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Hepatoprotective Activity of Pirdot Leaves (*Saurauia vulcani* Korth) Ethanol Extract in Laboratory Rats (*Rattus norvegicus*) and Characterization of Bioactive Compounds Using a Molecular Docking Approach

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Abstract

AIM: The purpose of the research was to determine the hepatoprotective activities of bioactive compounds pirdot by *in vivo* and *in silico* study.

METHODS: In this study, the completely randomized design non-factorial was experimentally to assess the value of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) and 24 adult male rats were divided into four groups: Group G_0, control group; group G_1, a treated group received 0.1 ml sheep red blood cell (SRBC); group G_2, a treated group received 500 mg ethanol extract pirdot; and group G_3, a group treated received 500 mg ethanol extract pirdot and 0.1 ml SRBC. On 31 days of treatment, the blood of all rats group was taken to value SGPT and SGOT using DiaLab kit. Furthermore, the molecular docking study was done to analyze molecular interaction of cyclooxygenase 2 (COX-2) and tumor necrosis factor alpha (TNF-α).

RESULTS: The results show SGOT and SGPT value significantly ($p < 0.05$) decreased on group G_2 and G_3. Moreover, the bioactive compounds of pirdot, such as pomolic acid and ursolic acid, tend to be the potential compound on liver protection. Moreover, pomolic acid has a good binding affinity –14.6 kcal mol^{-1} with COX-2 protein and the binding affinity of cis-3-O-p-hydroxycinnamoyl ursolic acid was –15.1 kcal mol^{-1} associated with TNF-α protein.

CONLUSION: Pirdot leaves (*Saurauia vulcani* Korth) ethanol extract showed hepatoprotective activity in rats (*Rattus norvegicus*). Molecular docking approach showed that pomolic acid has a good binding affinity with COX-2 protein and TNF-α protein.

Introduction

The liver has a very important role in the process of detoxification and excretion of various compounds in the circulatory system [1]. Liver interaction with these compounds induces an adaptive immune response that produces inflammatory cytokines such as interferon-γ and tumor necrosis factor (TNF)-α [2]. Liver damage is also caused by the use of drugs and chemical compounds that are toxic [3]. In the process of metabolic decompensation, liver function is related to the immune system [4]. Furthermore, hepatocytes and kupffer cells have an important role in antigen metabolism in the liver [5]. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) values are used as indicators in evaluating liver damage. SGOT is an enzyme found in the cytoplasm and mitochondria of liver cells. SGOT levels can be used to assess the extent of liver damage. Meanwhile, SGPT is an enzyme that is produced when there is damage to liver cells by seeing an increase in SGOT levels in blood serum [6]. The increase in SGPT or aspartate aminotransferase is due to changes in permeability or damage to the liver cell walls. Thus, it is used as a marker for impaired hepatocellular liver cell integrity. Secondary metabolites produced by plants are currently one of the alternatives in the treatment of liver damage [7]. *Saurauia vulcani* Korth is a family *Actinidiaceae* that has secondary metabolites of polyphenols, flavonoids, steroids, saponins, tannins [8], and triterpenoid [9]. According to a previous study, pirdot leaf flavonoid compounds are genistein isoflavones that function to reduce glucose levels and glucose tolerance in the body, heal wounds, and increase insulin levels in the body [10]. High flavonoids in pirdot leaves tend to be hepatoprotective. This study aims to determine the levels of SGOT, SGPT, and liver morphology from ethanol extract of pardon leaves and to predict molecularly the interaction of *Saurauia* secondary metabolites with proteins involved in oxidative stress and inflammation in the liver.

Materials and Methods

Preparation of test animals

Twenty-four laboratory rats (*Rattus norvegicus*) aged 3 months, weighing 150–200 g, were obtained from the Pharmacy Laboratory, Institut Teknologi Bandung. They were then acclimatized at a temperature of 24 ± 27°C for a week in the Pharmacy Laboratory, Universitas Padjajaran, Bandung. Then, the mice were treated for 30 days and given food and *ad libitum* water.

Preparation of pirdot leaf ethanol extract Saurauia leaf ethanol extract (EES)

Six km of pirdot leaves, obtained from North Tapanuli (North Sumatra, Indonesia), were dried and mashed to produce pirdot leaf flour. A 500 g of pirdot leaf flour were put into two places, 250 g each, and added with distilled ethanol (1.875 mL/place). Pirdot flour was then soaked for 5 s and was stirred twice a day. The filtrate obtained was separated using filter paper to obtain 2.1 l with the addition of ethanol further to get 3 l of solution. After that, the filter results were concentrated using a rotary-evaporator and left to stand in a water bath for 4 days. Fifty-one g of ethanol extract of pirdot were given orally to laboratory rats 500 mg/kg for 30 days [11].

Preparation of sheep red blood cells (SRBC)

Sheep blood as antigen was obtained from the Lembang Veterinary Laboratory, Bandung, Indonesia. Sheep blood was taken from the blood vessels in the neck as much as 5 mL and washed using phosphate buffered saline with a pH of 7.4 then centrifuged at 200 rpm for 15 min. Centrifugation was carried out 3 times. Blood specimens were stored in the freezer at –4°C.

Effect of pirdot leaf ethanol extract on SGPT and SGOT values

The laboratory rats were anesthetized after 30 days of treatment. Blood was collected from the treated group of laboratory rats through a vein from the neck of the laboratory rats. The serum was separated by centrifugation at 2500 rpm for 10 min. SGOT and SGPT were analyzed by spectrophotometric measurements (Dialab, 2006, Liquid Reagents of GOT, Austria: DIALAB Production von chemish-technishen.) [12].

Liver histopathology examination and degree of hepatocyte damage

Liver pieces were washed with 0.9% saline solution and preserved with 10% formalin solution at the fixation stage which was continued at the paraffin stage with hematoxylin and eosin staining [13], [14]. The histopathological assessment was carried out under a microscope with a magnification of 400 times. Then, the mean weight of the liver histopathological change score was calculated in five fields of each mouse using the *Manja Roenigk Histopathology Scoring model* [15]. The hepatic structures observed were normal hepatocytes and hepatocytes that have both necrosis, parenchymal degeneration, and hydropic degeneration. Then, recorded and counted the percentage of damage that occurred.

Molecular docking study

A research conducted by Pasaribu in 2020 reported the results of the phytochemical analysis of *Saurauia* having bioactive compounds, namely, 3.19-Dihydroxyurs-12-en-28-oic acid; cis-3-O-p-hydroxycinnamoyl ursolic acid; corosolic acid; maslinic acid; and genistein. These compounds were evaluated for molecular interactions between target proteins involved in the inflammatory process and oxidative stress. This was done to further analyze the molecular mechanism of the hepatoprotective activity of pirdot leaves [16].

The target proteins involved in the production of reactive oxygen species were TNF-α (PDB: 2AZ5), cyclooxygenase 2 (COX-2) with ibuprofen as an anti-inflammatory drug (PDB: 4PH9) [17], [18]. The ligand, a bioactive compound of pirdot leaves, was obtained from the PubChem database. Energy minimization was carried out in it using the mmff94, and docking with AutoDock Vina. Visualization of molecular interactions was carried out with the Chimera and Protein Plus database.

Data analysis

The experimental design used in this study was a completely randomized design. Twenty-four laboratory rats were grouped into four groups: The control group was given distilled water (G_0), the treatment group was given SRBC (G_1), the treatment group was given pirdot lead ethanol extract (EEP) (G_2), and the treatment group was given pirdot leaf extract (EEP) + SRBC (G_3). The obtained data were analyzed using Duncan's *post-hoc* test ($p < 0.05$), using Statistical Package for the Social Sciences version 24 software at an error rate of 5%.

Results

Analysis of SGOT and SGPT levels

The measurement of the SGOT and SGPT values is presented in Table 1. In this study, the SGOT

levels in the G_1, G_2, and G_3 treatments were lower than the control. SGOT levels in the control treatment laboratory rats were at 248.27 ± 58.75 with the administration of SRBC decreased to 189.77 ± 77.59, and in the G_3 and G_2 treatments were 191.55 ± 35.55 and 196.60 ± 24.37, respectively. In this study, SGPT levels decreased significantly. SGPT in the control treatment was 121.74 ± 7.62, and SGPT in the G_2 and G_3 treatments was 76.44 ± 11.38 and 55.50 ± 15.75. In the laboratory rats of the G1 treatment alone, the SGPT level increased to 214.00 ± 15.34.

Table 1: Effect of pirdot leaf ethanol extract on SGOT and SGPT values

Group	SGOT	SGPT
Control (G_0)	248.27 ± 58.75	121.74 ± 7.62*
SDMD (G_1)	189.77 ± 77.59	214.00 ± 15.34
EES (G_2)	196.60 ± 24.37	76.44 ± 11.38*
EES+SDMD (G_3)	191.55 ± 35.25	55.50 ± 15.75*#

*Significantly different to G1 group (p<0.05), #Significantly different to G2 group (p < 0.05), SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, EES: Ethanolic extract of pirdot leaves (*S. vulcani* Korth).

Effect of EES on liver histology and level of hepatocyte damage

According to the Kleiner research in 2018, the liver has a special pattern in indicating the condition of the liver tissue [19]. The pattern in the tissue indicates whether the degree of damage to the liver tissue was caused by the drug or not. Figure 1 visualizes that the histological observations of the liver of male laboratory rats found cell changes. The percentage of normal hepatocytes in the G1 group was the most compared to other treatments with a large number of cells experiencing swelling, hydropic, and necrosis.

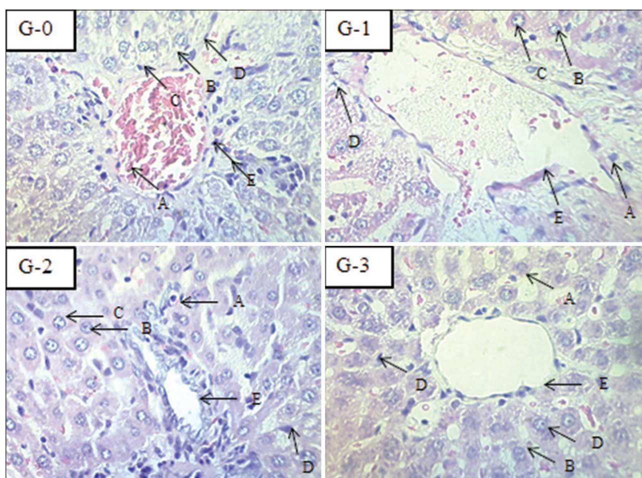

Figure 1: Histopathology of the liver of the test animal; G0: As a control (NA CMC), G1: Sheep red blood cells (SRBC), G2: Saurauia leaf ethanol extract (EES), and G3: EES + SRBC. (Magnification of 10 × 40) Description: (A) Normal, (B) parenchymptous degeneration, (C) hydropic degeneration, (D) necrosis, and (E) central vein

The results of observations made on the microscopic image of the liver given SRBC and EES found normal hepatocytes and hepatocytes that had changes in the form of parenchymal degeneration, hydropic degeneration, and necrosis.

In silico docking study

The results of molecular docking of bioactive compounds of pirdot on COX-2 protein (PDB: 4PH9) and TNF-α protein (PDB: 2AZ5) are shown in Figures 2 and 3. Table 2 shows the binding affinity of the molecular interactions of the compounds (ligand) on the target protein. The lowest and highest binding energies on the target protein of COX-2 (PDB: 4PH9) were found in pomolic acid (–14.6 kcal mol^{-1}) and genistein (–9.7 kcal mol^{-1}), respectively. Furthermore, the TNF-α protein target (PDB: 2AZ5) can be seen that the lowest binding energy was found in the cis-3-O-p-hydroxycinnamoyl ursolic acid (–15.1 kcal mol^{-1}) and the highest was in genistein (–8.6 kcal mol^{-1}). These findings were supporting previous studies [21], which stated that ursolic acid has significant potential in alcohol-induced liver protection as well as pomolic acid which is used as an anti-inflammatory [22].

Discussion

The data analysis presented in Table 1 shows that the decrease in SGOT levels was significant compared to the control group. The tendency of SGOT decreases in EES treatment showed the hepatoprotective properties of pirdot leaves. This is in line with a previous study conducted by Suryaningsih in 2017, where the administration of sambiloto extract did not affect hepatocyte function in laboratory rats [23]. This hepatoprotective property is shown by the decrease in SGOT in serum after administration of EES in serum.

SGPT levels in the G_2, $G_{3,}$ and G_0 treated laboratory rats in this study decreased significantly compared to the SGPT levels of the G_1 treated rats. The lowest SGPT was found in the G_3 and G_2 treatment rats. The least significant difference test showed that the SGPT level of the rats in the G_3 treatment was significantly lower than the SGPT level in the EES treatment and control rats. The SGPT level of the G_1 treatment rats was almost double the SGPT level of the control rats (Table 1).

The decrease in SGPT levels in the G_2 and G_3 treatments in this study showed the hepatoprotective properties of pirdot leaves. Thus, the liver function is well maintained and the immune system that is carried out by the liver runs well. The hepatoprotective properties of pirdot leaves are due to the presence of ursolic acid, which is closely related to liver protection. According to the research by Ma in 2020, administration of ursolic acid 20–80 mg kg^{-1}/day can reduce liver damage due to alcohol use [21].

The liver has an important function in innate and adaptive immunity. According to the research by Cai *et al*. in 2018, the liver contributes to the innate (non-specific)

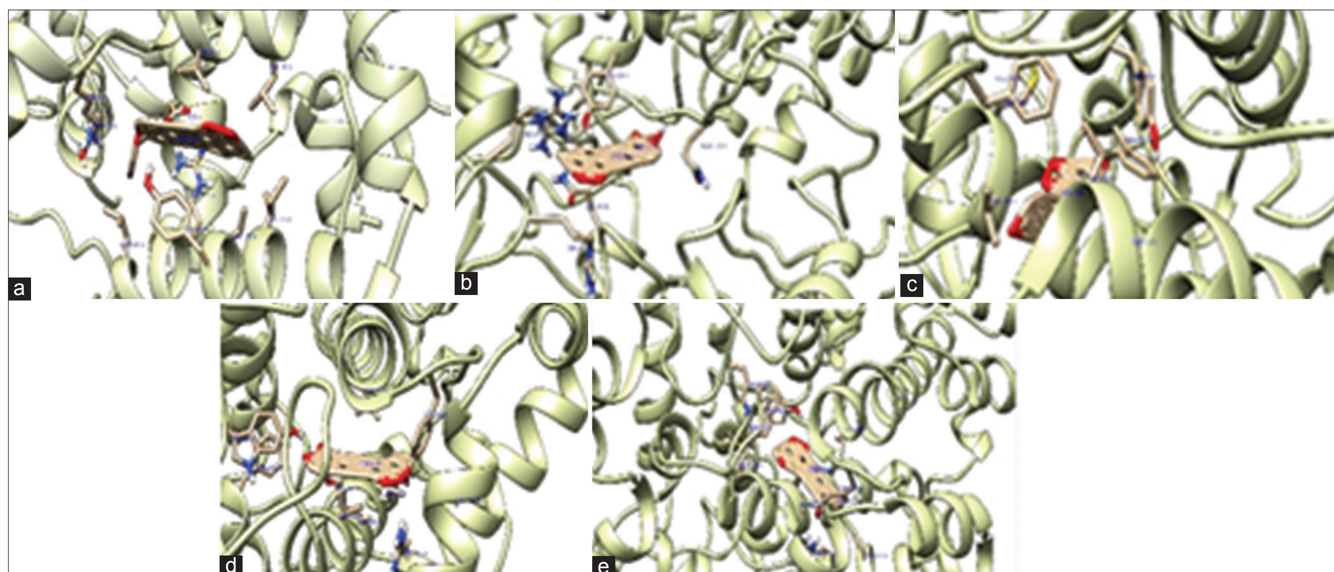

Figure 2: Molecular docking interaction between cyclooxygenase-2 protein (PDB: PDB: 4PH9) and bioactive compounds of pirdot leaf; (a) cis-3-O-p-hydroxycinnamoyl ursolic acid; (b) corosolic acid; (c) genistein; (d) maslinic acid; and (e) pomolic acid, visualized using Chimera 1.14

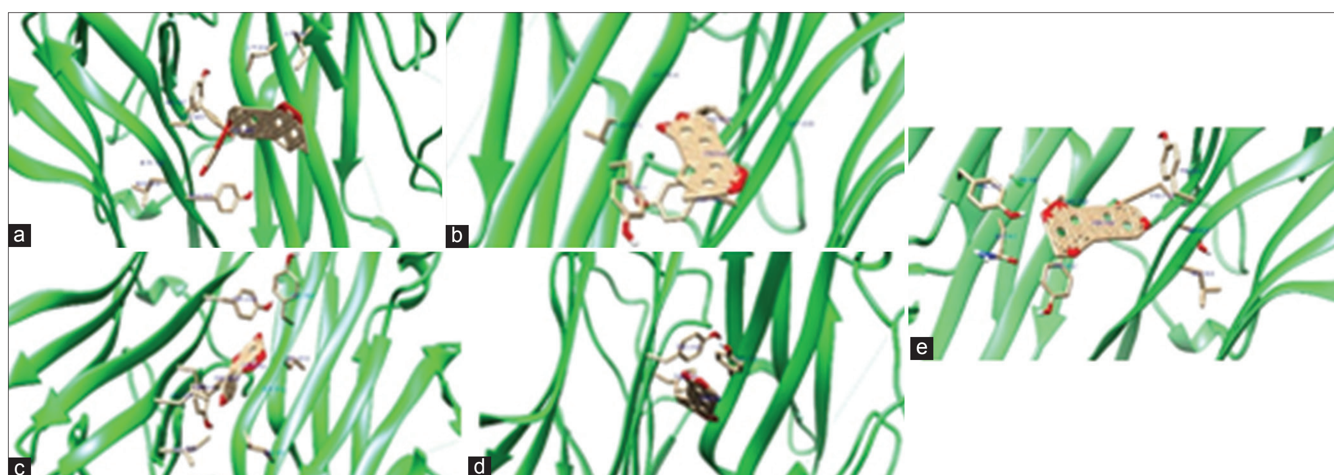

Figure 3: Molecular docking interaction between cyclooxygenase-2 protein (PDB: PDB: 4PH9) and bioactive compounds of pirdot leaf; (a) cis-3-O-p-hydroxycinnamoyl ursolic acid; (b) corosolic acid; (c) genistein; (d) maslinic acid; and (e) pomolic acid, visualized using Chimera 1.14

immune system including the acute protein phase, non-specific particle phagocytosis, non-specific molecular pinocytosis, and non-specific cell-killing [24].

Histological observation of the liver can be used to diagnose the level of damage to the liver, especially the level of damage to the hepatocytes [20]. The results

Table 2: The interaction of the protein bonds between COX-2 (PDB: 4PH 9) and TNF-α (PDB: 2AZ5) with bioactive compounds of pirdot

Bioactive compound	COX-2 (PDB: 4PH 9)			Bioactive compound	TNF-α (PDB: 2AZ5)		
	Binding affinity (kcal/mol)	Hydrophobic interaction	Hydrogen bond		Binding affinity [kcal/mol]	Hydrophobic interaction	Hydrogen bond
cis-3-O-p-hydroxycinnamoyl ursolic acid	–14.1	Pro84A, Val89A, Leu93A, Ile113A, Tyr116, and Leu124A	Lys83A, Arg121A, Phe471A, and Glu525A	cis-3-O-p-hydroxycinnamoyl ursolic acid	–15.1	Leu57A, Tyr59A, and Tyr119B	Leu120B
Corosolic acid	–13.6	Val350A, Leu353A, Tyr356A, Phe519A, Val524A, and Ala528A	His90A, Leu353A, Phe519A, and Ser531A	Corosolic acid	–13.6	Tyr59B, Tyr119A, and Tyr119B	Tyr119B, Gly121A, and Tyr151B
Genistein	–9.7	Leu353A, Phe519A, and Val524A	Tyr386A, Gly527A, and Ser531A	Genistein	–8.6	Tyr59D	Tyr119C
Maslinic acid	–14.0	Val350A, Tyr356A, Leu360A, Trp388A, Val524A, Ala528A, and Leu532A	Arg121A, Tyr356A, Tyr386A, and Ser531A	Maslinic acid	–13.7	Tyr59B, Tyr119A, and Tyr151B	Ser60A and Leu120B
Pomolic acid	–14.6	Val117A, Val350A, Tyr356A, Trp388A, Phe519A, and Ala528A	Tyr356A, Tyr386A, and Ser531A	Pomolic acid	–14.6	Tyr119C and Tyr119D	Ser60D, Leu120D, and Tyr151C

COX-2: Cyclooxygenase 2, TNF-α: Tumor necrosis factor alpha.

of observations on the level of hepatocyte damage in each treatment with SRBC and pirdot leaf extract were statistically presented in Table 3. The increase in cell death (necrosis) in the G_1 group with SRBC was 5.8%, followed by the G_3 group of 2.3%. According to the research by Vinken in 2021, hepatocytes are the type of cells that make up most of the liver [25]. Hepatocytes are responsible for the central role of the liver in metabolic processes. These cells are located between the sinusoids that fill with blood and the bile ducts. Furthermore, another study argues that liver cell damage is rarely caused by a substance but often by a metabolic toxic substance [26]. The cell contained in the liver might be deposited and might also change [27].

Table 3: Cross tabulation results in the liver treatment of laboratory rats (*Rattus norvegicus*)

Parameters	Group (%)				Total (%)
	G0	G1	G2	G3	
Normal	21.3	7.0	20.8	19.0	68.0
Degeneration of parenchymatose	1.8	5.8	1.5	1.8	10.8
Degeneration of hydropic	1.5	6.5	1.8	2.0	11.8
Necrosis	0.5	5.8	1.0	2.3	9.5
Total	25.0	25.0	25.0	25.0	100.0

According to the research by Kleiner in 2018, if the liver cells are damaged due to various factors, there will be a series of morphological changes in the liver cells. Some changes in the structure of the liver due to chemical compounds that can be seen under microscopic observation include inflammation, fibrosis, degeneration, and necrosis [28]. These changes can be sublethal, namely, degenerative or lethal in the form of necrotic [29]. After damaging the cell membrane, the toxic effects can also reach the nucleus and damage it, resulting in abnormal cell structure and eventually leading to necrosis [30]. Hepatocyte swelling only occurs in the mitochondria and endoplasmic reticulum due to stimulation that results in oxidation. In the treatment groups, the hepatocyte damage occurred possibly due to the influence of the treatment causing impairment of cell membrane permeability. The disruption of cell membrane permeability was due to the effect of the administration of pirdot leaf ethanol extract and SRBC where high content of pirdot leaf extract with triterpenoids [9] and flavonoids that interfere with free radicals [8] (Figure 1).

This is following the results of the *in silico* study using the molecular docking approach. Bioactive compounds from pirdot leaves which are triterpenoids such as ursolic acid, maslinic acid, and pomolic acid as well as genistein compounds which are included in the flavonoid class, have great potential in protecting the liver. These bioactive compounds have significant interactions on the target protein, namely, COX-2 and TNF- α. Pomolic acid compounds in pirdot leaves have the lowest binding energy of –14.6 kcal mol^{-1} in the bonding interaction with COX-2 protein (Table 2) which is shown in Figure 3. These compounds form hydrophobic interactions and hydrogen bonds in Tyr356A. Furthermore, the ursolic acid compounds in pirdot leaves also have a significantly low binding energy of –14.1 kcal mol^{-1} by interacting with Arg121A on COX-2 protein. This is supporting the interaction of ibuprofen as a drug that interacts with the active site of the binding of Tyr356A and Arg121A COX-2 [17].

TNF-α is a cytokine released by kupffer cells in the liver in response to toxic agents that enter the body [31]. In this study, bioactive compounds from pirdot leaves (Figure 3 and Table 2) had a significant interaction on the active side of TNF-α protein [32]. Ursolic acid in pirdot leaves has a significantly lower binding affinity of 15.1 kcal mol^{-1} by forming the hydrophobic interaction of Tyr59A and hydrogen bonding Leu120B on the binding site of TNF-α protein, which is following the finding of a previous study reported by Kumar *et al*. [33].

Conclusion

The potential hepatoprotective activity of pirdot leaf extract gave significant results both *in vivo* and *in silico*. A significant decrease in SGOT and SGPT values can be seen in the group given pirdot leaf ethanol extract. This is also supported by the *in silico* approach through molecular docking. Bioactive compounds from pirdot leaves such as pomolic acid and ursolic acid can inhibit the binding active side of COX-2 and TNF-α protein with significant binding affinity and molecular interactions, namely, hydrophobic interactions and hydrogen bonds formed on the binding active site of the target protein.

References

1. Ilyas U, Katare DP, Aeri V, Naseef PP. A Review on hepatoprotective and immunomodulatory herbal plants. Pharmacogn Rev. 2016;10(19):66-70. https://doi.org/10.4103/0973-7847.176544
   PMid:27041876
2. Ju C, Phillips E, Holt MP, Gao YR, Lammert C.The adaptive immune system and liver toxicity. In: Third E, editor. McQueen CABT-CT. Oxford: Elsevier; 2018. p. 346-67. https://doi.org/10.1016/b978-0-12-801238-3.95663-0
3. Villanueva-Toledo JR, Chale-Dzul J, Castillo-Bautista C, Olivera-Castillo L, Rangel-Méndez JA, Graniel-Sabido MJ, *et al*. Hepatoprotective effect of an ethanol extract of Tradescantia pallida against CCl4-induced liver damage in rats. S Afr J Bot. 2020;135:444-50. https://doi.org/10.1016/j.sajb.2020.09.031
4. Tarasenko TN, McGuire PJ. The liver is a metabolic and immunologic organ: A reconsideration of metabolic decompensation due to infection in inborn errors of metabolism (IEM). Mol Genet Metab. 2017;121(4):283-8. https://doi.org/10.1016/j.ymgme.2017.06.010
   PMid:28666653
5. Horst AK, Neumann K, Diehl L, Tiegs G. Modulation of liver tolerance by conventional and nonconventional antigen-presenting cells and regulatory immune cells. Cell Mol Immunol.

2016;13(3):277-92. https://doi.org/10.1038/cmi.2015.112

PMid:27041638

6. Shah VV, Shah VK, Sheth NR, Patel MM. Hepatoprotective and Anti-inflammatory activities of hydroalcoholic extract of bark of *Erythrina indica*. Res J Pharmacol Pharmacodyn 2017;9(4):189-94. https://doi.org/10.5958/2321-5836.2017.00033.7
7. Babu G, Shalima NK, Divya TA. Evaluation of hepatoprotective activity of rhizomes of *Curculigo orchioides* Gaertn. Res J Pharm Technol. 2013;6(10):1127-30.
8. Sitorus P. Characterization simplisia and ethanolic extract of pirdot (*Saurauia vulcani*, Korth) leaves and study of antidiabetic effect in alloxan induced diabetic mice. Int J Chemtech Res. 2015;8:789-94.
9. Situmeang BR, Suparman AR, Kadarusman M, Parumbak AS, Herlina T. Isolasi senyawa triterpenoid dari ekstrak etil asetat pirdot (*Saurauia vulcani*. Kurth). J Kim Valensi. 2018;4(2):93-7.
10. Lovena TN, Harahap U, Sitorus P, Satria D. Antioxidant activity and α-glucosidase inhibition effect of water extract Saurauia vulcani Korth leaves. J Innov Pharm Biol Sci 2018;5(3):47-51.
11. Sinaga E, Ilyas S, Hutahaean S, Sitorus P. Immunostimulatory activity from pirdot leaves ethanolic extract (*Saurauia vulcani* Korth.) in Rats (*Rattus norvegicus*). IOP Conf Ser Earth Environ Sci. 2019;305(1):012082. https://doi.org/10.1088/1755-1315/305/1/012082
12. Kumar Gupta R, Swain SR, Sahoo J, Gupta A, Chaudhary S. Hepatoprotective potential of *Trichosanthes dioica* Roxb in hepatotoxicity induced by simvastatin and its consequences on biochemical and haematological indices. Pharmacogn J 2018;10(4):720-4. https://doi.org/10.5530/pj.2018.4.120
13. Araya EM, Adamu BA, Periasamy G, Sintayehu B, Gebrelibanos Hiben M. *In vivo* hepatoprotective and *In vitro* radical scavenging activities of *Cucumis ficifolius* A. rich root extract. J Ethnopharmacol. 2019;242:112031. https://doi.org/10.1016/j.jep.2019.112031

PMid:31220599

14. Vasanthkumar T, Hanumanthappa M, Hanumanthappa SK. Hepatoprotective effect of curcumin and capsaicin against lipopolysaccharide induced liver damage in mice. Pharmacogn J. 2017;9(6):947-51. https://doi.org/10.5530/pj.2017.6.148
15. Rongngern P, Chularojanamontri L, Wongpraparut C, Silpa-Archa N, Chotiyaputta W, Pongpaibul A, *et al*. Diagnostic performance of transient elastography for detection of methotrexate-induced liver injury using Roenigk classification in Asian patients with psoriasis: A retrospective study. Arch Dermatol. 2017;309(5):403-8. https://doi.org/10.1007/s00403-017-1733-4

PMid:28303329

16. Pasaribu G, Budianto E, Herry C, Saepudin E. A review on genus saurauia: Chemical compounds and their biological activity. Pharmacogn J. 2020;12:657-66. https://doi.org/10.5530/pj.2020.12.97
17. Orlando BJ, Lucido MJ, Malkowski MG. The structure of ibuprofen bound to cyclooxygenase-2. J Struct Biol. 2015;189(1):62-6. https://doi.org/10.1016/j.jsb.2014.11.005

PMid:25463020

18. Khanal P, Patil BM. *In vitro* and *in silico* anti-oxidant, cytotoxicity and biological activities of Ficus benghalensis and Duranta repens. Chin Herb Med. 2020;12(4):406-13. http://doi.org/10.1016/j.chmed.2020.02.004
19. Kleiner DE. Histopathological challenges in suspected drug-induced liver injury. Liver Int. 2018;38(2):198-209. https://doi.org/10.1111/liv.13584

PMid:28865179

20. Roth NC, Qin J. Histopathology of alcohol-related liver diseases. Clin Liver Dis. 2019;23(1):11-23. https://doi.org/10.1016/j.cld.2018.09.001

PMid:30454825

21. Ma X, Zhang M, Fang G, Cheng C, Wang M, Han Y, *et al*. Ursolic acid reduces hepatocellular apoptosis and alleviates alcohol-induced liver injury via irreversible inhibition of CASP3 *in vivo*. Acta Pharmacol Sin. 2020;42(7):1101-10. http://doi.org/10.1038/s41401-020-00534-y

PMid:33028983

22. Dzoyem JP, Nganteng DN, Melong R, Wafo P, Ngadjui B, Allémann E, *et al*. Bioguided identification of pentacyclic triterpenoids as anti-inflammatory bioactive constituents of *Ocimum gratissimum* extract. J Ethnopharmacol. 2021;268:113637. https://doi.org/10.1016/j.jep.2020.113637
23. Suryaningsih NM, Dewi IA, Suksmawati NK, Putri NP, Febrianti NM, Warditiani NK. Pengaruh kadar SGOT SGPT dan morfologi hepar tikus putih betina wistar pada pemberian isolat andrografolid. J Farm Udayana. 2017;6(1):34-8.
24. Cai J, Zhang XJ, Li H. Role of innate immune signaling in non-alcoholic fatty liver disease. Trends Endocrinol Metab. 2018;29(10):712-22. https://doi.org/10.1016/j.tem.2018.08.003
25. Vinken M. Primary hepatocyte cultures for liver disease modeling. Curr Opin Toxicol. 2021;25:1-5. https://doi.org/10.1016/j.cotox.2020.08.003
26. Goodla L, Manubolu M, Pathakoti K, Poondamalli PR. Preventive and curative effects of *Cocculus hirsutus* (Linn.) Diels leaves extract on CCl4 provoked hepatic injury in rats. Egypt J Basic Appl Sci. 2017;4(4):264-9. https://doi.org/10.1016/j.ejbas.2017.10.004
27. Oke GO, Abiodun AA, Imafidon CE, Monsi BF. *Zingiber officinale* (Roscoe) mitigates CCl4-induced liver histopathology and biochemical derangements through antioxidant, membrane-stabilizing and tissue-regenerating potentials. Toxicol Rep. 2019;6:416-25. https://doi.org/10.1016/j.toxrep.2019.05.001

PMid:31193041

28. Yamakawa Y, Doi T, Naitou Y, Kawai H, Mitsumoto A, Kudo N, *et al*. A single pretreatment with clofibric acid attenuates carbon tetrachloride-induced necrosis, but not steatosis, in rat liver. Food Chem Toxicol. 2020;145:111591. https://doi.org/10.1016/j.fct.2020.111591
29. Rashidi N, Khatibjoo A, Taherpour K, Mohammad AG, Shirzadi H. Effects of licorice extract, probiotic, toxin binder and poultry litter biochar on performance, immune function, blood indices and liver histopathology of broilers exposed to aflatoxin-B1. Poult Sci. 2020;99(11):5896-906. https://doi.org/10.1016/j.psj.2020.08.034
30. Li M, Zhou Y, Zuo L, Nie D, Li X. Dietary fiber regulates intestinal flora and suppresses liver and systemic inflammation to alleviate liver fibrosis in mice. Nutrition. 2021;81:110959. https://doi.org/10.1016/j.nut.2020.110959

PMid:33059126

31. Galani BR, Owona BA, Chuisseu DP, Machewere E, Ngantchouko CB, Moundipa PF. Hepatoprotective activity of *Leptadenia hastata* (*Asclepiadaceae*) on acetaminophen-induced toxicity in mice: *In vivo* study and characterization of bioactive compounds through molecular docking approaches. Biomed Res Int. 2020;2020:3807234. https://doi.org/10.1155/2020/3807234

PMid:32953880

32. Youssef FS, Ashour ML, Sobeh M, El-Beshbishy HA, Singab AN, Wink M. *Eremophila maculata*-isolation of a rare naturally-occurring lignan glycoside and the hepatoprotective activity of the leaf extract. Phytomedicine. 2016;23(12):1484-93. https://doi.org/10.1016/j.phymed.2016.08.006

PMid:27765369

33. Kumar PP, Krishnaswamy G, Desai NR, Sreenivasa S, Kumar DB. Design, synthesis, PASS prediction, *in-silico* ADME and molecular docking studies of substituted-(Z)-3-benzylidine-5-aza-2-oxindole derivatives (Part-1). Chem Data Collect. 2021;31:100617. https://doi.org/10.1016/j.cdc.2020.100617