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In vivo Study of Uric Acid Inhibitory of Catechin from Gambir (*Uncaria gambir* (Hunter) Roxb) in Male Rats

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ABSTRACT

Hyperuricemia is a metabolic disease characterized by high levels of uric acid in the blood. In silico studies of catechin compounds using molecular docking were able to inhibit the activity of the xanthin oxidase enzyme so that it could reduce uric acid levels. One of the native Indonesian plants that contains catechin is gambier (Uncaria gambir (Hunter) Roxb). This research aims to obtain the efficacy and safety of gambier leaf catechins in reducing uric acid levels. In vivo efficacy testing at doses of 20, 40, and 40 mg/Kg BW with allopurinol as a comparison and the safety of gambier leaf catechin in hyperuricemic male mice through macroscopic and microscopic examination of the liver and kidney organs. The results of research on determining catechin levels using HPTLC obtained levels of 96.06%. Gambier leaf catechin at doses of 20, 40, and 40 mg/Kg BW was able to reduce uric acid levels in the blood serum of mice. The higher the dose of catechin, the better the anti-hyperuricemia effect. In the results of the safety parameters, gambier leaf catechins, there were no specific abnormalities in the liver and kidneys of mice both macroscopically and microscopically. Based on research, it can be concluded that gambier leaf catechin has great potential as an anti-hyperuricemia drug and has good safety if consumed for a long time.

Keywords: Catechin, Hyperuricemia, In Vivo, *Uncaria gambir*

INTRODUCTION

Indonesia is a country with diverse biodiversity that has plant potential and abundant medicine. People in ancient times have known and used it for a long time Medicinal plants as a means of traditional medicine either to prevent or to cure a disease. Traditional medicine is used by the Indonesian population, and is mostly based on experiences passed down from one generation to the next.[1]

Among the traditional medicines that have not been widely studied are traditional medicines for gout treatment. Uric acid is the final product of purine breakdown in humans. Purine products are converted to uric acid via xanthin in a reaction catalyzed by xanthine oxidase. Next, xanthin is oxidized to uric acid in the next reaction catalyzed by the xanthin oxidase. Thus, the enzyme xanthin oxidase is an essential location for pharmacological intervention in patients with hyperuricemia and gout.[2]

Hyperuricemia is a condition where the uric acid level in the blood is greater than the normal value. Men are said to have hyperuricemia if their uric acid levels are above 7 mg/dL and in women above 6 mg/dL. If left unchecked, hyperuricemia will trigger it kidney damage such as nephrolithiasis, nephropathy, and gout nephropathy.[3]

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The balance of uric acid production and excretion is the key to controlling uric acid in the blood. Excess production and lack of excretion of uric acid cause high levels of uric acid in the blood to increase.[4] Synthetic drugs used for hyperuricemia are allopurinol. Allopurinol works to reduce uric acid synthesis by inhibiting the activity xanthine oxidase enzyme. Hypoxanthine and xanthine are broken down by xanthine oxidase into uric acid, but in the presence of allopurinol, xanthine oxidase exerts its activity on this drug as a purine replacement.[5]

Allopurinol is a first-line therapeutic drug to inhibit the formation of uric acid, but this drug inevitably has some adverse side effects.[6] The side effects that are often found from using allopurinol are urticaria, leukopenia, skin rash, headaches, and potentially increased frequency of attacks and acute gout with initiation of therapy. Therefore further research is needed to find adjuvant allopurinol as a xanthine oxidase inhibitor.[7]

Several new in silico studies conducted on gambier catechin have the following effects antihyperuricemia through the mechanism of inhibiting the action of the xanthine oxidase enzyme.[8] There has been no research on the use of gambier leaves in vivo as an anti hyperuricemia carried out. Based on these data it is necessary to carry out in-depth studies to prove it efficacy and safety of gambier leaf extract in vivo in reducing uric acid levels in experimental animals.

METHODS

Place and Time of Research

The Research was conducted at the Pharmaceutical Laboratory, Ministry of Health, Jakarta and Bioanimalindo Depok Experimental Animal Laboratory. The research was conducted in March - May 2023.

Tools and Materials

The tools used in this research are High-Performance Thin Layer Chromatography (Camag), digital analytical balance (AND), oven (Memmert), sonicator (Ultrasonic Cleaner GB-928), syringe filter(Waters), Cobas m501 (Roche), Light Microscope (Canon). The materials used are standard catechin (Sigma Aldrich), gambier extract from Mungka Limapuluh Kota Regency, silica gel plate HPTLC (Merck), ethyl acetate (Merck), chloroform pa (Merck), formic acid pa (Merck), and methanol pa (Merck), hematology kit (Roche), UA kit (Roche), Creap kit (Roche), formalin, ketamine, and xylazine. The experimental animals used were male Wistar mice obtained from the laboratory Bioanimalindo, Depok.

Plant Determination

To carry out correct identification of plants used in research Plant determination was carried out in the Herbarium of the Department of Biology, Andalas University with determination number No. 024/ANDA/II/2023.

Preparation of Extract and Isolation of Catechins

The process of making gambier leaf extract is done by harvesting gambier leaves fresh obtained from Limapuluh Kota Regency, then the fresh gambier leaves put into a net and steamed using a stream of hot water steam. After it finishes steamed, lifted and then pressed using a hydraulic press until latex is produced gambier. After that, the gambier sap is drained and placed in a baking dish and cooled for a while 2 days until hard. The gambier sap that has hardened is then cut into squares. Next, drying is carried out using an oven at a temperature of 40-50^{o}C for 1 day to reduce water content.[9]

Determination of Levels of Catechin Isolates Using HPTLC

Determination of catechin levels begins with creating a standard curve with a series of levels of 50, 100, 150, 200, 250, and 300 µg/mL. A gambier catechin isolate test solution with a concentration of 200 µg/mL was prepared with replication 3 times. The standard and test solutions that have been prepared are then filtered using

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a syringe filter 0.45 µm to avoid potential contamination causing blockages in the injection process in HPTLC. After the filtering process, the solution. The standard and test samples are put into a vial and spotted on the TLC plate silica gel G_{F254} each 20 µL. The mobile phase used is a mixture chloroform: ethyl acetate: formic acid (5:4:1).[10]

Examination of Uric Acid Levels and Hematology in Rat Blood Serum

The research has received ethical approval from the Health Ethics Committee of the Research and Development Agency Health with number LB.02.01/2.KE.516/2022. A total of 30 mice were made hyperuricemic first by administering orally and separately preparing 4 ml chicken liver juice/Kg BW made by blending and urea 1 mg/Kg BW for 14 days. Hyperuricemia condition if levels uric acid have more than doubled the initial measurement level. After hyperuricemia was achieved, the test animals were divided randomly into 5 groups (6 animals each). Group I served as a negative control by administering distilled water solution. Group II was given allopurinol 10 mg/Kg BW as positive control. Group III-V is a group that was given gambier leaf extract at the doses of 20, 40, and 80 mg/Kg BW. On the 15th day to the 28th day, it started administering the test preparation orally. Blood as much as 1 mL was taken from the rat's tail to examine uric acid levels and hematology in rat blood serum.

Examination of The Safety of Rat Liver and Kidney Organs

Checking the safety of the liver and kidneys were carried out by observing macroscopic and microscopic histopathological preparations. Microscopic observations were carried out under a microscope with 200 times magnification.[11]

RESULTS AND DISCUSSION

Characterization examination of gambier leaf extract was carried out to test the quality of the extract gambier leaves before being used in therapy on experimental animals. Quality characteristics which include organoleptic tests, water content, ash content, and yield are listed in Table 1. According to organoleptic observations using the five senses, gambier leaf extract in the form of a dry extract, yellowish brown, and has a distinctive aroma.

Determination of water content aims to meet the maximum limit or range of the amount of water content in the material. This water content measurement was carried out to avoid the rapid growth of microbes and fungi in the extract, besides that, it is also to maintain the quality of test materials during storage. The water content results obtained were 4.72%. It shows that the water content is <14% and meets the quality requirements.[12]

Ash content is an indicator of contamination in the extract. The results of the total ash yield content the gambier leaf extract obtained were 0.24%. It shows that the total ash content still meets the quality standard, namely <0.5%. This small ash content figure shows very small mineral and inorganic compound content. Ash is a mixture of inorganic materials and minerals in the extract, when burned the organic material will burn out but the inorganic material will remain.[13]

Based on the calculation results, it can be determined that the level of gambier leaf catechin isolate obtained was 96.09%, it indicates that the sample truly contains catechins at quite high levels. Requirements for catechin levels in gambier leaf extract, based on the Indonesia Herbal of Pharmacopoeia, is determined that it cannot be less than 90%.

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Table 1. Characterization catechin isolates of gambier leaf

Characterization	Requirement	Result
Form	Powder	Powder
Color	Yellowish-brown	Yellowish-brown
Odor	Specific gambier	Specific gambier
Water content	< 14%	4.72%
Ash content	< 0.5%	0.24
Catechin assay	≥ 90%	96.09%

Table 2. Results of decreased uric acid and creatinine levels in rat blood serum after treatment

Group	Uric acid level (mg/dL ± SD)	Creatinine level (mg/dL ± SD)
Control -	0.2 ± 0.1	0.0 ± 0.0
Control +	2.4 ± 0.4*	0.1 ± 0.2*
Dose 20 mg/kgbb	1.8 ± 0.2*	0.3 ± 0.1*
Dose 40 mg/kgbb	3.4 ± 0.4*	0.4 ± 0.2*
Dose 80 mg/kgbb	4.5 ± 0.2*	0.4 ± 0.1*

The test of anti-hyperuricemia activity of gambier leaf extract with the effect of reducing acid levels urate and creatinine in mice induced by a diet high in purine bases for 14 days. Hyperuricemia mice were treated using test samples from day 14 to day 28. Results examination of uric acid and creatinine levels in mouse blood serum can be seen in Table 2.

The results of the analysis of the uric acid levels of the test samples show that both positive controls and gambier leaf catechin isolate at all three doses were able to reduce uric acid levels significantly ($p < 0.05$) when compared with the negative control that was only treated distilled water. Allopurinol is used as a comparison because its mechanism of action is capable inhibits the performance of the xanthine oxidase enzyme.[14] The use of varying dose concentrations of catechin isolate gambier leaves was carried out to determine the effect of increasing concentration on the inhibitory power and at what does inhibition of enzyme activity begins to occur xanthine oxidase. The mechanism of catechins in inhibiting the action of the xanthine oxidase enzyme is through competitive inhibitor between ligand and enzyme.[15]

These results are also in line with research conducted by Wu et al. which details the roles catechins inhibit xanthine oxidase activity to reduce excess acid production urate in the liver and in regulating the expression of uric acid transporters, URAT1, OAT1, OAT3, ABCG2, and GLUT9, to balance the levels of uric acid secretion and reabsorption, through the kidneys and intestines. In this way, uric acid levels in the blood can be regulated to normal values.[16]

According to Toragall et al. which states that creatinine uric acid levels are normal blood plasma in mice is 0.2-0.6 mg/dL. Providing high-purine-based foods can increase creatinine levels in the blood. The creatinine is the result of the breakdown of creatine phosphate muscle, produced by the body constantly depending on muscle mass.[17]

Creatinine levels are related to muscle mass, reflecting changes in creatinine and kidney function. An increase in levels of creatinine in the blood indicates a decrease in excretion caused by the presence of impaired kidney function. Creatinine is the anhydride form of most creatinine synthesized in muscle via the non-enzymatic dehydration process of creatinine phosphate. Creatinine is

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excreted entirely in the urine through glomerular filtration. Increased creatinine levels in the blood are an indication of kidney damage.[18]

The given of gambier leaf catechin isolate can reduce creatinine levels in the blood. This is due to the catechin compound which is the main compound in gambier leaf extract protects against kidney damage caused by uric acid and oxalate. Additionally, according to Wongmekiat et al., catechin compounds can inhibit kidney damage by inhibiting mitochondrial dysfunction supported by increased production of oxygen species mitochondrial reactivity, and decreased mitochondrial membrane potential. Treatment with catechins significantly weakens all changes caused by cadmium and uric acid. These findings suggest that catechins effectively protect the kidney against the toxic effects of cadmium, through antioxidant, anti-inflammatory, and mitochondrial cells protection mechanisms.[19]

Macroscopic observations of the vital organs of the test animals on day 28 showed no abnormalities. Administration of test preparations within a certain time does not indicate any organ damage macroscopic. Metabolism of most chemical compounds occurs in the liver. Some animals experience cell regeneration, especially in the periportal. Gambier leaf catechin isolate is safe for consumption and does not cause liver and kidney damage. This is caused by the content of catechins in gambier leaves have hepatoprotective properties that can protect liver cells against damage and help the process of liver cell regeneration. These results are in line with research from Mutia et al. which shows the hepatoprotective function of gambier leaf catechins was very high.[20]

Microscopic observation of the liver and kidneys of mice after surgery shows the same results. Liver and kidney organs in the aqua dest administration group, administration of 20, 40, 80 mg/Kg BW catechin isolate, indicating a normal liver shape which is arranged in radicals in the liver lobules and the cell boundaries are visible where liver cells have one or two round nuclei that are in the middle and the average diameter of the central vein starts from the smallest diameter to the largest respectively (Figure 1 and 2).

The group of allopurinol in the liver indicates there is a regeneration in the liver with a much larger average central vein diameter, some liver lobules suffer severe damage, and the damage occurs - the central vein endothelial cells undergo lysis, widening of the diameter of the central vein, blood clotting occurs, liver cells experience necrosis and sometimes obesity (steatosis) occurs.

Table 3. Results of macroscopic histopathological observations of gambier leaf catechin isolates in mice

Group	Liver	Kidney
Control -	NSAO	NSAO
Control +	NSAO	NSAO
Dose 20 mg/kg bb	NSAO	NSAO
Dose 40 mg/kg bb	NSAO	NSAO
Dose 80 mg/kg bb	NSAO	NSAO

NSAO = No Specific Abnormalities Occur

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In the liver in the administration group allopurinol indicates that the liver is regenerating with an average central vein diameter is much larger, some liver lobules experience severe damage, damage to the cells - central vein endothelial cells undergo lysis, widening of the diameter of the central vein occurs blood clots, liver cells experience necrosis and sometimes fat occurs (steatosis). Prolonged administration of allopurinol can cause organ damage to liver cells.[21]

On the other hand, there were no changes or damage to the kidney organs in all groups of cells. The kidney organ is said to be normal if there is a glomerulus, Bowman's capsule and cell nuclei are normal, and does not experience necrosis and cell infiltration, so it can be interpreted that there is administration of catechins and allopurinol after 28 days did not affect the histopathological picture kidney organs. Research by Permatasari shows that administering isolates of concentrated catechins did not have toxic effects through hematological and histopathological tests on mice.[22]

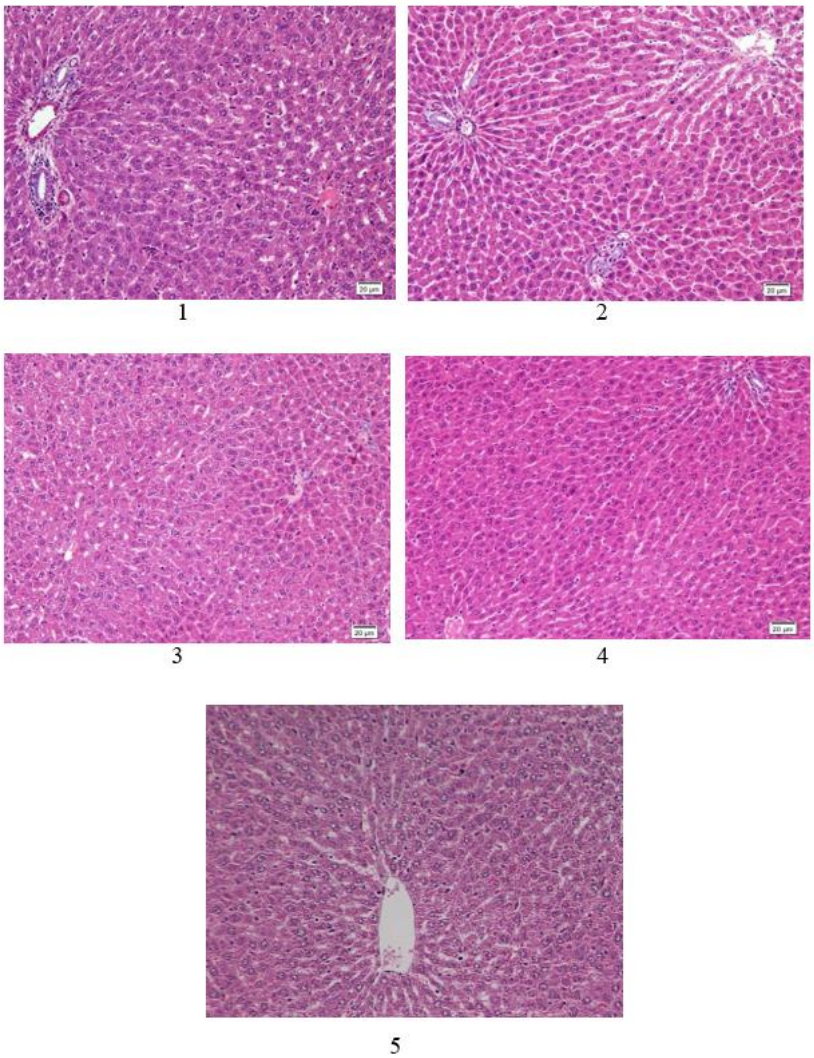

Figure 1. Microscopic histopathology of the liver with 200x magnification (1= aquadest, 2 = dose 80 mg/Kg BW, 3 = dose 40 mg/Kg BW, 4 = dose 20 mg/Kg BW, 5 = allopurinol)

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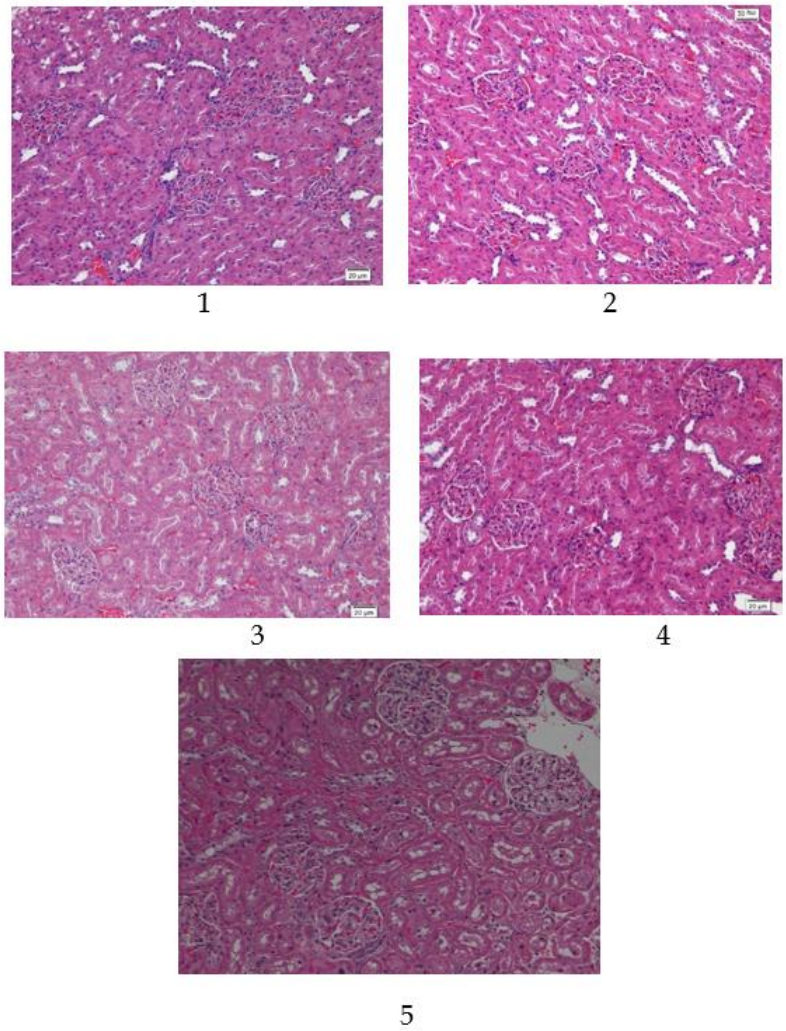

Figure 2. Microscopic histopathology of the kidneys with 200x magnification (1= aquadest, 2 = dose 80 mg/Kg BW, 3 = dose 40 mg/Kg BW, 4 = dose 20 mg/Kg BW, 5 = allopurinol)

CONCLUSION

Catechin isolates in 80mg/Kg BW dosage provide a decrease in acid levels best urate and creatinine in hyperuricemic male rats. Histopathological observations macroscopic and microscopic administration of gambier leaf catechin isolate at doses of 20, 40 and 80 mg/Kg BW is safe and does not cause specific abnormalities in the liver and kidneys of mice hyperuricemic males.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them. All authors contributed equally to this work.

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REFERENCES

1. Cahyaningsih R, Phillips J, Brehm JM, Gaisberger H, & Maxted N. Climate change impact on medicinal plants in Indonesia. Global Ecology and Conservation. 2021;30:e01752.
2. Artini NPR & Aryasa IWT. Effectiveness of Wijaya Kusuma Flowers (Epiphyllum oxypetalum) on reducing uric acid levels in wistar rats. The Journal of Muhammadiyah Medical Laboratory. 2019;2(2):36-45.
3. Ulya T, Masruroh IN, Muslichah S, & Ningsih IY. Uji aktivitas ekstrak metanol biji juwet (Syzygium cumini (L.) Skeels) sebagai antihiperurisemia pada mencit hiperurisemia. Pharmaceutical and Traditional Medicine. 2022;6(1):28-34.
4. Rachmania RA, Dwitiyanti D, Iriansyah QW, & Putri FF. Fraction potential secang wood (Caesalpinia sappan L.) on the inhibition of xanthine oxidase in reducing uric acid levels in hyperuricemia. PHARMACY: Jurnal Farmasi Indonesia. 2021;18(1):21-33.
5. Matsuo H, Ishikawa E, Machida H, Mizutani Y, Tanoue A, Ohnishi T, et al. Efficacy of xanthine oxidase inhibitor for chronic kidney disease patients with hyperuricemia. Clinical and Experimental Nephrology. 2020;24:307-13.
6. Stamp LK, Chapman PT, & Palmer SC. Allopurinol and kidney function: An update. Joint Bone Spine. 2016;83(1):19-24.
7. Rustamsyah A, Islami SN, Fitriana, Kusmiyati M. Inhibitory activity brewed xanthine oxidase enzyme and ethanol extract of the white (Camellia sinensis L.). Journal of Tea and Quinine Research. 2018;19(2):196-201.
8. Malik N, Dhiman P, & Khatkar A. In silico design and synthesis of targeted rutin derivatives as xanthine oxidase inhibitors. BMC chemistry;2019;13:1-13.
9. Yunarto N, Reswandaru UN, Sulistyowati I, Prameswari IO, Pinanditi QL, & Patadungan TM. Validation of spectrophotometry method for determination of (+)-catechin in ethyl acetate fraction of gambir extract (Uncaria gambir Roxb.). Jurnal Tumbuhan Obat Indonesia. 2021;14(2):127-36.
10. Yunarto N, Sulistyowati I, Reswandaru UN, Elya B, Sauriasari R, & Konadi L. Inhibitory activity of Uncaria Gambir Roxb extract, ethyl acetate fraction, and catechin isolate on lipase. In AIP Conference Proceedings. 2023;2606(1): 020011. AIP Publishing LLC.
11. Ningsih S, Agustini K, Nizar N, & Damayanti R. Uji toksisitas subkronik kombinasi ekstrak daun Uncaria gambir dan Caesalpinia sappan. Jurnal Kefarmasian Indonesia. 2017;7(1):34-45.
12. Ministry of Health of the Republic of Indonesia. Indonesian Herbal of Pharmacopeia. 2017, Jakarta.
13. Gruber L, Seidl L, Zanetti M, & Schnabel T. Calorific value and ash content of extracted birch bark. Forests. 2021;12(11):1480.
14. Sekine M, Okamoto K, Pai EF, Nagata K, Ichida K, Hille R, & Nishino T. Allopurinol and oxypurinol differ in their strength and mechanisms of inhibition of xanthine oxidoreductase. Journal of Biological Chemistry. 2023; 299(9):105189.
15. Choudhary DK, Chaturvedi N, Singh A, & Mishra A. Investigation of hypoglycemic effects, oxidative stress potential and xanthine-oxidase activity of polyphenols (gallic acid, catechin) derived from faba bean on 3T3-L1 cell line: insights into molecular docking and simulation study. Toxicology research. 2020;9(3):308-22.
16. Wu D, Zhang W, Lai X, Li Q, Sun L, Chen R, et al. Regulation of catechins in uric acid metabolism disorder related human diseases. Mini Reviews in Medicinal Chemistry. 2020;20(18):1857 66.
17. Toragall V, Jayapala N, Muthukumar SP, & Vallikanan B. Biodegradable chitosan-sodium alginate-oleic acid nanocarrier promotes bioavailability

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

and target delivery of lutein in rat model with no toxicity. Food chemistry. 2020;330:127195.
18. Rumondor R, Komalig R. Antioxidant activity test and effect of ethanol extract of nusa inda putih (Mussaenda pubescens) against creatinine, uric acid and ureum levels in white rats (Rattus novergicus). Indonesian Biodiversity Journal. 2020;2(1):30-5.
19. Wongmekiat O, Peerapanyasut W, & Kobroob A. Catechin supplementation prevents kidney damage in rats repeatedly exposed to cadmium through mitochondrial protection. Naunyn-Schmiedeberg's Archives of Pharmacology. 2018;391: 385-394.
20. Mutia L, Sinurat M, Syarifah S, Sedana D, Anggraini DR, Ichwan M. The hepatoprotector effect of Uncaria gambir Roxb extract in wistar rats induced by paracetamol. Journal of Drug Delivery and Therapeutics. 2020; 10(6):61-6.
21. Roncal-Jimenez CA, Sato Y, Milagres T, Andres Hernando A, García G, Bjornstad P, et al. Experimental heat stress nephropathy and liver injury were improved by allopurinol. American Journal of Physiology-Renal Physiology. 2018;315(3):726-33.
22. Permatasari D, Sinamar LP, Estera K, Ahmadin A. The impact of sub acute administration of purified gambier (Uncaria gambir Roxb.) to the liver and kidney functions and its reversibility in rats. Pharmacognosy Journal. 2021;13(1):44-51.