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Compounds and Histopathological Effect of *Terminalia Catappa L.* Leaves Extract for Anti-Bleeding Agent Tooth Extraction in Mice (*Mus Musculus*)

Ni Made Sirat[1], Asep Arifin Senjaya[2]

[1,2] Department of Dental Hygiene, Poltekkes Kemenkes Denpasar, Denpasar, Indonesia

Correspondence: Ni Made Sirat, Department of Dental Hygiene, Poltekkes Kemenkes Denpasar, Denpasar, Indonesia. E-mail: sirat_made@yahoo.com

Abstract
Complications in tooth extraction are such as bleeding, fracture, dry socket, swelling, shock, and several other complications. Ketapang (Terminalia catappa L.) is known to contain flavonoids which play a role in wound healing. The flavonoid content of *T. Catappa L.* can accelerate wound healing in the angiogenesis process by stimulating vascular endothelial growth factors. This study aims to examine the chemical sub-element of *T. Catappa L.* leaves extract and its toxicity by Lethal Dose (LD50) test in mice. This research was experiment in a laboratory of making ethanol extract from *T. Catappa L.* leaves. The LD50 test of ethyl acetate fractionated *T. Catappa L.* leaves extract was carried out on 20 mice divided into 5 treatment groups, namely 1, 2, 4, 8, and 16 g/kg body weight (BW). Graphical analysis of the results of the Liquid chromatography-mass spectrometry (LCMS) test was carried out to determine the detected chemical compounds and histopathology images were obtained. *T. Catappa L.* has tannin and flavonoid compounds. The results of the LD50 test show that no mice were poisoned and died. Histopathological tests on mice that were given a dose of 16gr/kg BW show that the liver has diffuse hydrophilic degeneration, while the normal kidneys have no abnormalities. Ethyl acetate fractionated ethanol extract of *T. Catappa L.* leaves is categorized as non-toxic. Based on the content of tannin and flavonoid compounds, it is suggested to use *T. Catappa L.* leaves as an anti-bleeding agent after tooth extraction.

Keywords: Herbal Plant, Histopathology, Ketapang Leaves, Terminalia Catappa L., Tooth Extraction

1. Introduction

Dental problems are a major challenge as they affect people around the world around 3.5 billion with dental caries dominating (Chandran & Usha, 2024). Dental caries is a pivotal reason for tooth extraction, which has now become the major treatment in dentistry (Rahmadhini & Wahyuni, 2023; Sharif et al., 2020). Tooth extraction often causes bleeding in the gingiva due to the creation of a tooth socket. The bleeding occurrence extends the damage to the tissue and can continue for 1 – 2 days, which causes uncomfortable moments for patients (Soltani et al., 2014). This situation is unavoidable as a consequence of unstable blood clots. Bleeding can cause

complications such as sluggishness, lethargy, and anemia (Ariestiana et al., 2023). Hence, the prevention of bleeding after tooth extraction is indispensable.

Tannins and flavonoids are compounds that contribute to blood clotting (Marcińczyk et al., 2022). Tannin and flavonoids are naturally occurring compounds that can be found in plants (Pouyfung & Sukati, 2021). One of the herbal medicines used by people in Indonesia to treat bleeding is Ketapang leaves. Ketapang leaves also known as Indian almond is a member of the Combretaceae family. It is native to Southeast Asia with the genus of Terminalia and species of T. catappa L . (Habibullah et al., 2023). Ketapang leaves are used by the community as a traditional herb for the treatment of several diseases because they have antimicrobial (Allyn et al., 2018; Dewi & Mardhiyani, 2021), antiparasitic (Nugroho et al., 2016), antibacterial (Terças et al., 2017), anti-inflammatory (Ngemenya et al., 2021), antidiabetic (Iheagwam et al., 2023), antioxidant (Vyn et al., 2020), hepatoprotective (Bhasker Shenoy, 2020), and anticancer activities (Dewi & Mardhiyani, 2021). Although herbal medicine is utilized for pain, it still has potential for harm due to self-medication and low awareness. Therefore, the toxicity of herbal medicine needs to be examined (Yuan et al., 2016).

A study of *T. Catappa L.* by Purwaningsih, et al. (2020) was used to examine the elucidation of the resistor efficacy against the growth of S. Aureus as the bacteria causing gingivitis. The results show that the higher the concentration of the extract (5%), the wider the zone of the resistor area. The extract of *T. Catappa L.* contains tannin, saponin, terpenoid, and flavonoid (Purwaningsih et al., 2020). Leaves of *T. Catappa L.* extract show a good function on wound healing activity than using povidone-iodine and vaseline in mice (Mus musculus) conducted by Nugroho, et al. (2019). Mice were grouped into control, Vaseline, provided iodine group and treatment groups consisting of ethanolic extract either green (GLE) or brown leaves (BLE), and water extract green (GLW) and brown (BLW) leaves of Terminalia catappa L as an ointment. The mice were previously prepared with excision. The mice treated with GLE and GLW were better on day 12. Another research by Nguy, et al (2023) who used *T. Catappa L.* seed oil (TCO) for examining the physiological effects in mice proves that by using a high oral dose of 21780 mg/kg of mice, all instinctive behavior of mice is normal and the survival rate is 100%. Prolonging the intake of TCO makes the mouse coat is thick, soft, and smooth compare to just in short time use (72-h) (Nguy et al., 2023). However, little research was conducted in the integrated evaluation of chemical compounds, toxicity and histopathological effect of *T. Catappa L.* leaves.

The investigation consists of a test on the acute lethal dose (LD50) ethanol extract of *T. Catappa L.* As *T. Catappa L.* is accessible in Indonesia, this plant has the potential for the preparation of bleeding after tooth extraction. Given the benefit of *T. Catappa L.* as a medicine for bleeding, this study aims to investigate the chemical compounds, toxicity, and histopathological effects of its plant in mice (Mus musculus).

2. Method

This research has received approval of ethical clearance No. LB.02.03/EA/KEPK/0254/2022 from KEPK (Ethical Committee) of Poltekkes Kemenkes Denpasar, Bali.

This research method is an experiment in the laboratory with three variables: the existence of chemical compounds, toxicity defined by mice's (Mus musculus) behavior for 2x24 hours after being given *T. Catappa L.* extract, and histopathological abnormality defined by the changes that occurred in the liver and kidneys of mice after 2x24 hours of being given *T. Catappa L.*

2.1 Population and Sample

The Ketapang leaves used in the study were numbered 3 – 6 from the base, at a tree height of 6 meters. Ketapang leaves were picked at about 1 kilogram.

2.2. Steps of Research

The processes that are carried out as shown in Figure 1.

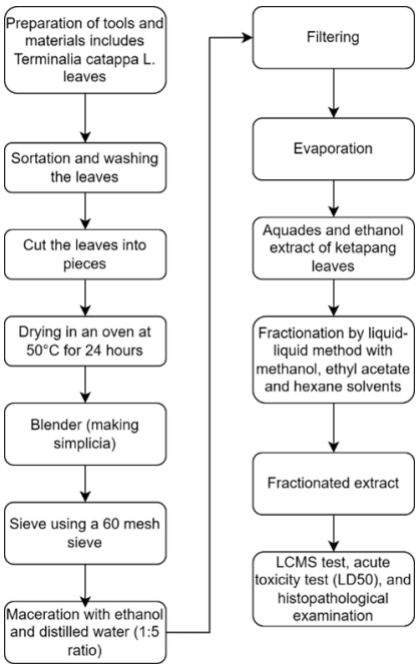


Figure 1: Research flowchart

1) Preparation of Ketapang leaves extract (*T. Catappa L.*)

Before conducting the acute lethal dose (LD50) toxicity test, the researchers prepared the leaf extract to be tested. The weight of Ketapang leaves to be extracted was 1 kilogram and the powder obtained was 500 gr. The active compounds of *T. Catappa L.* In general, will be maximally produced if a polar solvent is used, namely the ethanol extract of Ketapang leaves which is fractionated with ethyl acetate. The mature leaves are used because the older the leaves, the more the effect on the secondary metabolite content. Older leaves are indicated by a dark green color. The leaves were subsequently washed under running water (Sirat & Senjaya, 2021).

2) Making simplicia

The dark green Ketapang leaves were used. Older leaves affect the content of secondary metabolites. The leaves were washed under running water. Next, the Ketapang leaves were cut into smaller pieces. Then it was dried at 50°C for 24 hours. After the leaves dried, the simplicial was made by blending the dried Ketapang leaves. The blended Ketapang leaves were sieved with a 60-mesh sieve.

3) Ethanol and aquadest extract making

Ketapang leaf powder was then macerated with ethanol and distilled water (ratio of 1:5) for 3 days at room temperature. The filtrate was obtained by filtering with Whatman No.1 filter paper. The dregs obtained were then macerated again with 1000 mL of ethanol 2 times (Purwaningsih et al., 2020). The filtrates obtained were combined and then evaporated using a vacuum foam evaporator (Iwaki, Japan) at 400°C. Evaporation results obtained Ketapang leaves ethanol crude extract (*T. Catappa L.*) and Ketapang leaves aquades crude extract (*T. Catappa L.*). Crude extract is obtained in the form of a paste which is assumed at a concentration of 100% (Purwaningsih et al., 2020).The macerate obtained was filtered and evaporated using a rotary evaporator. The rotary evaporator helped the extract not damaged by high temperatures (Dhora, 2017).

4) Fractionation

The ethanol-condensed extract or distilled water condensed extract (coarse extract) of Ketapang leaves was then partitioned using ethyl acetate with a composition of 4 mg of crude extract and 200 ml of ethyl acetate (Purwaningsih et al., 2020). The mixture was shaken in a separatory funnel. Then, it was left for a while until the

ethanol and the hexane phases were seen. The two phases were separated, and the solvent for each phase was evaporated in a vacuum rotary evaporator to obtain ethanol of the ethyl acetate fraction. The ethanol extract of the ethyl acetate fraction of Ketapang leaves obtained was then used as an acute lethal dose (LD50) toxicity test material against mice (Mus musculus).

5) Procedure for acute lethal dose toxicity test (LD50)

The acute lethal dose (LD50) toxicity test procedure was carried out according to applicable regulations of the statute of Indonesia Food and Drugs Agency Supervisor Number 7, 2014 about Guidelines for In Vivo Nonclinical Toxicity Testing (Pedoman Uji Toksisitas Nonklinik Secara In Vivo (Guidelines for In Vivo Nonclinical Toxicity Testing), 2014). This procedure is adopted by researchers with toxicity test studies.(Dhora, 2017; Herli & Wardaniati, 2019; Munira et al., 2018; Pambudi et al., 2015; Purwaningsih et al., 2020). This procedure is as follows (Pambudi et al., 2015).

a. Preparation of extract solution.

Ethyl acetate fractionated ethanol extract was dissolved using 0.3% Na-CMC. Preparation of 0.3% Na-CMC solvent, namely, as much as 300 mg of Na-CMA dissolved with distilled water to a volume of 100 ml. Stir gently until homogeneous.

b. Preparation of extract solution

The dosages used were 1, 2, 4, 8, and 16 g/kg BW Mice (5 treatments). The average weight of mice is 22 grams.

c. Preparation of experimental animals

A total of 20 experimental animals were involved in this research with the following criteria: Balp/c strain mice, male, aged 2 to 2.5 months, weighing 21 to 24 grams were prepared as many as 20. The mice were divided into 5 treatment groups, randomly. Each group consisted of 4 mice. Then labeled/marked in each treatment group to differentiate between groups. The mice were fasted overnight for about 12 – 18 hours before the induction of anesthesia.

d. Treatment preparation

The 5 treatment groups in this research are according to the dose used, i.e. 1) the dose group is 1 g / kg BW, 2) the dose group is 2 g / kg BW, 3) the dose group is 4 g / kg BW, 4) the dose group 8 g / kg BW, 5) The dose group is 16 g / kg BW. As many as experimental animals, each was given 1 cc of extract solution orally (gastric sonde). Then observed for 2 x 24 hours.

6) Procedure for histopathological examination

Experimental animals were euthanized using cervical dislocation techniques. After the experimental animal has died, then disinfect the external abdominal area with 70% alcohol, then perform surgery on the abdominal area. Once the internal organs are exposed, the kidney and liver tissue are taken. Liver and kidney tissues were washed with 0.9% NaCl, then fixed with 10% buffered formalin. (in a 50-cc plastic pot). The tissue was then cut (trimming), with a thickness of 5mm. The tissue that had been cut earlier (specimen) was placed on the embedding cassette, then inserted into the tissue processor with the time setting of fixation NBF 10% 2 hours (Chandran & Usha, 2024; Rahmadhini & Wahyuni, 2023), fixation NBF 10% 2 hours, and dehydration (Sharif et al., 2020): 70% alcohol 2 hours, 95% alcohol 2 hours, 100% alcohol 2 hours, 100% alcohol 3 hours. The next process was clearing using toluol for 3 hours 2 times. Impregnation was performed afterward with paraffin for 2 hours 2 times. The preparations were put into the incubator and left overnight. The preparations were then stained with Harris-Haematoxyllin-eosin dye. After the staining process was complete, an examination was carried out under a microscope with 10x and 40x magnification, to see tissue histopathological changes.

3. Results

UPLC-MS was used for the analysis of the metabolite profile of the ethanol extract of the aquades fraction. UPLC can lower the consumption of mobile phase in the amount of 80%, which is a shorter time than using HPLC. Metabolite profile analysis was commenced by sample injection and it will be entered. The analysis of the metabolite profile of the ethanol extract from the aquades fraction of Ketapang leaves starts with injecting the sample, which is then introduced into the column. C18 column or octadecyl silica was used as the stationary phase. The benefit of using octadecyl silica as the stationary phase is its ability to separate compounds with varying polarities, from low to medium to high (Herli & Wardaniati, 2019).

Masslynx 4.1 application was used to process chromatograms to predict the molecular formula and the content of compounds. The results of the metabolite profile are shown in Figure 2.

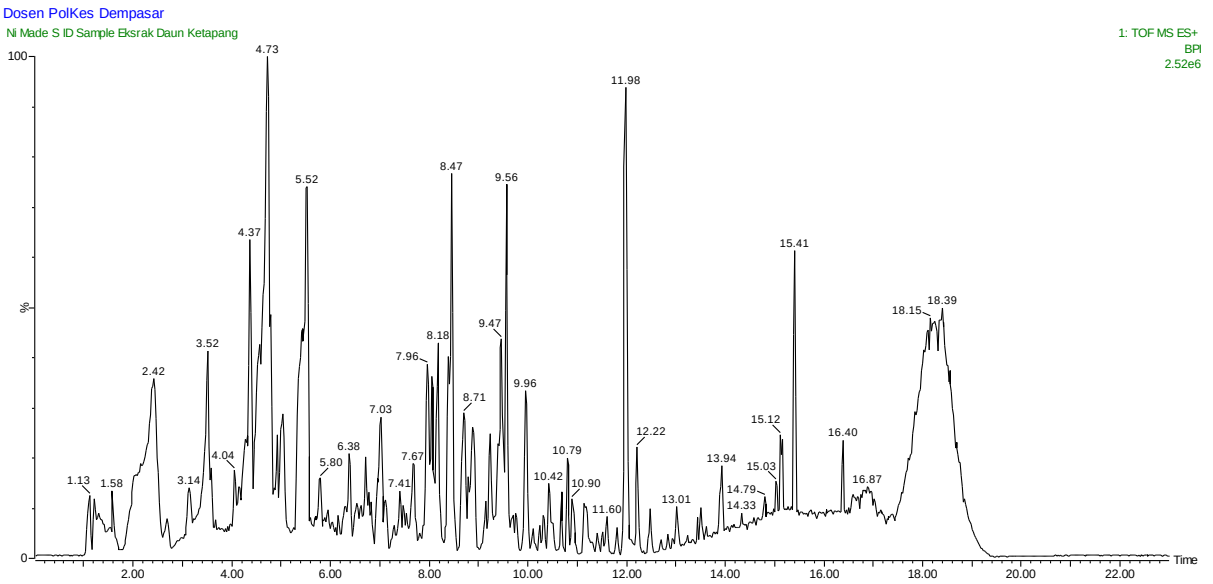


Figure 2: UPLC-MS chromatogram of the ethanol extract of the aquadest fraction of *Terminalia catappa L.*

Each peak in the chromatogram represents a distinct compound. The mass values obtained from the measurements, along with the calculated mass values in the spectra, allow for the prediction of the molecular formula. The measured and calculated mass values must be adjusted by subtracting the mass of one hydrogen atom (1.0078) because the addition of hydrogen atoms occurs during the separation process in the column, as a result of the ESI (+) ionization. The molecular formula is then determined based on the difference between the measured and calculated mass, which is within a ± 0.0005 range. The molecular formula is further confirmed using the www.chemspider.com website.

The results found that there are 19 compounds in the ethanol extract of the aquadest fraction (Table 1).

Table 1: Toxic category

Retention Time	**Measured Mass**	**Calculated Mass**	**Formula**	**Compound**
1.13	151.0352	151.0395	C_8H7O_3	Mandelate
1.58	130.0873	130.0868	$C_6H_{12}NO_2$	6-Aminohexanoate
2.42	120.0814	120.0813	$C_8H_{10}N$	1-Allylpyridinium
3.14	1102.1033	1102.1029	$C_{40}H_{16}N_{25}O_{14}S$	Unknwon
3.52	188.0720	188.0745	$C_8H_{14}NO_2S$	2-methoxy-1-(2-methyl-4H-thiazol-5-yl)propan-1-ol
4.37	449.1087	449.1084	$C_{21}H_{21}O_{11}$	Cyanidin-3-glucoside
4.73	433.1144	433.1135	$C_{21}H_{21}O_{10}$	**Pelargonidin 3-O-glucoside**
5.52	585.1256	585.1244	$C_{28}H_{25}O_{14}$	Unkown
5.80	197.1178	197.1178	$C_{11}H_{17}O_3$	3-Hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptane-1-carboxylate
6.38	261.1128	261.1127	$C_{15}H_{17}O_4$	7-Hydroxy-4-(methoxycarbonyl)-2-(2-methyl-2-propanyl)chromenium
7.03	309.0872	309.0875	$C_{17}H_{13}N_2O_4$	3-Carbamoyl-1-[2-oxo-2-(2-oxo-2H-chromen-3-yl)ethyl]pyridinium

7.41	570.2218	570.2187	$C_{26}H_{36}NO_{13}$	1-[(4-methoxyphenyl)methyl]-2-methyl-1,2,3,4,5,6,7,8-octahydroisoquinolin-2-ium;(2R,3R)-2,3,4-trihydroxy-4-oxo-butanoate
7.67	648.4308	648.4345	$C_{35}H_{62}N_5O_2S_2$	Unknown
7.96	275.2017	275.2011	$C_{18}H_{27}O_2$	(9E,11E,13E,15E)-9,11,13,15-Octadecatetraenoate
8.18	645.2926	645.2924	$C_{35}H_{41}N_4O_8$	Unknown
8.47	345.0617	345.0610	$C_{17}H_{13}O_8$	5,7-Dihydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-4-oxo-4H-chromen-3-olate
8.71	181.1230	181.1229	$C_{11}H_{17}O_2$	2-(5-Hexen-1-yl)-5-hydroxy-3,4-dihydropyranium
9.47	343.0454	343.0454	$C_{17}H_{11}O_8$	Unknown
9.56	343.1188	343.1188	$C_{19}H_{19}O_6$	(3R)-3-(2,3-Dihydro-1,4-benzodioxin-6-yl)-3-(3,4-dimethoxyphenyl)propanoate
9.96	345.1337	345.1338	$C_{19}H_{21}O_6$	(1R,2R,5S,8S,9S,10R,11S,12S)-5,12-Dihydroxy-11-methyl-6-methylene-16-oxo-15-oxapentacyclo[9.3.2.1^{5,8}.0^{1,10}.0^{2,8}]heptadec-13-ene-9-carboxylate
10.42	214.2535	214.2535	$C_{14}H_{32}N$	tetradecylammonium
10.79	627.2828	627.2819	$C_{35}H_{39}N_4O_7$	3-{(3S,4S)-5-{2-[(3-Ethyl-5-formyl-4-methyl-1H-pyrrol-2-yl)methyl]-5-(methoxycarbonyl)-3-methyl-4-oxo-1,4-dihydrocyclopenta[b]pyrrol-6-yl}-3-methyl-2-[(3-methyl-5-oxo-4-vinyl-2,5-dihydro-1H-pyrrol-2-y l)methyl]-3,4-dihydro-2H-pyrrol-4-yl}propanoate
10.90	271.1692	271.1692	$C_{18}H_{23}O_2$	(17β)-17-Hydroxyestra-1(10),2,4-trien-3-olate
11.60	277.2166	277.2166	$C_{18}H_{29}O_2$	linolenate
11.98	601.5199	601.5148	$C_{22}H_{65}N_{16}OS$	unknown
12.22	425.3632	425.3632	$C_{27}H_{45}N_4$	unknown
13.01	425.3607	425.3644	$C_{27}H_{45}N_4$	unknown
13.94	423.3973	423.3991	$C_{31}H_{51}$	unknown
14.33	423.3975	423.3991	$C_{31}H_{51}$	unknown
14.79	423.3984	423.3991	$C_{31}H_{51}$	unknown
15.03	419.3139	419.3140	$C_{27}H_{39}N_4$	5-Ethyl-2-methyl-1-[3-({4-[(E)-phenyldiazenyl]-5,6,7,8-tetrahydro-1-naphthalenyl}amino)propyl]piperidinium (Alkaloid piperidin)
15.12	423.3979	423.3991	$C_{31}H_{51}$	unknown
15.41	423.3986	423.3957	$C_{19}H_{51}N_8S$	unknown
16.40	423.3958	423.3991	$C_{31}H_{51}$	unknown
16.87	423.3968	423.3991	$C_{31}H_{51}$	unknown

The major compound in the ethanol extract of the aquadest fraction is Pelargonidin 3-O-glucoside with an iFit percentage of 97.56%. Pelargonidin 3-O-glucoside is a type of anthocyanin including the flavonoid compound (Firdaus Kamal et al., 2014). Spectra and chemical structure of the compound can be seen in Figure 3.

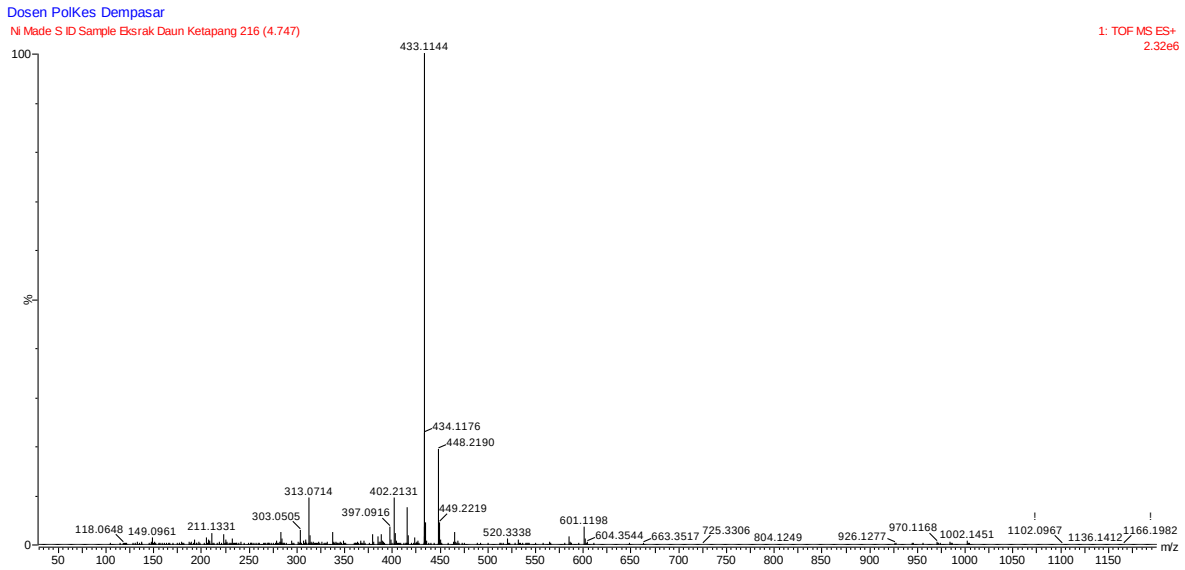
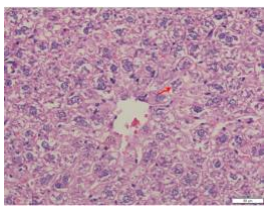


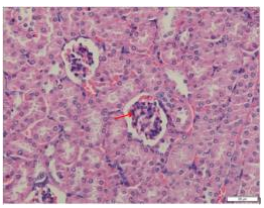
Figure 3: Spectra and chemical structures of major compounds

The ethanol extract of the aquadest fraction of Ketapang leaves contains several secondary metabolites such as alkaloids, i.e. 1-Allylpyridinium and 5-Ethyl-2-methyl-1-[3-({4-[(E)- phenyldiazenyl]-5,6,7,8-tetrahydro-1-naphthalenyl} amino) propyl] piperidinium and the flavonoid group, namely Cyanidin-3-glucoside and Pelargonidin 3-O-glucoside. Hence, the ethanol extract of the aquadest fraction can be utilized as a medicine to control bleeding after tooth extraction.

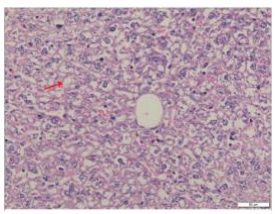
The results of the acute toxicity test (LD50), it was found that during the 2x24 hour observation period, all (100%) mice in the five treatment groups did not show signs of poisoning, mice moved agilely, and mice also kissed/smelled each other. In all treatment groups (100%), after 2 x 24 hours of administration of ethyl acetate fractionated Ketapang leaf ethanol extract, no deaths were found. The histopathology images of the mice's liver and kidney after LD50 test were carried out as shown in Figure 4.



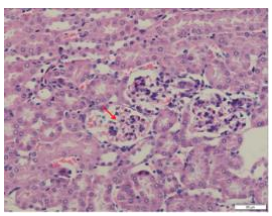
(a) Liver of mice treated with 1g/kg BW. Severe damage occurs in the form of hydrophic degeneration in the liver in all places (diffuse).



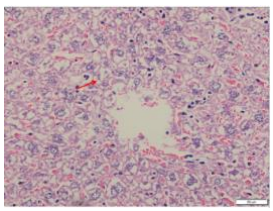
(b) Mice kidneys treated with 1g/kg BW. The kidneys look normal, there is no glomerular reduction.

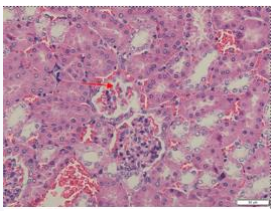


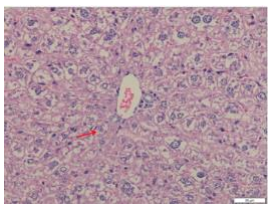
(c) Liver of mice treated with 2g/kg BW. Severe damage occurs in the form of hydrophic degeneration in the liver in all places (diffuse).

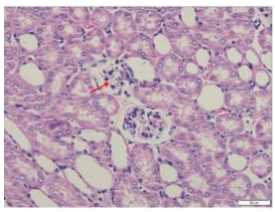


(d) Mice kidneys treated with 2g/kg BW. Damage occurs in the form of moderate glomerular reduction in the kidneys in one place (focal).







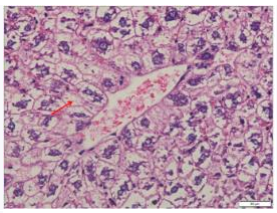


(e) Liver of mice treated with 4g/kg BW. Severe damage occurs in the form of hydrophic degeneration in the liver in all places (diffuse).

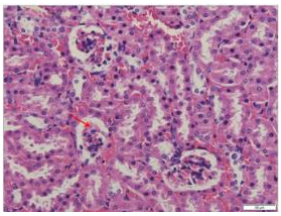
(f) Mice kidneys treated with 4g/kg BW. Damage occurs in the form of moderate glomerular reduction in the kidneys in one place (focal).

(g) Liver of mice treated with 8g/kg BW. Severe damage occurs in the form of hydrophic degeneration in the liver in all places (diffuse).

(h) Mice kidneys treated with 8g/kg BW. Damage occurs in the form of moderate glomerular shrinkage in the kidneys in several places (multifocal).



(i) Liver of mice treated with 16g/kg BW. Severe damage occurs in the form of hydrophic degeneration in the liver in all places (diffuse).



(j) Mice kidneys treated with 16g/kg BW. Damage occurs in the form of moderate glomerular shrinkage in the kidneys in several places (multifocal).

Figure 4: Histopathology images of the liver and kidney of the mice after LD_{50} test of ethanol extract of *T. Catappa L.*

The liver in the doses either 1g/kg BW, 2 g/kg BW, 4 g/kg BW, 8 g/kg BW, and 16 g/kg BW shows severe damage in the liver. The kidney with 1 g/kg BW shows normal condition. Meanwhile, in 2 g/kg BW, 4 g/kg BW shows focal light damage, and 8 g/kg BW as well as 16 g/kg BW shows multifocal moderate damage in the kidney. The results of the histopathology condition in the liver and kidney and the statistical calculation of the damage show that in all treatment groups, mice were found whose hepatocyte cells experienced a severe degree of hydropic degeneration shown in Table 2.

Table 2: Histopathology results in mice liver

No. of mice	**Dose Group**					**p-value**
	1 g/kg BW	2 g/kg BW	4 g/kg BW	8 g/kg BW	16 g/kg BW	
Liver						
1	3	3	0	3	3	0.331
2	0	3	3	3	3	
3	3	3	3	3	3	
4	3	3	3	3	3	
Kidney						
1	0	0	0	2	2	0.002
2	0	1	2	2	2	
3	0	1	1	2	2	
4	1	1	1	2	2	

Score 0 shows the normal condition without damage, score 1 shows the focal (moderate) damage in one place, score 2 shows multifocal (moderate) damage in several places, and score 3 shows diffuse (severe damage) in all places. The damage is hydropic degeneration in the liver and glomerulus reduction in the kidney. The result of the p-value in Table 2 of histopathology in the liver shows 0.331, meaning that there is no significant difference in liver damage of the mice in treatment groups. Meanwhile, the kidney damage of the Kruskal Wallis test resulted in a p-value of 0.002 shown in Table 2, meaning that there is a significant difference in kidney damage in the mice.

4. Discussion

Based on the results of examination and analysis of LCMS results, 20 compounds can be identified and 15 compounds whose compound names are unknown. Compounds that can be identified include the types of flavonoids, and tannin compounds also belong to the flavonoid group. The presence of tannins can precipitate

blood proteins and constrict the narrow blood vessel network. Tannins can act as an astringent that causes the closing of skin pores hardens the skin, and stops exudate and light bleeding. Meanwhile, flavonoids can act as an anti-inflammatory which can reduce inflammation and pain.

The results of the acute lethal dose (LD50) toxicity test of the ethanol extract of *T. Catappa L.* in mice showed that the ethanol extract of ketapang leaves is safe, and not toxic. In general, the smaller the LD50 value, the more toxic the compound is (Raj et al., 2013). The results obtained (in mg/kg BW) can be divided into several classes according to the potential for acute toxicity of the test compounds, i.e super toxic is with LD50 5 mg/kg BW or less, extremely toxic with 5 – 50 mg/kg BW, very toxic with 50 – 500 mg/kg BW, medium toxic with 0.5 – 5 g/kg BW, fairly toxic with 5- 15 g/kg BW, non-toxic>15 g/kg BW.

Ethanol extract of Ketapang leaves and aquadest is concluded in the practically non-toxic category. Histopathological examination of the liver and kidney organs of mice with the highest dose (16gr/kg BW), showed diffuse hydropic degeneration (spreading) in the liver. These results were slightly different from the studies of Astawan, et al. (2005) which histomorphological showed degeneration of cells in the liver and kidneys, especially in the 4, 8, and 16 g/kg BW treatment groups (Astawan et al., 2005). While in this study mice were fed 16g/kg BW of *T. Catappa L.* extract, there was no liver degeneration. The results are in line with Nugroho, et al. (2020) in which the mice were extracted with *T. Catappa L.* of green and brown leaves doses of 125 mg/kg, 250 mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg shows the toxic effect on the liver of mice in terms of necrosis and degeneration damage (Rudy et al., 2020). The dose at 1000 mg/kg BW shows the most significant liver degeneration among all the doses in the aforementioned study. This is in line with the present study that shows at 16 gr/kg BW shows the degeneration of cells in the liver and kidney. The studies show also that the color of the leaves either green or brown can cause toxic effects on the liver of mice at certain doses, specifically at more than 1000 mg/kg BW.

The highest dose used of ethyl acetate fraction of the peel Kandis acid (Garnicia cowa Roxb) shows dangerous active damage. Besides, the use of ethyl acetate is observed to have the significant effect on the activity of Serum Glutamic Pyruvic Transaminase (SGPT) in white mice (Wahyuni et al., 2017). The present study performed the testing on the mice with *T. Catappa L.* extract once for 2x24 hours and extracting ethyl acetate in the fractionation process. The extract which is given repeatedly and for a long time, can cause an accumulation of dangerous active ingredients toxic to the tested animal it is proven that repeated intake of Hibiscus rosa-sinensis L. extract in high doses of 800 mg/kg for 14 days can cause liver and kidney toxicity in mice (Nath & Yadav, 2015). However, in fact, the factor which affects the tested animal was proven by Wahyuni, et al. (2017) is the dose ($p < 0,05$) and the duration of administration of the extract does not significantly affect ($p>0,05$) (Ridzwan et al., 2014; Wahyuni et al., 2017). This is in line with research by Deshpande, et al. (2015) which shows that the exposure of 90-day repeated dose oral administration of Urban leaves (Centella asiatica) produced no significant toxic effect in rats with LD50 > 2000 mg/kg (Deshpande et al., 2015). However, the study suggests that a low dose of Hibiscus rosa-sinensis L. extract i.e. 400 mg/kg can be considered safe for traditional medicine (Nath & Yadav, 2015). This is in line with the present study in which the dose of 1 g/kg, 2 g/kg, 3 g/kg, and 4 g/kg is proven safe, even in 8 g/kg. Although 16 g/kg dose is toxic somehow, particularly for liver cell degeneration.

The liver is crucial for nutrient metabolism, glucose and lipid synthesis, and the detoxification of drugs and foreign substances. It is commonly the primary organ affected by chemical damage. Various compounds can harm liver cells, with oxidative stress being a major contributor, as an excess of pro-oxidants can damage cells, often leading to cell death. The main function of the kidneys is to eliminate waste products, during the reabsorption process, potentially toxic chemicals can predispose the kidneys to injury.

The kidney's vulnerability to toxic damage is linked to the complexity of its structure and function. Nephrotoxicity can arise as a serious complication from drug treatments or chemical exposure. This study demonstrates that, after the LD50 test, the liver experiences more severe damage than the kidneys in mice. This is understandable considering the function of the liver as the main organ of detoxification. Considering that the cause of hydropic degeneration is diet or toxicity, and based on the results of the LD50 test, this study shows that the ethanol extract

of *T. Catappa L.* is not toxic or safe. The cause of hydropic degeneration may be diffuse in the liver of mice, namely the diet from the ethanol extract of *T. Catappa L.* given as treatment.

Many plant families harbor potentially toxic alkaloids (Griffiths et al., 2021). Secondary metabolites in plants, such as alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids, can be toxic to both plants and animals (Paiva et al., 2023). In some plants, these compounds serve as a defense mechanism against threats, but at specific doses, they may also have medicinal properties. The potential liver and kidney damage observed in mice after the LD50 test could be attributed to the secondary metabolites present in the ethanol extract of *T. Catappa L.* leaves.

This study presents the histopathological experiment of T. catappa L which is exposed to mice with the limitation of dose to 1 g/kg, 2 g/kg, 3 g/kg, 4 g/kg and 8 g/kg and 16 g/kg BW. The results show there is diffuse hydropic degeneration in the liver with dose of 16 g/kg BW. Meanwhile, in the kidney, there is no degeneration occurred. T. catappa L. is categorized as a non-toxic substance. Regarding the importance of safe pain curing using plants, an extension for the near future is the use of the T. catappa L. to be tested in real as an anti-bleeding agent after tooth extraction.

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