Osmoregulation and Toxicity Test Ethanolic Extracts of Andaliman Leaves (*Zanthoxylum acanthopodium* D. C.) Against Physiological of Goldfish (*Cyprinus carpio* L.)

M. S. Batubara[1*], N. Ginting[1], I. Safri[1], S. Tua[1] and K. Umar[2]

[1] Biology Study Program, Universitas Muhammadiyah Tapanuli Selatan, Padangsidimpuan, Indonesia

[2] Mathematics Education Study Program, Universitas Muhammadiyah Tapanuli Selatan, Padangsidimpuan, Indonesia

*melvarianisyari@um-tapsel.ac.id

Abstract. The purpose of this study was to determine the osmoregulation of goldfish (Cyprinus carpio L.) treated with ethanolic extracts of andaliman leaves (Zanthoxylum acanthopodium DC) and to determine the toxicity level of ethanolic extracts of andaliman leaves (Zanthoxylum acanthopodium DC) on physiological goldfish (Cyprinus carpio L.). The method used in this study is included in experimental research with a significant correlation method to determine osmoregulation and toxicity testing ethanolic extracts of andaliman leaves (Zanthoxylum acanthopodium D. C.) on physiological goldfish (Cyprinus carpio L.). Measurements are made once at the same time. Quantitative chemical test results of the ethanol extract of andaliman leaves (Zanthoxylum acanthopodium D. C.) are phenol, hydroquinone, and amide compound. The results of this study indicate that there was a decrease in body weight of goldfish samples treated with ethanolic extracts of andaliman leaves compared with controls, and statistically showed significantly different. Then the results were also obtained that the higher the dose ethanolic extracts of andaliman leaves was given, the more the number of dead goldfish samples, where the 100% mortality dose was > 0.25 g/100 ml distilled water, and the length of time the goldfish died was also faster at time is 1-4 hours. For data on the number of erythrocyte goldfish samples that were treated with ethanolic extracts of andaliman leaves also decreased compared to controls, but statistically showed results that were not significantly different.

1. Introduction

Andaliman plant (*Zanthoxylum acanthopodium* D. C.), is a herbaceous plant of the family Rutaceae that is often found in North Sumatra, and its fruit is widely used as a spice for traditional cooking by the Batak tribe [1] [2]. Information was obtained that the Dairi community used Andaliman leaves as a tuba to make it easier to catch fish, so the fish fainted. Thus, based on empirical observations in the field of andaliman leaves are toxic to the digestive system of fish. Besides that, the Malay people have also used andaliman leaves as a spice in cooking and deodorizing fish and meat. But until now there has been no information about the use of andaliman leaves scientifically [3].

The people of Dairi Regency in particular recognize two types of andaliman plants as "tuba sihorbo" and "tuba siparjolo". The difference is in the length of the mother flower stalk or fruit. Mother stem "tuba sihorbo" is relatively shorter than "tuba siparjolo". Sometimes the length of a series of "tuba sihorbo" fruit is shorter than the sticky thorn found on the stem or branch and this usually makes it difficult for farmers to harvest. The fragrance of the two types is also different. "Tuba sihorbo" has the nature of retention or bitter taste that is longer lost and more spicy than "tuba siparjolo". This research is still being conducted in a limited area and there are still other villages supplying andaliman plants in North Sumatra so that there may still be a diversity of andaliman plants in the area. Therefore, further studies to enrich the germplasm information of andaliman plants are still needed [3].

From the research results it is known that the highest chemical content of andaliman leaf extract is obtained from alkaloids and steroids, while the saponin substance is found in low contents. But this research is still limited to testing the chemical content of andaliman leaf extract qualitatively only, not yet tested quantitatively to determine the concentration of each substance [3]. Alkaloids and saponins are commonly used in medicines. Alkaloids are toxic, while saponins are used to lower the surface tension of body fluids and can demolish blood cells [4].

Currently the andaliman plant is considered to be a source of aromatic compounds and essential oils. The fruit contains aromatic compounds with a characteristic spicy and bitter taste. If eaten, it leaves a trembling effect on the taste buds and causes the tongue to feel invulnerable. Several studies have shown that the terpenoid content in the form of steroids has antioxidant and antimicrobial activity, and also has immunostimulatory effects. This gives an opportunity for andaliman plants as raw material for antioxidant or antimicrobial compounds for the food industry and the pharmaceutical industry [5]

Based on the description above and based on the curiosity of researchers in revealing an investigation of the implications of andaliman leaves to be treated for goldfish, researchers are interested in presenting and choosing research topics namely Osmoregulation and Toxicity Tests Ethanol Extract of Andaliman Leaves (*Zanthoxylum acanthopodium* DC.) on Physiological of Goldfish (*Cyprinus carpio* L.)

2. Methods

This research was included in an experimental study with the method to be used is a significant correlation method to determine osmoregulation and toxicity test of ethanol extract of andaliman leaves (*Zanthoxylum acanthopodium* DC.) on physiological of goldfish (*Cyprinus carpio* L.). Measurements are made once at the same time.

2.1. Making ethanol extract of andaliman leaves

Simplicia drying: andaliman leaves are cleaned, dried and air dried, then blended with a blender. Making of andaliman extract: andaliman leaf powder macerated with 96% ethanol for ± 1 night. The results of maceration and simplicia are isolated until a clear liquid is obtained. The results of the percolation were concentrated with an evaporator until a concentrated extract was obtained [6] [7].

2.2. Research Design

The method used in this study is a non-factorial Completely Randomized Design (CRD) method consisting of 2 groups: the control group and the treatment group. The treatment consisted of 1 factor, namely the dose of the material used.

- Control distilled water at a dose of 0 g/100 ml distilled water (P0)
- Treatment with a dose of 2,500 ppm (0.25 g in 100 ml of distilled water) (P1)
- Treatment with a dose of 5,000 ppm (0.5 g in 100 ml of distilled water) (P2)
- Treatment with a dose of 10,000 ppm (1.0 g in 100 ml of distilled water) (P3)
- Treatment with a dose of 20,000 ppm (2.0 g in 100 ml of distilled water) (P4)
- Treatment with a dose of 40,000 ppm (4.0 g in 100 ml of distilled water) (P5)

The number of repetitions for each treatment group was determined using the Federer formula, namely: $(t-1)(n-1) \geq 15$, where:

t = number of treatments

n = number of repetitions [5].

Provision of test material with a volume of 0.1 ml/10 g body weight is given based on the assumption that the human body weight is 50,000 g with a dose of 200 cc.

2.3. Treatment of Research Animals

2.3.1. Measurement of Fish Osmoregulation Against Ethanol Extract of Andaliman Leaves

Goldfish weighed initially and then put into a glass jar containing a solution of test material in the form of ethanol extract of andaliman leaves. After 15 minutes samples were taken, namely carp from the test solution in the form of ethanol extract of andaliman leaves. Samples namely carp then weighed and recorded the final weight. The treatment was repeated 4 times.

2.3.2. Measurement of Goldfish Toxicity to Ethanol Extract of Andaliman Leaves

Goldfish put into a glass jar containing a solution of test material in the form of ethanol extract of andaliman leaves. After 5 hours it was observed how many samples were dead goldfish. Then note how long each fish died. The treatment was repeated 4 times.

2.4. Examination of Goldfish Blood Given Treatment of Andaliman Leaf Ethanol Extract

To calculate the number of erythrocytes: Goldfish blood is sucked using an erythrocyte pipette to the mark of 0.5 or 1.0. Suction Hayem diluent solution to the mark 101 quickly and without causing air bubbles. The aspirator pipe is released. Stirring is done until the part that is mixed is only the enlarged part of the pipette. A blood suspension is dropped on the edge of the haemositometer and closed with a glass cover. The number of erythrocytes is calculated in the middle box in the middle of the haemositometer. Calculation formula:

$JE = X \times 10 \times Y$

Information

JE = Amount of erythrocytes per mm^3

X = Amount of erythrocytes in 1 intermediate box

Y = Dilution (up to 0.5 = 200, up to 1.0 = 100)

3. Results and Discussion

Table 1. Results of Andaliman Leaf Extract Chemical Test

Substance	% Area	SI
1,3-Benzenediamine	1,51	89
Phenol	3,34	80
Hydroquinone	9,05	86

From Table 1. it can be seen that the highest chemical content of andaliman leaf extract is obtained from phenol, hydroquinone, and amide compounds. Based on the results of spectrometry analysis, it can be identified that the target component is a substituted phenol, quinone, and amide compound. In accordance with IUPAC rules, the name of the target compound is 2-methylpropyl-dodekatetraenamida [5].

Phenol compounds include a variety of compounds derived from plants, which contain one or two hydroxyl boilers. Phenolic compounds tend to dissolve easily in water because generally they often bind to sugar as glycosides, and are usually present in cell vacuoles. Plant phenol compounds can cause great disturbances because of their ability to form complexes with proteins through hydrogen bonds. When plant cell content is mixed and the membrane becomes damaged during the process of isolation, phenol compounds rapidly form complexes with proteins. As a result, there are often obstacles to the action of enzymes in crude plant extracts. Conversely, phenol itself is very sensitive to enzyme oxidation and may be lost in the isolation process due to the action of the phenolase enzyme found in plants. Extraction of plant phenol compounds with boiling ethanol usually prevents enzyme oxidation, and this procedure should be carried out routinely [4].

Phenol-derived compounds have antioxidant activity. Phenolic antioxidants are usually used to prevent damage due to oxidation reactions in food, cosmetics, pharmaceuticals and plastics. The function of phenols as free radical scavengers and binders from damage to metal ions. The group is very soluble in water and fat and can react with vitamins C and E [5].

The colors of natural quinone pigments vary from pale yellow to almost black, and the structures that are known to number are more than 450. Although quinones are widespread and have very diverse structures, their contribution to the color of small relative plants. So, this quinon is often found in the skin, roots, or in other tissues such as leaves, but the tissue is covered in other pigments. Its spread in tall plants has been investigated mainly because of certain anthraquinone, efficacious laxatives. Quinones are colored compounds and have basic chromophores like the chromophores on benzokuinone, which consists of two carbonyl groups conjugating with two carbon double bonds. For identification purposes, quinones can be divided into four groups: benzokuinone, naphthaquinone, anthraquinone, and isoprenoid quinone. The first three groups are usually hydroxylated and are "phenol compounds" and may be present in vivo in the form of a combination with sugar as glycosides or in the form of quinols, sometimes also in the form of dimers. In such case, acid hydrolysis is needed to release its free quinone. Isoprenoid quinones are involved in cellular respiration (ubiquinone) and photosynthesis (plastoquinone) and thus the universe is spread in plants [4].

Biosynthesis with transaminases from suitable aldehydes has been shown to be the main pathway for the synthesis of aliphatic amines in about 50 plants. The most widely distributed plant amines can be very well divided into three groups, namely: aliphatic monoamines, aliphatic polyamines, and aromatic amines. Aliphatic amines are volatile compounds, ranging from simple compounds such as methylamine, to n-hexylamine. These amines are widespread in tall plants and fungi. If there is a high concentration, it gives an unpleasant odor such as the smell of fish. It functions in flowers as an insect puller by mimicking the smell of carcasses. In contrast to monoamines and diamines, polyamine is less volatile even though it still has a pungent odor. Widespread polyamines include putresina, agmatina, spermidina, and spermina. Some of the distribution of diamines is more limited, for example cadaverine. Polyamines are an interesting research material because their activities stimulate growth

associated with influences on ribosomal RNA. The aromatic amine of the plant that is best known is probably meskalina, the active compound from the flowering end (peyote) of the cactus. It is a strong hallucinogenic compound. In fact many aromatic amines have been known to be physiologically active and for this reason they are classified as alkaloids. Three substances that are very important in animal physiology, for example in brain metabolism are noradrenaline, histamine, and serotonin. Noradrenalin, for example, is found in bananas and potatoes. Perhaps the most widespread aromatic amine is thyroid, which has been detected in 50 of the 77 plant tribes [4].

Table 2. Difference in Weight and Amount of Erythrocytes of Goldfish Treated by Andaliman Leaf Extract Treatment

Dose (g)	Parameter	
	Difference of weight (g) Goldfish	Amount of erythrocytes (million cells/mm^3) Goldfish
0	0 a	2,05 a
0,25	20,25 b	0,29 b
0,5	21 b	0,41 b
1	26,75 Ba	0,29 b
2	33 cA	0,20 b
4	42,5 c	0,37 b

From Table 2. it can be seen that the administration of andaliman leaf extract can increase the difference in body weight of goldfish in the treatment group when compared to the control group. In general the administration of andaliman leaf extract can be said to increase the difference in body weight of goldfish, where the higher the dose given, the difference in body weight of goldfish increases. The increase in goldfish body weight difference is due to a decrease in initial body weight compared to the final body weight of goldfish after being treated with andaliman leaf extract. This situation is probably caused by the chemical content of andaliman leaf extract, namely quinone. Quinone compounds are mainly due to certain anthraquinones, which have efficacious laxatives [8].

From Table 2. it can also be seen that administration of andaliman leaf extract can reduce the amount of goldfish erythrocytes in the treatment group when compared to the control group. In general the administration of andaliman leaf extract can be said to reduce the amount of goldfish erythrocytes, where the higher the dose given, the number of goldfish erythrocytes decreases. This situation is probably caused by the chemical content of andaliman leaf extract, namely phenol. Plant phenol compounds can cause great disturbances because of their ability to form complexes with proteins through hydrogen bonds. When plant cell content is mixed and the membrane becomes damaged during the process of isolation, phenol compounds rapidly form complexes with proteins. As a result, there are often obstacles to the action of enzymes in crude plant extracts. This event is often known as the process of hemolysis of blood cells, especially red blood cells or erythrocytes [8]. Hemolysis is the event of the release of cell contents from the red blood cells to the surrounding fluid caused by the rupture of the red blood cell membrane [9].

Table 3. LD and LT of Goldfish Given Treatment of Andaliman Leaf Extracts

% Mortality	Parameter	
	LD (g)	LT (hour)
10	-1,63	0,36
50	0,0012	2,55
90	3,97	4,73

In Table 3. shows the LD50 of ethanol extract of andaliman leaves against goldfish toxicity level is 0.0012 g/100 ml aquadest. Whereas LT50 from goldfish treated with andaliman leaf ethanol extract was 2 hours 55 minutes. In general the administration of ethanol extract of andaliman leaves can be said to be the higher the dose given, the average percentage of goldfish mortality increases and the time of mortality of goldfish is also faster. The higher number of goldfish deaths and the faster goldfish death time is likely due to the dosage and volume of isoprenoid amine compounds because this substance is very important in animal physiology, for example in brain metabolism, in addition it is a strong hallucinogenic compound [10].

4. Conclusion

The conclusions in this study are: (1) Data on osmoregulation of goldfish (*Cyprinus carpio* L.) treated with ethanol extract of andaliman leaves (*Zanthoxylum acanthopodium* DC.), where there is a decrease in sample weight compared to control, and statistically shows the results that significantly different, (2) Data on the level of toxicity of the ethanol extract of andaliman leaves (*Zanthoxylum acanthopodium* DC.) on physiological of goldfish (*Cyprinus carpio* L.), where the higher the extract dose given, the more the number of dead fish samples, where the death dose is 100% is > 0.25 g / 100 ml of distilled water and the time of death of the fish is also getting faster, ie at 1-4 hours. For data on the number of erythrocyte goldfish samples that were treated with andaliman leaf extract also decreased compared to controls, but statistically showed results that were not significantly different.

Aknowledgement

This research was funded by the APB 2018 Universitas Muhammadiyah Tapanuli Selatan. The researcher expressed his appreciation to the Institute of Research and Community Service (LPPM) of the Universitas Muhammadiyah Tapanuli Selatan for technical assistance.

References

[1] L. A. P. Putri, A. O. Y. Sinaga, I. M. S. Sembiring, and A. Sinaga, "Assessing genetic diversity of Indonesian Andaliman (Zanthoxylum acanthopodium DC .) germplasm based on 7 RAPD markers," no. i, pp. 131–133, 2015.

[2] Y. R. Meutia, N. I. A. Wardayanie, Rienoviar, T. Mahardini, and I. Wirawan, "Pengaruh Suhu dan Waktu Maserasi terhadap Komponen Volatil yang Terlibat pada Ekstraksi Andaliman (Zanthoxylum acanthopodium DC)," *J. Agro-based Ind.*, vol. 32, no. 1, pp. 9–15, 2015.

[3] M. S. Batubara, E. Sabri, and M. Tanjung, "PENGARUH PEMBERIAN EKSTRAK ETANOL DAUN ANDALIMAN (Zanthoxylum acanthopodium DC.) TERHADAP GAMBARAN MORFOLOGI OVARIUM MENCIT (Mus musculus L.) STRAIN DDW.," *Klorofil*, vol. 1, no. 1, pp. 5–10, 2017.

[4] R. E. Kristanty and J. Suriawati, "The indonesian Zanthoxylum acanthopodium DC.: Chemical and biological values," *Int. J. PharmTech Res.*, vol. 8, no. 6, pp. 313–321, 2015.

[5] S. Indariani, C. Hanny Wijaya, M. Rahminiwati, and M. Wien Winarno, "Antihyperglycemic activity of functional drinks based on java tea (orthosiphon aristatus) in streptozotocin induced diabetic mice," *Int. Food Res. J.*, vol. 21, no. 1, pp. 349–355, 2014.

[6] M. J. P. Yuni Andriani, Desi Sagita, "Efek Paparan Asap Rokok Pada Model Mencit selama Fase Organogenesis dan Pertumbuhan," *Semnasffua.Com*, pp. 6–7, 2015.

[7] Y. Sari, W. Munir, and Y. Allen, "Uji Teratogenitas Ekstrak Bungo Timah (Peperomia

pellucida L. Kunth.,) Terhadap Organ Viseral Embrio Mencit Putih (Mus musculus L.)," *Biog. J. Ilm. Biol.*, vol. 2, no. 2, pp. 115–118, 2014.

[8] N. W. Diah Wulandari Rousdy, "PROFIL HEMATOLOGI DAN PERTUMBUHAN IKAN MAS (Cyprinus carpio Linn.) PADA PEMBERIAN ASAM HUMAT TANAH GAMBUT KALIMANTAN," pp. 135–144, 2015.

[9] S. Hendry Yanto, Hastiadi Hasan, "Studi Hematologi Untuk Diagnosa Penyakit Ikan Secara Dini di Sentra Produksi Budidaya Ikan Air Tawar Sungai Kapuas Kota Pontianak Hematologycal Study for Fish Disease Early Diagnosis in the Production Center of Freshwater Fish Farming Kapuas River , Ponti," *J. Akuatika Vol.VI*, vol. 2015, no. 1, pp. 11–20, 2015.

[10] R. Fredi Susandi, Mulyana, "Peningkatan Imunitas Benih Ikan Gurame (Osphronemus gouramy Lac.) Terhadap Bakteri Aeromonas hydrophila Menggunakan Rosella (Hibiscus sabdariffa L.). Increased," *J. Chem. Inf. Model.*, vol. 3, no. 2, 2017.