Hendri Satria Kamal Uyun *et al*, International Journal of Pharmaceutical Sciences & Medicine (IJPSM), Vol.8 Issue. 2, February- 2023, pg. 1-5

ISSN: 2519-9889
Impact Factor: 5.721

Cytotoxic Activity Ethanol Extract Rind of Arumanis Mango (*Mangifera indica* Linn.) and Ethanol Extract Rind of Kweni Mango (*Mangifera odorata* Griff.) by Brine Shrimp Lethality Test Method

Hendri Satria Kamal Uyun[1*]; Dwi Dinni Aulia Bakhtra[1]; Anzharni Fajrina[1]; Ica Bela Octavia[1]; Meilinda Mustika[1]

[1]School of Pharmaceutical Science (STIFARM) Padang, Indonesia
Email: hendrisatria@stifarm-padang.ac.id

DOI: 10.47760/ijpsm.2023.v08i02.001

Abstract

Mango is a plant that has many health benefits such as helping the body to ward off free radicals. Research on the cytotoxic test of the ethanol extract rind of arumanis mango (*Mangifera indica* Linn.) and rind of kweni mango rind (*Mangifera odorata* Griff.) with the brine shrimp lethality test method was to determine the cytotoxic activity of the rind. Simplicia powder of mango arumanis rind and mango kweni rind was macerated with 70% ethanol. Cytotoxic testing used various concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 100 µg/mL, 50 µg/mL. From the results of phytochemical screening, the ethanol extract of the arumanis mango rind contains alkaloids, flavonoids, phenols, tannins, terpenoids, and ethanol extract of kweni mango rind (Mangifera odorata Griff.) Containing alkaloids, saponins, flavonoids, phenols, tannins, and terpenoids. The results of the cytotoxic test obtained LC_{50} values of 41.6869 µg/mL for arumanis mango and 29.4238 µg/mL for kweni mango with a very toxic category of <30 µg/mL and toxic of 30-1000 µg/mL. From the results of the study, it can be concluded that the ethanol extract rind of arumanis mango and the ethanol extract rind of kweni mango have cytotoxic activity.

Keywords: Rind, *Mangifera indica* Linn., *Mangifera odorata* Griff, cytotoxic activity, Brine Shrimp Lethality Test

1. Introduction

Arumanis mango (Mangifera indica Linn.) is a plant that has many benefits for the health of the body, mangoes have good vitamin A, where vitamin A is needed to maintain healthy skin. In addition, mangoes have vitamin B6, vitamin C, and vitamin E. Consuming foods rich in vitamin C helps the body to ward off free radicals (Maldonado, et al., 2019).

Secondary metabolites found in arumanis mango (Mangifera indica Linn.) are alkaloids, tannins, steroids, saponins, terpenoids, and flavonoids. Flavonoids in the skin of the arumanis mango showed a three-fold higher amount compared to the flesh of the arumanis mango (Kim et al., 2010). Flavonoids in arumanis mango have biological activities such as anti-microbial, anti-oxidant and anti-cancer (Ajila et al., 2007).

ISSN: 2519-9889
Impact Factor: 5.721

Based on the research results of Chowdhury et al., (2017) stated that the results of the Brine Shrimp Lethality Test (BSLT) from the methanol extract of arumanis mango skin had an LC50 value of 2.04 µg/mL which indicated a high cytotoxic effect. The results of Abdullah et al., (2014) showed that the ethanol extract of Mangifera indica seeds had anticancer activity on MCF-7 and MDA-MB-231 cells with IC50 values of 15 µg/mL and 30 µg/mL.

One method for testing cytotoxic activity is by using the Brine Shrimp Lethality Test (BSLT) method. The Brine Shrimp Lethality Test is a screening method for test material that uses Artemia salina Leach which is an animal that has high sensitivity, where shrimp larvae have thin skin, so that the death of a larva due to the cytotoxic effect of bioactive compounds can be analogous to the death of a cell in an organism. (Fenton & Longo, 2011). From a literature search, it is known that relatively few have examined the cytotoxic test of the ethanol extract of arumanis mango rind (Mangifera indica Linn.) and kweni mango rind (Mangifera odorata Griff.)

2. Material

Arumanis manggo (Mangifera indica Linn.), Kwenni (Mangifera odorata Griff.), hexane (Brataco), aquadest (Brataco), seawater, dimethyl sulfoxide (DMSO) (Merck), hydrochloric acid (HCl) (Merck), mercury (II) chloride (HgCL 2) (Merck), bismuth nitrate (Merck), chloroform (Brataco), nitric acid (Merck), potassium iodide (KI) (Merck), iodine (Merck), Ferric chloride ($FeCl_3$) (Merck), ethyl acetate (Brataco),magnesium (Merck) powder, Acetic anhydrous (Merck), sulfuric acid (H_2SO_4) (Merck), TLC plate (60F 254), shrimp eggs (Artemia Salina Leach)

3. Method

3.1Collection of Arumanis mango (Mangifera indica Linn.) and Kweni mango (Mangifera odorata Griff.)

Arumanis mango plants (Mangifera indica Linn.) were taken in the Griya Durian Ratus Housing area, Kurao Pagang, Nanggalo District, Padang City, West Sumatra Province. The part of the plant taken was 8 kg of arumanis mango (Mangifera indica Linn.). Kweni mangoes (Mangifera odorata Griff.) were collected from the Unib Rear area, Muara Bangkahulu District, Bengkulu City, Bengkulu Province. The part of the plant taken was 8 kg of kweni mango (Mangifera odorata Griff.).

3.2 Extract Preparation

Arumanis mango rind powder (Mangifera indica Linn.) and kweni mango rind powder (Mangifera odorata Griff.) were weighed as much as 200 grams, macerated by soaking simplicia using 70% ethanol solvent as much as 2 liters, soaked for the first 6 hours while stirring occasionally. , then let stand for 18 hours. The macerate is separated by filtration, the extraction process is repeated 3 times, using the same type and amount of solvent. All maserate is collected, then evaporated using a rotary evaporator (Ministry of Health of the Republic of Indonesia, 2017).

3.3 Phytochemical screening of Mango Rind

1. Test for Phenolic

2 mL of the extract was put into a test tube, a solution of iron (III) chloride was adde,d and a change in the color of the solution was observed. If a blue or black-purple solution is formed, it indicates the presence of phenolic compounds (Harbone, 1987)

2. Test for Flavonoid

2 mL of extract was put into a test tube, then it was added concentrated hydrochloric acid and a few grains of magnesium powder. The formation of orange to red indicates the presence of flavonoid compounds (Harbone, 1987)

3. Test for Alkaloid

2 mL of extract was put into a test tube, added 2 mL of HCl and 1 mL of Mayer reagents. If the solution becomes turbid or the formation of white precipitate, it indicates the presence of alkaloid compounds (Harbone,1987).

ISSN: 2519-9889
Impact Factor: 5.721

4. Test for Saponin

2 mL of extract was put into a test tube and shaken. Then a few drops of concentrated hydrochloric acid were added. If foam forms and does not disappear after the addition of concentrated hydrochloric acid, it indicates the solution contains saponin compounds (Harbone, 1987).

5. Test for Triterpenoid and Steroid

Triterpenoid and steroid tests were carried out by dripping the extract on a drop plate, then adding concentrated sulfuric acid and acetic anhydride. If red or purple color is formed, indicates the sample contains triterpenoid compounds and if a green or blue-green ring is formed, indicates the sample contains steroid compounds (Harbone, 1987).

3.4 Testing Cytotoxic Test

The extract was weighed as much as 80 mg, then dissolved in 8 mL of ethanol. This solution is a mother liquor of 10,000 ppm. The test was carried out by means of 5 concentration variations, namely 1000, 500 and 250, 100, 50 ppm then each concentration was made in 3 copies. The test solution was prepared by pipetting each 500 µL, 250 µL, 125 µL, 50 µL, and 50 µL from the main solution, then the solution was left for 24 hours to evaporate the solvent. The solution was then added with seawater up to a final volume of 5 ml and 10 Artemia salina larvae were added to each. After 24 hours, the dead larvae are counted, and once the percent is known, the LC_{50} value is determined (Darweni, 2015).

4. Result and Discussion

The results of the phytochemical test of the ethanol extract of arumanis mango rind showed that the extract contained alkaloids, flavonoids, phenols, and terpenoids. While the results of the phytochemical test of the ethanol extract of kweni mango contain alkaloids, saponins, flavonoids, phenols, and terpenoids. Phytochemical screening aims to determine the presence of secondary metabolites contained in the extract. Phytochemical screening result see in Table 1.

Table 1. Phytochemical screening result

Number	Secondary metabolite	Reagent	Observation	Ethanol Extract Rind of Manggo Arumanis	Ethanol Extract Rind of Manggo Kweni
1	alkaloid	Mayer	White precipitation is formed	+	+
2	Flavonoid	Shianode test	An orange-red solution is formed	+	+
3	Phenolic	FeCl3	A blue or black-purple solution is formed	+	+
4	Saponin	H2O/ HCl concentrate	Formed foam does not disappear with the addition of concentrated chloric acid	–	+
5	Triterpenoid	Liebermann Burchard	A red or purple ring is formed	+	+
6	Steroid	Liebermann Burchard	A green/Green-blue ring is not formed	-	–

ISSN: 2519-9889
Impact Factor: 5.721

The ethanol extract of arumanis mango rind (Mangifera indica Linn.) and the ethanol extract of kweni mango rind (Mangifera odorata Griff.) were tested for cytotoxicity using various concentrations, namely 1000 µg/mL, 500 µg/mL, 250 µg/mL , 100 µg/mL, 50 µg/mL and was repeated three times. The solvent used to dissolve the extract is Dimethyl Sulfoxide (DMSO), which is a solvent that can dissolve almost all polar, semipolar and nonpolar compounds and the use of DMSO has no effect on cell proliferation (Ersita & Kardewi, 2016).

In the results of the research that was carried out, the LC50 value obtained from the ethanol extract of arumanis mango (Mangifera indica Linn.) was 41.6869 µg/mL in the toxic category. Meanwhile, the LC50 value obtained from the ethanol extract of kweni mango rind (Mangifera odorata Griff.) was 29.4238 µg/mL with a very toxic category. According to (Meyer et al., 1982) the LC50 value <30 µg/mL is in the very toxic category, 30 -1000µg/mL is in the toxic category, >1000 µg/mL is not toxic. Cytotoxic Test Results (LC_{50}) see in Table 2.

It is suspected that the secondary metabolites of the ethanol extract of arumanis mango rind (Mangifera indica Linn.) and the ethanol extract of kweni mango rind (Mangifera odorata Griff.) which contain flavonoid compounds have cytotoxic activity against artemia salina leach larvae present in the ethanol extract of mango rind arumanis (Mangifera indica Linn.) and ethanol extract of kweni mango rind (Mangifera odorata Griff.) are derived from flavonoid compounds. The class of flavonoid compounds has a mechanism as an anticancer because flavonoids act as antioxidants through the mechanism of activating the apoptotic pathway of cancer cells. The mechanism of cell apoptosis in this theory is due to DNA fragmentation. This fragmentation begins with the release of the proximal DNA chain by reactive oxygen compounds such as hydroxyl radicals. Another effect is that flavonoids act as inhibitors of tumor or cancer proliferation, one of which is by inhibiting protein kinase activity thereby inhibiting the signal transduction pathway from the membrane to the cell nucleus. Flavonoids inhibit receptor tyrosine kinase activity because receptor kinase activity increases and contributes to cancer cell malignancy (Ren et al., 2003).

Table 2. Cytotoxic Test Results (LC_{50})

Number	Sample Extract	Concentration (µg/mL)	Repetition			Percentage of shrimp larvae mortality (%)	LC_{50} (µg/mL)
1	Manggo Arumanis	1000	9	8	6	76.7 %	41.6869
		500	7	8	5	66.7 %	
		250	6	6	7	63.3%	
		100	5	7	5	56.7%	
		50	5	6	5	53.3 %	
2	Manggo Kweni	1000	9	10	10	96.7%	29.4238
		500	9	9	*8*	86.7%	
		250	*8*	*9*	*7*	80.0%	
		100	*8*	*7*	*8*	76.7%	
		50	*7*	*6*	*5*	60.0%	

ISSN: 2519-9889
Impact Factor: 5.721

5. Conclusion

The ethanol extract of arumanis mango rind (Mangifera indica Linn.) and the ethanol extract of kweni mango rind (Mangifera odorata Griff.) have cytotoxic activity using the Brine Shrimp Lethality Test. The LC_{50} value of the ethanol extract of mango arumanis rind (Mangifera indica Linn.) was 41.6869 µg/mL and the ethanol extract of mango kweni rind (Mangifera odorata Griff.) was 29.4238 µg/mL.

References

[1]. Ajila, C. M., Naidu, K. A., Bhat, S. G., Prasada Rao, U. J. S., (2007) Bioactive Coumponds and Antioxidant Potential of *Mangifera indica* Rind Extract. *Food Chemistry*. 105: 982-988

[2]. Chowdhury, S., Poddar, S. K., Zaheen, S., Noor, F. A., Najneen Ahmed, N., Haque, S., Sukul, A., Sunjida, S. B., Mazumder, Md. M. U., & Akbar, N., (2017). Phytochemical Screening And Evaluation of Cytotoxic And Hypoglycemic Properties of *Mangifera indica* Rinds. *Asian Pac J Trop Biomed* 7(1), 49–52.

[3]. Darweni, Yul Tri., (2015). Toxicity Test of Cinnamon Bark Essential Oil (Cinnamomum burmanii BI.) Against Artemia Salina Leach Larvae. With the Brine Shrimp Lethality Test (BSLT) Method. Surakarta : Digilib UNS

[4]. Ersita & Kardewi. (2016). Antibacterial effectiveness test of the active fraction of soursop leaves (Anona Murikata Linn) against Escherichia coli bacteria. Bina Husada Health Journal, 11, (4), 1-9

[5]. Fenton, R. G., & Longo, D. L. (2011). *Cancer Cell Biology & Angiogenesis. Harrison'sprinciples Of Internal Medicine* (18th ed.). New York: Mc Graw-Hill Medicinal Publishing Division.

[6]. Harbone, J.B. 1987. Phytochemical Methods: Guiding Modern Ways of AnalyzingPlant. Edition 4. Translation by Kosasih P., and Soediro L. Bandung:Bandung Institute of Technology

[7]. Maldonaldo, M. E., Yahya E. M., Bedoya. R., Landazuri, P., Loango, N., Restrepo B., & Ospina, J. C. G., (2019). Chemical Composition of Mango (*Mangifera indica* L.) Fruit: Nutrional and Phytochemical Compounds. *Frontiers in Plant Science* 10(1), 52-73.

[8]. Ministry of Health of the Republic of Indonesia, (2017). Indonesian Herbal Pharmacopoeia. (Edition 1). Jakarta: Directorate General of Drug and Food Control, Directorate of Traditional Medicine Control.

[9]. Kim, H., Yong, J,. Kim, H., Lee, D., Cho, M., Choi, H., Suk, Y., Mosaddik, A., & Kim, S., (2010). Antioxidant and Antiproliperative Activities of mango (*Mangifera indica* L.) Flesh and Rind . *Food Chemistry*., 121(2), 429-436.

[10]. Ren, W., Qiao, Z., Wang, H., Zhu, L., & Zhang, L. (2003). Flavonoids: promising anticancer agents. *Medicinal Research Reviews*, 23(4), 519–534.

