Pharmacogn J. 2022; 14(5): 649-654
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Phytochemical Test and Acute Safety Evaluation of Oral Purple Leaves (*Graptophyllum Pictum* L. Griff) Extract in Rats

Feda Makkiyah*, Eldiza Puji Rahmi, Yuni Setyaningsih

Feda Makkiyah*, Eldiza Puji Rahmi, Yuni Setyaningsih

Faculty of Medicine, UPN Veteran Jakarta, Indonesia.

Correspondence

Feda Makkiyah

Faculty of Medicine, UPN Veteran Jakarta, INDONESIA.

E-mail: fedaanisah@upnvj.ac.id

History

- Submission Date: 16-07-2022;
- Review completed: 12-08-2022;
- Accepted Date: 30-08-2022.

DOI : 10.5530/pj.2022.14.148

Article Available online

http://www.phcogj.com/v14/i5

ABSTRACT

Background: Purple leaves (*Graptophyllum pictum (L) Griff*) is a native plant from Indonesia (Polynesia) which is empirically common used to treat hemorrhoids, diabetes, and many other diseases. Despite its massive development globally, there are few studies have written about the toxicity of this herbal medicine. **Aim:** The aim of this study is to describe the acute toxicity of this herbal medicine, as the basic ground of the further safe development of this medicine. **Method**: Each purple leaf dry powder (2 kg) was extracted with 15 L of 70% ethanol and 15 L of hexane by maceration method for 3 days at room temperature and then filtered to obtain macerate liquid. The study was conducted on 36 male 6-8 weeks, Sprague–Dawley (SD) rats. The result was presented as the mean value ± standard deviation (SD). Data were evaluated for homogeneity using Saphiro Wilk. The comparisons between pre dan post treatment body weight were measured by paired student t test and hematological measurements were analyzed by ANOVA. P value less than 0.05 was considered significant. **Results:** Acute toxicity of ethanol and hexane extract were assessed following a single dose administered by gavage at a dose of 50, 2000 and 3000 g/kg bw. The mean body weight of rats increased from 150 ± 2.22 g to 161 ± 6.68 g during the 14 days. The difference of body weight between before and at 14 days amongs groups were significantly different with *P* value <0.01. **Conclusion:** The present study showed that hexane extract contains more valuable components for medical treatment purposes. The acute toxicity on 50, 2000,3000 kg/BW oral ethanol and hexane extract of *Graptophyllum pictum* showed no significant influence on hematological blood parameters of rats. It is safe to administered orally ethanol or hexane extract of G. *pictum* below 3000mg/kgBW rats.

Key words: Acute, purple leaves, Safety, Toxicity.

INTRODUCTION

Purple leaves (*Graptophyllum pictum (L) Griff*) is a native plant from Indonesia (Polynesia) which is empirically common used to treat hemorrhoids, diabetes, and many other diseases.[1,2] The nano technology of this product also have been widely developed.[3-5] Despite its massive development globally, there are few studies have written about the toxicity of this herbal medicine. The aim of this study is to describe the acute toxicity of this herbal medicine, as the basic ground of the further safe development of this medicine.

MATERIALS AND METHODS

The process of making purple leaf extract

Fresh purple leaves (*Graptophyllum pictum (L)griff*) harvested from the Herbal Plants Resource Garden of Indonesian Medicinal and Aromatic Crops Research Institute (IMACRI), Bogor, Indonesia. They were washed with running water, sorted wet to sort the fresh leaves and then drained. Fresh purple leaves were dried using an oven (50°C) and then the moisture content was measured using a moisture balance device. The dried purple leaves were blended and sieved using a mesh sieve no.40. Each purple leaf dry powder (2 kg) was extracted with 15 L of 70% ethanol and 15 L of hexane by maceration method for 3 days at room temperature and then filtered to obtain macerate liquid. Each process was repeated 2 times and each macerate was concentrated using a rotary evaporator and followed by freeze-drying to produce 70% ethanol extract and purple leaf hexane extract.

Phytochemical qualitative analysis

The ethanol extract of *G. pictum* was tested using standard procedures with specific reagents to detect the presence of secondary metabolites such as alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, and glycoside.

Alkaloid test: The presence of alkaloids was tested using three reagents namely, Dragendorff's reagent, Wagner's reagent, and Mayer's reagent. Each test tube was filled with 500 µL of extract solution. Subsequently, a few drops of reagent were added into the tube. A reddish-brown precipitate indicated the presence of alkaloids.[6]

Saponins: Few drops of Na2HCO 3 were added to the extract solution (500 µL) and shaken for 5 minutes. The presence of saponin was indicated by the formation of froth or lather.[6]

Tannins: A few drops of 5% w/v ferric chloride solution were added to 1-2 mL of the extract. A greenish color indicated the presence of gallotannins, while brown color indicated the presence of pseudotannins.[6]

Triterpenoids: The dry crude plant extract (5 mg) was dissolved in chloroform (2 mL) and then acetic anhydride (1 mL) was added to it. Concentrated sulphuric acid (1 mL) was added to the solution. Formation of reddish violet colour shows the presence of triterpenoids.

Cite this article: Makkiyah F, Rahmi EP, Setyaningsih Y. Phytochemical Test and Acute Safety Evaluation of Oral Purple Leaves (*Graptophyllum Pictum* L. Griff) Extract in Rats. Pharmacogn J. 2022;12(5): 649-654.

Steroids: Chloroform was added to the extract solution (500 µL) followed by conc. H2SO4 added slowly through the sides of the test tube. The lower sulphuric acid fraction turned brownish yellow and the upper layer turned reddish orange which indicated presence of steroids.

Glycoside: Few drops of aqueous NaOH were added to the extracts (500 µL). Yellow colored solution indicated presence of glycosides.

Phenolics: To the test solution (500 µL), a few drops of FeCl3 were added. Presence of phenols was indicated by formation of a blue or blue-green colored solution (500 µL).[7]

Flavonoids: A few drops of NaOH solution was added to the extract solution (500 µL) followed by diluted HCl. The solution turned yellow and then colorless, indicating the presence of flavonoids.[6]

Quantitative analysis of chemical constituents

Total saponins: Five µL extracts (1 mg/ml) were separately applied (samples and standard) to the plate. The mobile phase consisted of hexane-ethyl acetate-methanol (4:4:1) and 20 ml of mobile phase was used per chromatography. Linear ascending development was carried out glass chamber saturated with the mobile phase. The developed plate was scanned at 301 nm using CAMAG TLC scanner equipped with win-CATS- software.

Total flavonoids: Total flavonoid content was estimated by the method of Sathish Kumar *et al*. using Quercetin as reference compound.[8] A volume of 10 mL of extract was added to 0.5 ml of sodium acetate 0.5% solution, 2 ml AlCL3 (2%), added acetic acid up to 50 ml, and kept at room temperature for 25 minutes. The absorbance for the extract and the standard solutions was measured with a UV/Visible spectrophotometer at 425 nm. The standard solution was prepared using a quercetin solution (0-1 mg/mL) to generate a standard curve (r 2 = 0.999). The amount of flavonoids in the extract was presented as per cent quercetin.

GC-MS analysis of extracts: GC-MS analysis was employed for the identification of chemical constituents in PLE and PLH. An Agilent 7890 series GC coupled with a 5975 mass selective detector was equipped with an Agilent HP ultra 2 capillary columns (5%-phenyl)-methylpolysiloxane phase, 30 m x 0.20 mm I.D. with 0.11 µm film thickness) as stationary phase for compound separation. Helium gas at a constant flow rate of 1.2 mL/min. was used as carrier gas. Five µL of each extract was subjected to GC-MS analysis in 8:1 split mode with GC inlet temperature of 250 °C. The GC oven temperature program was started at 80 °C. Subsequently, it was gradually increased at the rate of 3°C/min until reaching 150 °C. The oven temperature was then maintained at 150°C for another 1 min. Finally, the temperature gradually increased at the rate of 20°C/min to 280°C/min and hold for 26 minutes. MS detection was carried out in electron impact (EI) mode with ionization energy of 70 eV. The temperature of transfer line, MS source, and MS quadrupole were kept constant at 280, 230, and 140°C, respectively. A full scan acquisition mode was performed for MS analysis in the mass range from 30 to 600 m/z. The identification of constituents was accomplished by matching their mass spectral data with those in the Chemstation Data System.

Acute toxicity study of purple leaves extract in rats

36 male Sprague-Dawley (SD) rats, aged 6 to 8 weeks, were used in the investigation. Every day, the rats have free access to food and water. During the experiment, the animal room's temperature was roughly 22+3 °C, relative humidity was around 80%, and there was a 12-hour light-dark cycle. Every day, all rats have free access to food and water from the faucet.

The three ethanol extract groups and the three hexane extract groups were split up into seven groups of rats at random. Procedures set by the Organization for Economic Co-operation and Development are followed for conducting toxicology tests (OECD). The doses of the test preparations given were 50, 300 and 2000 mg/kg of the test extract given to each test animal in the treatment group orally. The extract was mixed with water and amount of 20 cc/kg was fed by oral tube thrice daily. The rat fasted in the following 3 hours after administration of extract. Test animals were observed intensively for indications of toxic effects during the first 6 hours, 12 hours, 24 hours after administration of the extract and then observed every day for 14 days. Rats were weighed and observed every day for death, behavioral patterns, physical changes, pain, injuries and signs of illness. On day 14th, blood was withdrawn *via* tail veins, then, rats were sacrificed, and vital organs such as liver, kidney, heart, lungs and spleen were taken and examined for lesions. All organs are weighed individually. The liver and kidneys were then used for histopathology. The procedure of necropsy and sampling of the liver and kidney was as described in Fiette.[9] Blood samples were used to assess the hematological profiles (including the number of white blood cells, red blood cells, hemoglobin, hematocrit, MCH, MCHC, and platelets) and blood chemistry (including the levels of glucose, urea, creatinine, total protein, ALT, and AST) to assess the health of the liver and kidneys.

Statistical analysis

The results were displayed as the mean standard deviation (SD). The homogeneity of the data was determined using Saphiro Wilk. The paired student t test was used to compare pre- and post-treatment body weight, whereas ANOVA was used to examine hematological data. P values less than 0.05 were considered statistically significant. The statistical analysis was evaluated by Jamovi 2.2.2 and displayed in a table.

RESULTS

The purpose of the qualitative phytochemistry is to determine at an early stage which bioactive compounds are suitable for *simplicia* and its extracts, as well as to establish the plant's potential drug activity. This study demonstrated that *Graphtophyllum pictum L. Griff* extracts etanol and hexane contained metabolite seconder such as alkaloid, flavonoid, fenol, saponin, and tannin (Table 1).

Chemical compositions of *Graptophyllum Pictum L Griff* was identified by spectophotometer, flavanoid is higher in hexane extract than ethanol. However, extract etanol has better effect of antioxidant than Hexane (table 1). Results from GCMS yielded different components, such as (2E)-3,7,l l,15-TETRAMETHYL- 3,40 2-HEXADECEN-1-OL high in ethanol than hexane. However, Gamma tocopherol and gamma. – Sitosterol were more in hexane than ethanol extract.

Acute toxicity of extract ethanol and hexane in rats

Acute toxicity of ethanol and hexane extract were assessed following a single dose administered by gavage at a dose of 50, 2000 and 3000 g/kg bw. All rats were survived for 14 days and all animals gained more weight and appeared active. The mean body weight of rats increased from 150 ± 2.22 g to 161 ± 6.68 g during the 14 days. The difference of body weight between before and at 14 days among groups were significantly different with *P* value <0.01.

The observation from macroscopic and gross pathology performed at necropsied yielded no visible lesions in any animals. Therefore, no evidence of acute toxicity of ethanol and hexane extract of G. *pictum* in rats was found. The oral LD 50 values for male rats must be greater than 3000 g/kgBW.

All hematological parameters measured (haemoglobin, haematocryte, leucocyte) were not significantly different. The thrombocyte level was significantly different amongst group (*P* value 0.033). The results of

Table 1: Chemical compositions of *Graptophyllum Pictum L. Griff.*

	Extract Ethanol	Extract Hexane	Method
Saponin (%)	2.76	2.89	TLC scanner
Flavanoid as Quersetin (%)	2.66	6.24	Spectophotometer
Antioxidant IC 50%	698	639	DPPH/Spectophotometer
Tanin(%)	0.13	0.82	
Phytochemistry test			
Alkaloid	+	+	
Saponin	+	+	
Tanin	+	+	
Fenolik	+	+	
Flavanoid	+	+	
(2E)-3,7,l l,15-TETRAMETHYL- 3,40 2-HEXADECEN-1-OL	3.4	1.14	
Hexadecanoic acid, methyl ester	1.71		
HEXADECANOIC ACID	13.71		
9,12-Octadecadicadienoic acid	64.06		
Methyl 8,11,14- 31.354 94 heptadecatrienoat	1.32		
cis,cis,cis-7, 10, 13- 4,44 Hexadecatriena	4.44		
Gamma tocopherol	2.98	5.3	
Stigmastan-3,5-diene	1.11		
NEOPHYT ADIENE		3.26	
Phytol		8.99	
ETHYL (9Z,12Z,15Z)-9,12,15-OCTADECATRIENOATE		2.11	GCMSD (Gas Chromatography Spectrometry Mass)
9,12-Octadecadienoic acid		5.43	
Pyridine-3-carboxamide, ox.ime, N-(2-trifluoromethvlohenvl) -		2.61	
Phenol, 2-methyl		3.5	
Hexanoic acid, heptadecyl ester		4.61	
DELTA.l.DELTA.-CYCLOHEXANEBUT ANOL, .ALPHA.-ETHYNYL		6.2	
.Squalene		7.42	
CELIDONIOL, DEOXY		2.95	
Vitamin E		4.6	
Stigmasterol		15.66	
.gamma.-Sitosterol	1.19	16.87	
ALPHA-AMYRIN		1.55	

Table 2: The results of body weight measurements and hematological measurements of rats administered orally by ethanol and hexane extract of *G. pictum*.

	BW before	BW at 14 day	Hb	Ht	Leucocyte	Thrombocyte	Ureum	Creatine	SGOT	SGPT
CONTROL-50	153±2.22	157±2.5	13.6±1.02	35.3±2.91	4.2±2.52	382±266	31.8±7.41	1±0.115	20.9±7.53	38.9±14.5
CONTROL-2000	159±6.06	161±6.86	14.1±1.83	37.3±5.26	4.1±238	191±97.6	28.3±8.66	0.9±0.141	16.9±6.22	36±7
CONTROL-3000	158±5.23	161±5.56	13.6±0.404	35.3±1.27	2.25±1.33	426±80.3	31.5±0.577	0.8±0.115	19.4±5.31	34.6±11.7
ETANOL-50	158±2.94	161±2.94	13.6±1.02	35.3±2.91	4.2±2.52	382±266	31.8±7.41	1±0.115	20.9±7.53	38.9±14.5
ETANOL-2000	155±4.57	158±5.07	14.1±1.83	37.3±5.26	4.1±2.38	191±97.6	28.3±8.66	0.9±0.141	16.9±6.22	36±7
ETANOL-3000	160±3.1	163±3.86	13.5±0.506	35.2±1.09	2.13±1.2	367±176	33.5±4.36	0.9±0.163	20.8±7.26	29.5±10.2
HEXANE-50	159±2.06	161±2.16	13.3±1.36	34.3±3.68	4.38±2.42	382±266	26.3±7.41	0.925±0.126	16.3±6.01	41.3±11.8
HEXANE-2000	158±4.11	161±3.86	14.4±1.31	38±4.15	4.05±2.4	250±111	31.8±5.32	0.875±0.171	20.1±5.54	38.7±7.99
HEXANE-3000	156±1.29	159±1.29	13.5±0.435	35.6±1.12	1.7±1.13	460±69.5	31.3±0.5	0.65±0.379	17.1±4.58	29.6±10.1
P value	<0.01*		0.976	0.946	0.394	0.033**	0.909	0.523	0.962	0.883

* paired t test

** ANOVA

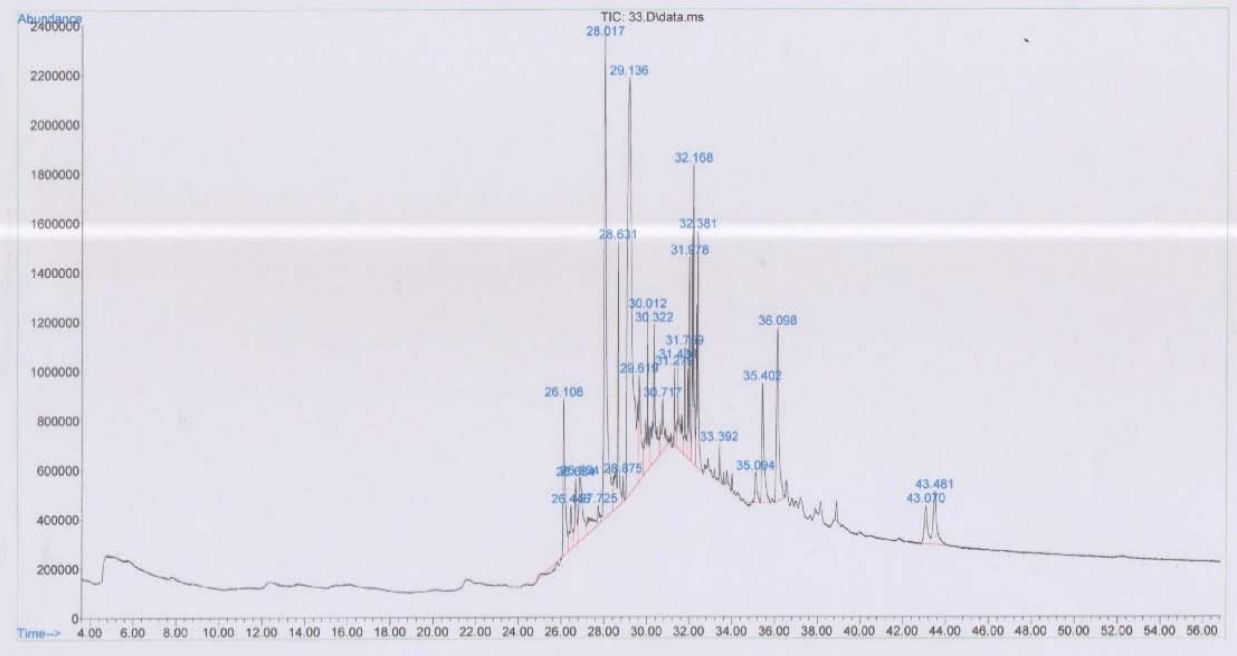

Figure 1: GC-MS chromatogram of ethanol extract of *Graptophyllum pictum* leaves.

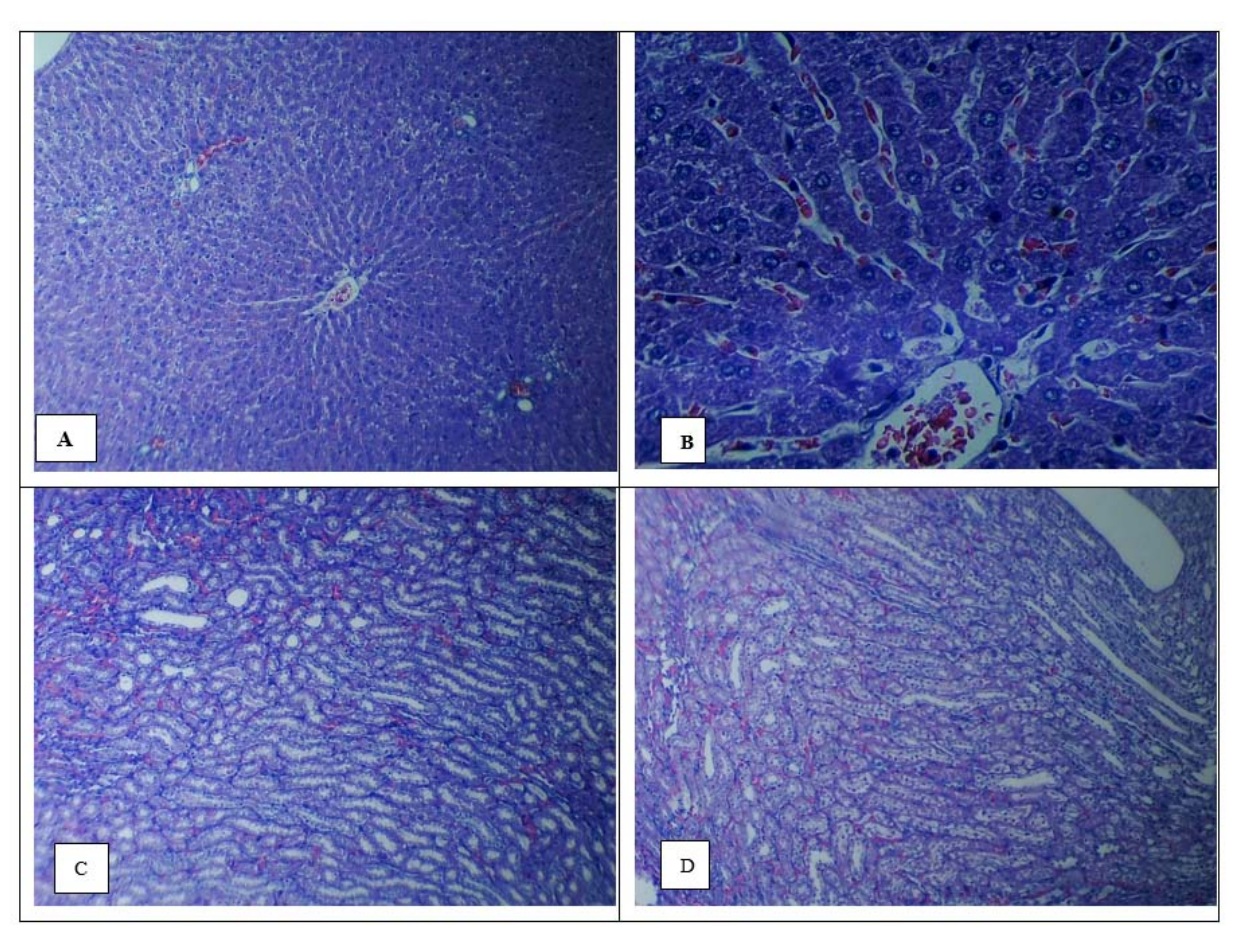

Figure 2: A and B; Rat liver from the hexane-3000 group demonstrating normal histological structure of the central vein (CV) and surrounding hepatocytes (H&E stain, 400x) C and D; Anatomy histologically normal of the capilare and collecting tubules in the kidney of an etanol-3000-group rat.

urea, creatinine, SGOT and SGPT were within normal range and with not significantly different between control and treatment groups (Table 2).

DISCUSSION

Natural products, including minerals, plants and animals, have been used as drugs since ancient times. There are so many plants used for traditional medicine, such as in Ayurvedic Medicine, Chinese Traditional Medicine, Unani Medicine, etc., which probably possess therapeutic effects and need to be proven for its efficacy and safety to fulfil the modern standards of therapeutic agents.[10] The increased use of herbal plants as remedy for various diseases is gaining massive attention due to their lower allergenicity.[11] However, the potential toxicity in many of bioactive substances must be investigated to claim the safety of the herbal.

In this study, phytochemical screening qualitatively showed the presence of flavonoids, tannins, saponins, alkaloids and phenolics in both ethanol and hexane extracts of *G. pictum*. Recent studies on the chemistry of *G. pictum* have shown the occurrence of phenol, flavonoids, tannin, alkaloid, saponin, terpenoid and steroid.[1] Estimation of amount of phytochemical demonstrated that PLH (Purple Leave Hexane) contained more flavonoids, saponin, and tannin than PLE. The DPPH method showed that PLE (Purple Leave Ethanol) and PLH possessed low antioxidant activity with IC_{50} greater than 500 µg/mL. Interestingly, in contrast with these findings, Rustini & Ariati[12] found that PLE possessed high antioxidant activity compared to other extracts.

The use of *G. pictum* preparation as a treatment of disease is very common. In Indonesia, it has been popularly used as treatment of haemorrhoid. Some of these usages have been studied *in vitro* and *in vivo*. Recently many commercial products in form of *jamu,* powder and capsule containing *G. pictum* extract have become available in the market, especially in Indonesia.

The results of acute toxicity study indicated that PLE and PLH (Purple Leave Hexane) did not cause visible signs of toxicity or mortality. The body weight changes have been observed for 14 days of experiment. However, the results showed that PLE and PLH did not significantly affect body weight as compared to corresponding baseline values. These results suggest that the extracts did not disturb rat growth; this is with the agreement to the toxicity classification reported by Loomis and Hayes.[13]

The macroscopic examination of vital organs showed no abnormality. The histological evaluation did not reveal any pathology after PLE and PLH treatment. Furthermore, haematological biochemical values were found normal in PLE and PLH treated groups compared to normal group.

This result indicated the evidence of safety at the tested doses when administered orally. The safety of treatment was also confirmed by the absence of behavioural changes and absence of difference in feed consumption between the treated and normal control groups. Hence, the results clearly showed that oral administration of PLE and PLH are safe at the evaluated doses (50, 2000 and 3000 mg/Kg) in Sprague Dawley rats. In this context, the data presented in this report would further in utilizing the traditional medicinal and health benefit attributes of *G. pictum*, which is finding increasing use in several herbal products that are currently being marketed in Indonesia.

CONCLUSION

The current study showed that hexane extract contains more valuable components for medical treatment purposes. The acute toxicity on 50, 2000,3000 kg/BW oral ethanol and hexane extract of *Graptophyllum pictum* showed no significant influence on hematological blood parameters of rats. It is safe to administered orally ethanol or hexane extract of G. *pictum* below 3000mg/kgBW rats.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was derived from UPN Veteran Jakarta commission ethics, Number: 2770/IX/2020/KEPK**.**

CONSENT FOR PUBLICATION

This study does not relate to patients, there is no need consent for publication.

AVAILABILITY OF DATA AND MATERIALS

Authors are ready to give the data when it is needed.

COMPETING INTERESTS

Authors affirm they have no competing interests.

FUNDING

FAM has a national grant from (Ministry of Education, Culture, Research, and Technology) No 013/UN.61.4/2022.

AUTHORS' CONTRIBUTIONS

FAM wrote the manuscript, EPR analysed the result and wrote the discussion, YS documented the results.

ACKNOWLEDGEMENTS

Special appreciation to Rector of UPN Veteran Jakarta Dr Ernawati, Dean Taufiq Fredrik Pasiak for their tremendous support and collaboration in providing infrastructure for research.

REFERENCES

1. Makkiyah F, Makkiyah FA, Rahmi EP, Revina R, Susantiningsih T, Susantiningsih T, *et al*. Graptophyllum pictum (L.) Griff. (Syn: *Justicia picta* Linn.) and its Effectiveness: A Well-Known Indonesian Plant. Pharmacogn J. 2021;13(3):835-8.
2. Pranoto H. Kualitas Spermatozoa Dan Indeks Fertilitas Tikus Putih Dewasa (*Rattus novergicus* L.) Setelah Pemberian Ekstrak Daun Wungu (*Graptophyllum pictum*. L.Grifft). Jurnal Biologi Lingkingan Industri Kesehatan. 2018;4(2).
3. Sari IP, Yulizar Y. Green synthesis of magnetite (Fe3O4) nanoparticles using Graptophyllum pictum leaf aqueous extract. IOP Conference Series: Materials Sci Eng. 2017.
4. Osman MS, Ismail MH, Khairuddin K, Azis N, Salim NS, Zaini NA, *et al*. Bioynthesis of silver nanoparticles using Graptophyllum pictum leaves extracts. IOP Conference Series: Materials Sci Eng. 2019.
5. Wattimena SC, Silooy DR, Patty PJ. Characterization of green silver nanoparticles of Graptophyllum pictum leaf extract: From the localized surface plasmon resonance to the antimicrobial activity. J Physics: Conference Series. 2021.
6. Dewi, NP. Uji Kuantitatif Metabolit Standar Ekstrak Etanol Daun Awar-Awar (*Ficus Septica* Burm. F) Dengan Metode Kromatografi. Acta Holistica Pharmaciana. 2020;2(1):16-24.
7. Ningsih DS, Henri H, Roanisca O, Mahardika RG. Skrining Fitokimia dan Penetapan Kandungan Total Fenolik Ekstrak Daun Tumbuhan Sapu-Sapu (*Baeckea frutescens* L.). Biotropika: J Tropical Biol. 2020;8(3):178-85.
8. Kumar TS, Shanmugam S, Palvannan T, Kumar VB. Evaluation of antioxidant properties of Elaeocarpus ganitrus Roxb. Leaves. Iranian J Pharm Res. 2010;3(1):211-5.
9. Fiette L, Slaoui M. Necropsy and sampling procedures in rodents. Methods Mol Biol. 2011;691:39-67.

10. Farnsworth NR, Soejarto DD. The Conservation of Medicinal Plants: Global importance of medicinal plants. 1991;26:25-51.
11. Unno T, Sugimoto A, Kakuda T. Xanthine oxidase inhibitors from the leaves of *Lagerstroemia speciosa* (L.) Pers. J Ethnopharmacol. 2004;93(2-3):391-5.
12. Rustini NL, Ariati NK. Identification of Active Antioxidant Compounds from Ungu Leaf Ethanol Extract (*Graptophyllum pictum* L. Griff). J Health Sci Med. 2018;2(1):4.
13. Hayes AW, Loomis TA. Loomis's essentials of toxicology. Elsevier. 1996.

ABOUT AUTHORS

Feda Makkiyah is a fulltime lecture, neurosurgeon in school of medicine UPN Veteran Jakarta that has special interest in herbal medicine.

Eldiza Puji Rahmi is a lecture and pharmacist that has the special interest in herbal medicine.

Yuni Setyaningsih is a fulltime lecture and parasitologist-science that collaborated in the herbal medicine research cluster.

Cite this article: Makkiyah F, Rahmi EP, Setyaningsih Y. Phytochemical Test and Acute Safety Evaluation of Oral Purple Leaves (Graptophyllum Pictum L. Griff) Extract in Rats. Pharmacogn J. 2022;12(5): 649-654.