other parties who have helped researchers.

REFERENCES

Abusufyan, S., Ibrahim, M., & Mohib, K. 2018. Comparative in Vitro Antidiabetic and Antioxidant Activity of Various Extracts of Ficus Species. *Pharmacognosy Journal*, 10(2), 349–354. DOI: 10.5530/pj.2018.2.59

Ahmed, F., & Urooj, A. 2010. Traditional Uses, Medicinal Properties, and Phytopharmacology of Ficus Racemosa: A review. *Pharmaceutical Biology*, 48(6), 672–681. DOI: 10.3109/13880200903241861

Albarano, L., Ruocco, N., Lofrano, G., Guida, M., & Libralato, G. 2022. Genotoxicity in Artemia spp.: An Old Model with New Sensitive Endpoints. *Aquatic Toxicology*, 252. DOI: 10.1016/j.aquatox.2022.106320

Al-Shabibi, M. H. S., Al-Touby, S. S. J., & Hossain, M. A. 2022. Isolation, Characterization and Prediction of Biologically Active Glycoside Compounds Quercetin-3-Rutinoside From the Fruits of Ficus Sycomorus. *Carbohydrate Research*, 511. DOI: 10.1016/j.carres.2021.108483

Antonyan, A., Sharoyan, S., Harutyunyan, H., Barboni, L., Lupidi, G., & Mardanyan, S. 2016. Protection of Hippocampal and Islet Beta Cells In Vitro by Emodin From Leaves of Rumex Confertus. *International Journal of Pharmacognosy*, 3(10), 437-444.

Carabelly, A. N., & Aspriyanto, D. 2020. Pythochemical Screening of Musa Acuminata Stem Water Extract. *Dentino: Jurnal Kedokteran Gigi*, 5(1), 77. DOI: 10.20527/dentino.v5i1.8127

Chan, W., Shaughnessy, A. E. P., Van Den Berg, C. P., Garson, M. J., & Cheney, K. L. 2021. The Validity of Brine Shrimp (Artemia Sp.) Toxicity Assays to Assess the Ecological Function of Marine Natural Products. *Journal of Chemical Ecology*, 47, 834–846. DOI: 10.1007/s10886-021-01264-z/Published

Clemen-Pascual, L. M., Macahig, R. A. S., & Rojas, N. R. L. 2022. Comparative Toxicity, Phytochemistry, and Use of 53 Philippine Medicinal Plants. *Toxicology Reports*, 9, 22–35. DOI: 10.1016/j.toxrep.2021.12.002

Farahani, Z. K. (2021). The Effect of Extraction Method (Ultrasonic, Maceration and Soxhlet) and Solvent Type on The Extraction Rate of Phenolic Compounds and Extraction Efficiency of Arctium Lappa L. Roots and Polygonum Aviculare L. Grass. *Food & Health,* 4(2), 28-34.

Firnanda, F., Herupradoto, E. B. A., Rahmawati, K., Kurnijasanti, R., Sukmanadi, M., & Hidajati, N. 2021. Toxicity Testing of White Pomegranate (Punica granatum L.) Fruit Extracts Using Brine Shrimp Lethality Test Method as A Candidate of Anti-Cancer Drug. *Journal of Basic Medical Veterinary*, 10(2), 45-50.

Fecker, R., Buda, V., Alexa, E., Avram, S., Pavel, I. Z., Muntean, D., ... & Danciu, C. 2020. Phytochemical and Biological Screening of Oenothera Biennis L. Hydroalcoholic Extract. *Biomolecules*, 10(6), 818.

Golla, U. R., Gajam, P. K., Mohammad, A. R., Kumar, K. A., & Raj, B. S. S. 2011. Assessment of Bioactivity of Desmostachya Bipinnata (L.) Stapf Using Brine Shrimp (Artemia Salina) Lethality Assay. *Pharmacologyonline*, 3, 982-990.

Jain, R., Rawat, S., & Jain, S. C. 2013. Phytochemicals and Antioxidant Evaluation of Ficus Racemosa Root Bark. *Journal of Pharmacy Research*, 6(6), 615–619. DOI: 10.1016/j.jopr.2013.06.004

K, K., & AN, A. 2018. The Phytoconstituents and Pharmacological Actions of Ficus racemosa Linn (Family: Moraceae) - An updated review. *PharmaTutor*, 6(12), 55. DOI: 10.29161/pt.v6.i12.2018.55

Khan, A., Afzal, M., Rasool, K., Ameen, M., & Qureshi, N. A. 2023. In-vivo Anticoccidial Efficacy of Green Synthesized Iron-Oxide Nanoparticles Using Ficus Racemosa Linn Leaf Extract. (Moraceae) Against Emeria Tenella Infection in Broiler Chicks. *Veterinary Parasitology*, 321. DOI: 10.1016/j.vetpar.2023.110003

Kumar Dubey, P., Yaduwanshi, P., & Gouttam, V. 2018. Ficus Racemosa Lin-Gooler A RevieW. *Prashant et al. World Journal of Pharmaceutical Research*, 7, 325. DOI: 10.20959/wjpr201812-12653

Kusmana, C., & Hikmat, A. 2015. The biodiversity of flora in Indonesia. *Journal of Natural Resources and Environmental Management*, 5(2), 187-198.

Marinho, T. A., Oliveira, M. G., Menezes-Filho, A. C. P., Castro, C. F. S., Oliveira, I. M. M., Borges, L. L., Melo-Reis, P. R., & Silva-Jr, N. J. 2022. Phytochemical characterization, and antioxidant and antibacterial activities of the hydroethanolic extract of anadenanthera peregrina stem bark. *Brazilian Journal of Biology*, 82. DOI: 10.1590/1519-6984.234476

Marliza, H., & Oktaviani, D. 2021. Uji Sitotoksik Ekstrak Etanol Daun Kemumu (Colacasia Gigantea Hook. F) dengan Metode Brine Shrimp Lethality Test (BSLT). *Bencoolen Journal of Pharmacy*, 1(1), 38-45.

Musyarrifah, M., Ilyas, A., & Baharuddin, M. 2014. Identifikasi Metabolit Sekunder Ekstrak Etil Asetat Biji Alpukat (Persea americana Mill.) dan Uji Toksisitas Terhadap Larva Udang Artemia salina Leach. *Al-Kimia*, 2(1), 45-51.

Mohiuddin, A. K. 2020. Phytochemical Screening & Biological Investigations of Ficus Racemosa. *Open Access Journal of Complementary & Alternative Medicine*, 2(4). DOI: 10.32474/oajcam.2020.02.000145

Muthukrishnan, S., Senthil Kumar, T., & Rao, M. V. 2017. Anticancer Activity of Biogenic Nanosilver and its Toxicity Assessment on Artemia Salina- Evaluation of Mortality, Accumulation and Elimination: An experimental report. *Journal of Environmental Chemical Engineering*, 5(2), 1685–1695. DOI: 10.1016/j.jece.2017.03.004

Ntungwe N, E., Domínguez-Martín, E. M., Roberto, A., Tavares, J., Isca, V. M. S., Pereira, P., Cebola, M.-J., & Rijo, P. 2020. Artemia species: An Important Tool to Screen General Toxicity Samples. *Current Pharmaceutical Design*, 26(24), 2892–2908. DOI: 10.2174/1381612826666200406083035

Ojah, E. O., Oladele, E. O., & Chukwuemeka, P. 2021. Phytochemical and Antibacterial Properties of Root Extracts from Portulaca Oleracea Linn. (Purslane) Utilised in The Management of Diseases in Nigeria. *Journal of Medicinal Plants for Economic Development*, 5(1). DOI: 10.4102/jomped.v5i1.103

Pahari, N., Majumdar, S., Karati, D., & Mazumder, R. 2022. Exploring the Pharmacognostic Properties and Pharmacological Activities of Phytocompounds Present in Ficus Racemosa Linn.: A concise review. *Pharmacological Research - Modern Chinese Medicine*, 4. DOI: 10.1016/j.prmcm.2022.100137

Parbuntari, H., Prestica, Y., Gunawan, R., Nurman, M. N., & Adella, F. 2018. Preliminary Phytochemical Screening (Qualitative Analysis) of Cacao Leaves (Theobroma cacao L.). *EKSAKTA: Berkala Ilmiah Bidang MIPA*, 19(2), 40-45.

Permana, R., & Andhikawati, A. 2021. Secondary Metabolites Identification and Antioxidant Activity Determination of Crude Extract of Barringtonia asiatica L.(kurz) Leaves. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, 8(3), 51-58.

Rahamouz-Haghighi, S., Bagheri, K., Sharafi, A., Tavakolizadeh, M., & Mohsen-Pour, N. 2022. Phytochemical screening and Cytotoxicity assessment of Plantago lanceolata L. Root Extracts on Colorectal Cancer Cell Lines and Brine Shrimp Larvae and Determination of The Median Lethal Dose in Mice. *South African Journal of Botany*, 149, 740–747. DOI: 10.1016/j.sajb.2022.06.058

Sharma, H., Pathak, R., Jain, S., Bhandari, M., Mishra, R., Reena, K., & Varshney, P. 2023. Ficus Racemosa L: A Review on Its Important Medicinal Uses, Phytochemicals and Biological Activities. *Journal of Population Therapeutics and Clinical Pharmacology*, 30(17), 213-227.

Shiksharthi, A. R., & Mittal, S. 2011. Ficus Racemosa: Phytochemistr y, Tr aditional Uses and Phar macological Pr oper ties: A Review. *International Journal of Recent Advances in Pharmaceutical Research*, 4, 6–15.

Singh, R., Ali, A., Jeyabalan, G., Kakar, S., & Semwal, A. 2013. Development of quality control parameters for the standardization of fruit of Ficus racemosa Linn. (M). *Journal of Acute Disease*, 2(3), 207–213. DOI: 10.1016/s2221-6189(13)60128-6

Sivakumar, P., Manimekalai, I., Sivakumari, K., & Ashok, K. 2019. Anticancer effect of fig fruit Ficus racimosa extract against human hepatocellular canrcinoma (HepG-2) cell line. *Int. J. Res. Anal. Rev*, 6(1), 767-783.

Stamenković, J., Đorđević, A., Stojanović, G., Mitić, V., & Petrović, G. 2021. Phytochemical Analysis of Volatiles and Biological Activities of Chaerophyllum bulbosum L. Essential Oils. *Journal of the Serbian Chemical Society*, 86(3), 257-267.

Sukandar, D., Hermanto, S., & Lestari, E. 2009. Uji Potensi Aktivitas Anti Kanker Ekstrak Daun Pandan Wangi (Pandanus amaryllifolius Roxb.) dengan Metode Brine Shrimp Lethality Test (BSLT). *Jurnal Kimia Terapan Indonesia*, 11(1), 32-38.

Suryani, N., Munawar, F., & Hajaroh, S. 2022. Phytochemical Screening of Active Secondary Metabolites and Antibacterial Activity Kaffir Lime Leaf (Citrus hystrix) and Tumeric Leaf (Curcuma longa Linn.) Against Escherichia coli. *ALKIMIA: Jurnal Ilmu Kimia Dan Terapan*, 5(2), 150–158. DOI: 10.19109/alkimia.v5i2.11264

Suryawanshi, V. S., Yadav, A. R., Mohite, S. K., & Magdum, C. S. 2020. Toxicological Assessment using Brine Shrimp Lethality Assay and Antimicrobial Activity of Capparis Grandis. *Journal of University of Shanghai for Science and Technology,* 22(11), 746-759.

Twaij, B. M., & Hasan, M. N. 2022. Bioactive Secondary Metabolites from Plant Sources: Types, Synthesis, and Their Therapeutic Uses. *International Journal of Plant Biology*, 13(1), 4–14. DOI:10.3390/ijpb13010003

Uswatun, U., & Wijayanti, E. D. 2020. Toksisitas Akut Kombucha Daun Tin (Ficus Carica) Dengan Metode Brine Shrimp Lethality Test (Bslt). *Jurnal Farmasi Medica/Pharmacy Medical Journal (PMJ)*, 3(1), 11-15.

Vera Zambrano, M., Dutta, B., Mercer, D. G., MacLean, H. L., & Touchie, M. F. (2019). Assessment of Moisture Content Measurement Methods of Dried Food Products in Small-Scale Operations in Developing Countries: A review. *Trends in Food Science and Technology*, 88, 484–496. DOI: 10.1016/j.tifs.2019.04.006

Yani, D. F., Ramadan, N., Athiah, R., Maghpiroh, A., & Sunarsih, T. 2023. UJI Toksisitas Ekstrak Etanol Daun Kerai Payung (Filicium decipiens) dengan Metode Brine Shrimp Lethality Test (BSLT). *Spin Jurnal Kimia & Pendidikan Kimia*, 5(1), 27-36.

Zamzami, A. D. R. A., Isnaini, I., & Yasmina, A. 2021. Uji Toksisitas Ekstrak Metanol Kulit Kayu dan Daun Galam dengan Metode BSLT. *Homeostasis*, 4(1), 43-48.