Immunomodulator Potential of *Phyllantus Acidus* Leaf Extract in Type 1 Diabetes Mellitus (T1DM) Models

Firas Khaleyla[1*], Nur Kuswanti[1], Nur Qomariyah[1], and Erlix Rakhmad Purnama[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Surabaya-60231, Indonesia

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ABSTRACT

Type 1 diabetes mellitus (T1DM) is characterized by high level of blood sugar, or hyperglycemia. The hyperglycemia condition in T1DM patients results in complication that made patients have higher chance to be infected with various infectious disease. *Phyllantus acidus* is a local plant usually consumed for its fruit, but other parts of the plant still saw lack of use locally. This study was aimed to examine the potential of *Phyllantus acidus* leaf extract to be immunomodulator in T1DM-induced animal model. Leaves of *P. acidus* was extracted using maceration and its content was analyzed using LC-HRMS. T1DM was induced in mice models using alloxan. *P. acidus* leaf extract was given for 10 days and in the 11th day, mice were injected with Staphylococcus aureus to induce macrophage activity. Phagocytic activity of peritoneal macrophages was evaluated from Giemsa-stained smear, while spleen was prepared into 4 µm histological section using paraffin method and stained with hematoxylin-eosin, then examined of its structure. Results showed that phagocytic activity increased significantly in mice treated with extract compared to negative control, however extract did not significantly improve spleen structure in T1DM mice compared to normal control. The secondary metabolites identified in extract including sorbic acid, L (-)-Pipecolinic acid, and Y-Aminobutyric acid (GABA). Metabolites contained in the extract possibly affects the innate response of the mice models, however the short period of treatment resulted in insignificant effect to adaptive immunity. Thus, it can be concluded that *P. acidus* leaf extract was able to increase innate immune response in T1DM mice models, however further study is needed to examine longer period of treatment.

Keywords: *Phyllantus acidus*, diabetes, innate immunity, macrophage, spleen

INTRODUCTION

Diabetes mellitus (DM) is a degenerative disease characterized by high level of blood glucose or known as hyperglycaemia. According to International Diabetes Federation (IDF), diabetes mellitus (DM) is currently the global health emergency with fastest development in the 21st century, including in Indonesia. Indonesia is ranked 5th in the world for the number of DM patients, with a total of 19.5 million patients in 2021 and an estimated 28.6 million in 2045 [1]. Generally, DM is categorized as type I DM (T1DM) which is caused by lack of insulin and type 2 DM (T2DM), characterized by insulin resistance.

Type 1 DM is characterized by low insulin levels due to damage to pancreatic beta cells. Damage to pancreatic beta cells in the pathogenesis of T1DM is still not fully understood to date, but it is suspected that this damage is caused by an autoimmune response initiated by a combination of genetics and environmental triggers, such as infection [1]. Under normal conditions, pancreatic beta cells are protected from autoimmune responses by the expression of regulatory T cells. However, based on previous research, inadequate immune regulation by regulatory T cells is hypothesized to trigger an autoimmune reaction leading to the destruction of pancreatic beta cells [2].

DM patients are known to have a higher risk of contracting infectious diseases. DM is also a risk factor increasing the chance of severe infections and risk of mortality from coronavirus disease 2019 (COVID 19) [3,4]. Both T1DM and T2DM patients are known to have an increased risk of infection, but this risk is twofold

higher in T1DM patients [5]. Studies have shown that T1DM patients produced lower levels of pro-inflammatory cytokines compared to normal adult. In addition, the primary antibody response to antigen-dependent T cells and the antigen-dependent T cell response to antigen proteins were also found to be reduced in T1DM patients [6,7]. This results to lower immune response in T1DM patients during infection.

Majority of infection are eliminated via phagocytosis mechanism by macrophages, which are part of the innate immune system. Macrophage phagocytosis is widely used as immunological parameter to evaluate immune function [8]. On the other hand, the spleen is a lymphoid organ that functions to destroy old red blood cells and as a reservoir for white blood cells, especially T cells and B cells. When an infection occurs, antigens will be presented in the white pulp of the spleen which initiates the activation of T cells and B cells, initiating antibody production and adaptive immune responses [9].

The hyperglycaemic condition suffered by T1DM patients causes a decrease in the immune response, resulting to susceptibility to infectious diseases. Immunomodulators can help to regulate the immune system to overcome infections. Various species from genus *Phyllantus* have been widely studied for its immunomodulatory properties due to their secondary metabolite contents, for example geraniin, gallic acid, and phyllanthin [10]. *P. acidus* or known as *ceremei* locally has been studied to regulate macrophage activity through downregulation of nitric oxide (NO) release, decreasing excessive inflammatory responses [11]. In Indonesia, *P. acidus* is ubiquitous and widely planted by the community, however its use is still limited to the consumption of its fruit. In addition, no study has been performed to examine the effect of *P. acidus* leaf extract on the immune system of T1DM models.

EXPERIMENT

Animal Model

The experimental animals used were male *Mus musculus* strain Balb/C aged between 3-4 months weighing 25-30g. Before given treatment, all experimental animals were acclimated for 1 week in stable condition. Feed and water were given daily ad libitum.

P. acidus Leaf Extraction

Extraction of *P. acidus* leaves were performed using maceration method. Leaves were dried and then ground into fine powder. A total of 500 g leaf powder was macerated in ethanol at 1:1 (w/v). Solvent was evaporated in rotary evaporator and resulting filtrate was stored at -20ºC until being used. The filtrate was dissolved in 1% Na-CMC according to concentration given to animal models.

Induction of T1DM

Mice were induced T1DM using a single-dose alloxan method. Alloxan was dissolved in 0.9% NaCl and injected at 130 mg/kg BW intraperitoneally. Glucose levels were measured 3 days after injection [20]. Mice with fasting blood glucose levels ≥120 mg/dL were used as T1DM models.

Oral Treatment of *P. acidus* Leaf Extract

The experimental animals were divided into 6 groups, each group consisted of 5 animal models. Treatment performed consisted of negative control group (NC), diabetes control (DC), positive control using Metformin (MC), dose I extract of 215 mg/BW (D1), dose II extract of 230 mg/kg BW (D2), and dose III extract of 245 mg/kg BW (D3). Doses used in the experiment were based on previous study [11]. Extract was given per oral to experimental animals daily for 10 days.

Evaluation of Macrophage Phagocytic Activity

Immunomodulatory activity was determined based on the phagocytic activity of mouse peritoneal macrophage cells. Mice were injected 0.5 mL of *Staphylococcus aureus* suspension intraperitoneally on the 11th day of treatment, then incubated for 1 hour. After that, the mice were anesthetized and dissected. Intraperitoneal fluid

was taken using syringe, smeared into object glass. The slides were fixed in methanol, then stained with Giemsa and examined using light microscope. The ratio of active to total macrophages was calculated. The proportion of active macrophages was calculated from observations of the number of active and inactive macrophages with a total of 300 macrophage cells per individual (n = 5 per group). Percentage of active macrophage was then analyzed statistically using one-way ANOVA and post-hoc Duncan test.

Evaluation of Spleen Histopathology

Histological slides of spleen were made using paraffin method. Dissected spleen was fixed in 10% neutral buffer formalin fixative solution for at least 24 hours. The spleen samples were processed in graded ethanol for 30 minutes each and then cleared in xylene overnight. The spleen samples were then infiltrated and embedded in paraffin medium, then sectioned using a rotary microtome at 4 µm thickness. Sections were stained with Hematoxylin-Eosin staining and covered with a cover slip using entellan. Evaluation of the structure of the spleen tissue slices was carried out based on scoring from previous studies [12] and analyzed statistically using Kruskal-Wallis test with post-hoc Mann-Whitney test.

Analysis of *P. acidus* Extract Content

Leaf extract was analyzed using Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS) (Thermo Scientific Dionex Ultimate 3000 RSLCnano). Extract diluted with ethanol was vortexed, then spun down. The supernatant was taken and filtered using a 0.22 µm syringe filter, then put into a vial. The sample was inserted into autosampler and then injected into LC-HRMS. A total of 10 µl of solution was injected into the column with temperature of 30oC. Separation was carried out on Hypersil GOLD aQ 50 x 1 mm x 1.9 u particle size. The mobile phase was 0.1% formic acid in water (v/v) (A) and 0.1% formic acid in Acetonitrile (v/v) (B). The elution gradient lasted for 30 min, at a flow rate of 40 µl/min.

RESULTS

Immune Response of T1DM Experimental Models to P. Acidus Extract

The results of the evaluation of macrophage phagocytic activity and splenic tissue evaluation after induction of *P. acidus* leaf extract presented in Table 1. Based on the results, significant difference of phagocytic activity was found between DC and other groups. In addition, the group given P. acidus leaf extract with all three doses experienced an increase in the number of macrophage activities compared to DC (Figure 1). The results of statistical tests showed that the active macrophage activity of the extract treatment group was not significantly different from the NC or MC, indicating that *P. acidus* extract was able to increase the number of active macrophages in hyperglycemic immunocompromised animal models. On the other hand, significant difference was found between NC and other groups in regards to splenic tissue structure. After given extract treatment of *P. acidus* leaf for 10 days, splenic tissue structure was found to have not improved yet back to its normal level (Figure 2). NC showed well-defined white pulp, while DC showed distortion at the edge of follicle. Spleen from the groups given extract treatment groups showed repairs to more defined follicles, but several diffused white pulps still persisted.

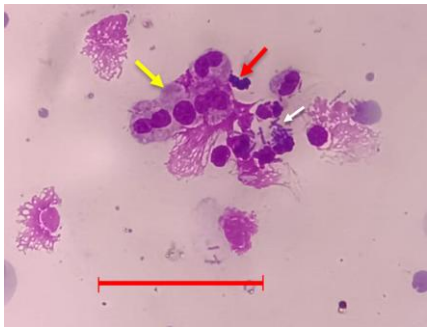

Figure 1. Phagocytic activity of intraperitoneal macrophages on *S. aureus*. Yellow arrows indicate active macrophages, red arrows indicate inactive macrophages, and white arrows indicate S. aureus bacteria.

Scale bar = 25 µm.

Table 1. Immune response in T1DM experimental animals after administration of P. acidus leaf extract.

Group	Fasting blood sugar (mg/dL)*	Percentage of active macrophage (%)*	Splenic tissue structure*
NC	81,40±26,29[a]	49.40±11.28[a]	0,2±0,45[a]
DC	152,40±26,48[b]	22.50±11.12[b]	2,4±0,89[b]
MC	143,80±14,45[b]	40.00±3.54[a]	1,6±0,89[b]
D1 (extract 215 mg/kg bw)	168,80±48,49[b]	37.80±5.59[a]	1,6±0,55[b]
D2 (extract 230 mg/kg bw)	143,40±14,88[b]	39.75±5.9[a]	1,4±0,55[b]
D3 (extract 245 mg/kg bw)	156,60±13,81[b]	39.80±7.50[a]	1,5±0,58[b]

*) Different subscript indicate significant differences based on statistical test (α=0.05).

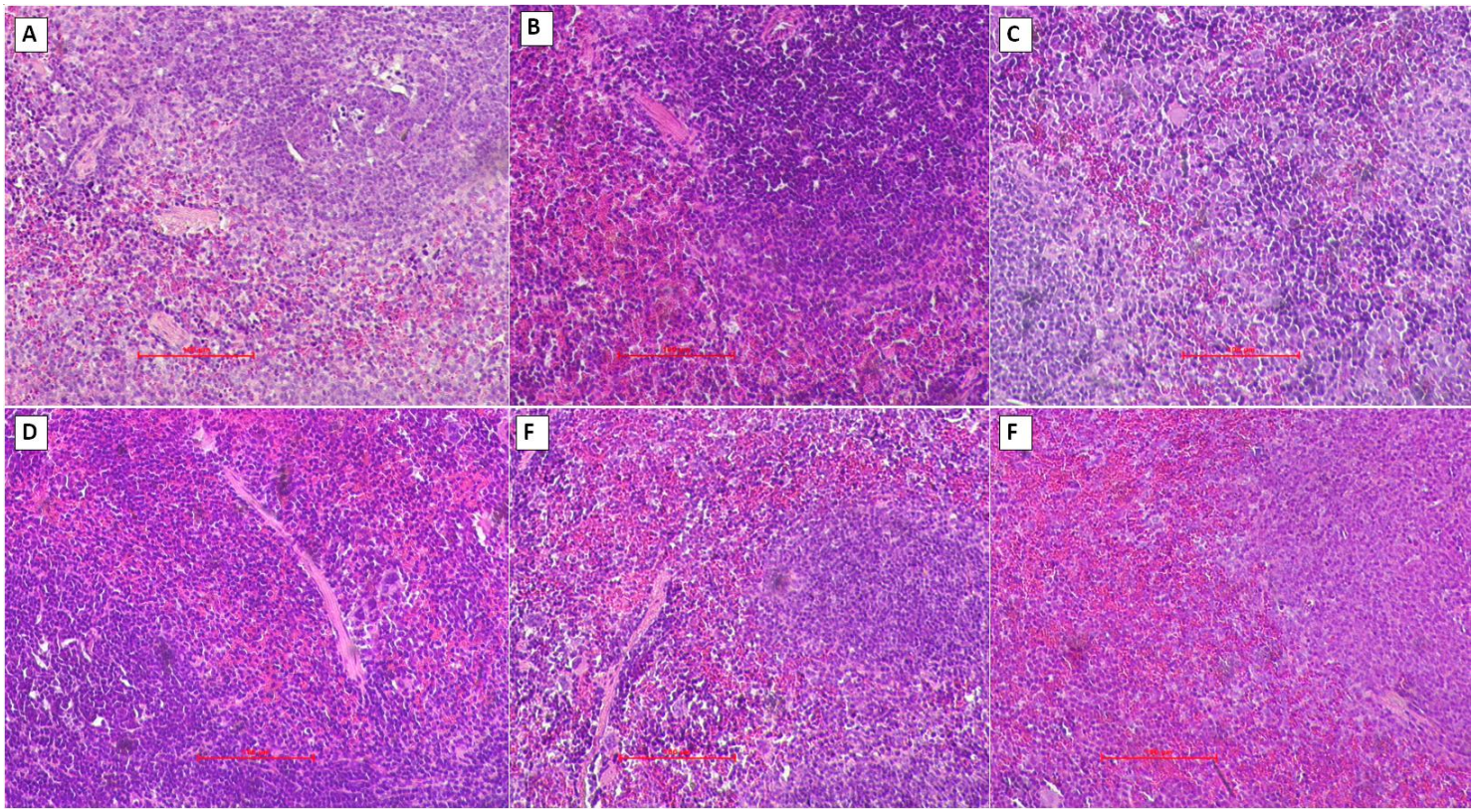

Figure 2. Section of spleen slide from each group. A: Normal control. B: Diabetic group. C: Metformin control. D: D1 group (215 mg/kg bw extract). E: D2 group (230 mg/kg bw extract). F: D3 group (245 mg/kg bw extract). Scale bar = 100 µm.

Identification of Bioactive Substances in P. acidus Leaf.

Table 2 presents the results of LC-HRMS analysis of *P. acidus* leaf content with a best match limit above 90. From the results of this analysis, the most abundant content was sorbic acid, L(-)-pipecolinic acid, ɣ-Aminobutyric acid (GABA), choline, and L-phenylalanine.

Tabel 2. Secondary metabolites content of *P. acidus* leaf extracted using ethanol solvent

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Name	Formula	Calc. MW	RT [min]	Total content (%)	Best Match
Sorbic acid	C6 H8 O2	112.0524	2.482	33%	97.7
L (-)-Pipecolinic acid	C6 H11 N O2	129.07894	2.64	16%	98.5
Y-Aminobutyric acid (GABA)	C4 H9 N O2	103.06358	2.933	13%	97.3
Choline	C5 H13 N O	103.10002	3.343	12%	98.1
L-Phenylalanine	C9 H11 N O2	165.07892	2.558	3%	99.6
DL-Tryptophan	C11 H12 N2 O2	204.09041	3.47	1%	99.2
Adenine	C5 H5 N5	135.05441	2.522	1%	99.3
Adenosine	C10 H13 N5 O4	267.10089	2.471	1%	99.1
Trigonelline	C7 H7 N O2	137.04761	2.457	1%	99.6
D- (+)-Proline	C5 H9 N O2	115.06345	3.542	1%	97.7

DISCUSSION

The hyperglycemic condition characterizes DM causes activation of protein kinase C. Protein kinase C decreases neutrophil migration, phagocytic activity, superoxide production that helps degrade foreign cells, and the destruction of pathogenic microorganisms [13]. This causes a reduced immune response, including macrophage activation as observed in the diabetic control (Table 1), leading to increased susceptibility to infection. Metformin is more widely used for the treatment of T2DM as a drug that can increase sensitivity to insulin. However, research shows that metformin is able to regulate macrophage function in atherosclerosis [14] so that it can help control the immune system in hyperglycemic conditions. Macrophages play a role in the primary immune response that directly acts nonspecifically to destroy foreign objects that enter the body, one of which is by producing reactive oxygen species (ROS) to degrade foreign cells [15]. Macrophages also help regulate other immune cells by producing various important cytokines as a first line of defense [15].

Based on the results of the study, *P. acidus* leaf extract was able to increase the number of active macrophages to level similar to NC or MC. The number of active macrophages in extract-treated mice were also significantly different from DC (Table 1). This is possibly due to extract content have the effect to increase phagocytic responses, sorbic acid specifically was able to increase IGF-1 in previous study [16]. However, extract had not been able to improve the structure of spleen tissue. DC spleen sections showed distorted follicle structure, in where diffused white pulps to red pulp were observed. More defined follicle structures were observed in mice given leaf extracts; however, repairs of structure were still not significant compared to normal control. Although there was indication of amelioration in the spleen tissue after *P. acidus* leaf extract treatment, histopathological scores were not significantly different to DC. Improvement on innate immunity but nor to adaptive immunity was likely due to the short period of extract treatment.

Previous study has also examined the effect of *P. acidus* in diabetic condition. *P. acidus* bark showed strong antioxidant activity against free radicals, while its leaves showed anti-inflammatory activity [17]. Other studies had also shown *P. acidus* as hepatoprotective agent [18] and inhibitor of α-glucosidase in DM models [19]. The general effect of *P. acidus* in diabetic condition is the reduction of blood glucose level and elevation of inflammatory level.

Analysis of the content of ethanol extract of *P. acidus* was carried out using LC-HRM showing that the highest content in the extract was sorbic acid (33%) (Table 2). Sorbic acid is generally known as a preservative and has antimicrobial activity that induces degradation of microorganism cells [20]. In previous studies, sorbic acid was indicated to be able to increase the concentration of insulin-like growth factor 1 (IGF-1) but reduce cortisol levels in the blood by regulating the expression of the IGF-1 gene in pigs [21]. IGF-1 signals were known to increase macrophage activation in compromised immunometabolic conditions, for example due to helminth worm infections [20].

On the other hand, pipecolinic acid content has been studied to be able to reduce fat accumulation, reduce oxidative stress and inflammation, and have anti-tumor activity. Pipecolinic acid has also been indicated to increase during pathogen infection and is associated with inherited systemic resistance [22]. Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter that has biological activity as an anti-inflammatory by suppressing the production of pro-inflammatory cytokines to increase the activation of NF-kB which plays an important role in the immune system response in both animals and humans [23].

Other secondary metabolites identified including choline and L-phenylalanin. Choline supplementation has been studied to be able to increase the immune response of Sprague-Dawley rat offspring. Lymphocyte cells were observed to develop better, T cells expressed more activation markers, and a decrease in the number of B cells leading to improved T cell function [24]. The decrease in L-phenylalanine in the body results in suppression of T cell proliferation. L-phenylalanine also affects the production of oxidative H2O2 from phenylalanine oxidase. Phenylalanine oxidase itself is encoded by interleukin 4 induced gene 1 (IL4I1) which is expressed by antigen-presenting cell (APC) dendritic cells [25]. In addition, the extract also found to have alkaloid and flavonoid content which have been widely studied to have the ability as immunomodulators through various mechanisms [26].

This study examined the effect of *P. acidus* extract on the immune response of T1DM and found that extract affected the innate response of T1DM mice models, however due to the short time of the treatment, the adaptive immunity, as indicated by spleen tissue structure was not affected significantly. Further study should be performed to examine various immunity markers, such as levels of inflammatory cytokines an antibody after extract treatment at longer period of treatment.

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REFERENCES

1. International Diabetes Federation (2021). Diabetes Atlas. IDF.
2. Roep, B. O., Thomaidou, S., van Tienhoven, R., & Zaldumbide, A. (2021). Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). Nature Reviews Endocrinology, 17(3), 150–161.
3. Milionis, C., & Milioni, S. O. (2020). A brief analysis and hypotheses about the risk of COVID-19 for people with type 1 and type 2 diabetes mellitus. Journal of Diabetes & Metabolic Disorders, 19(2), 2035–2039.
4. Shang, L., Shao, M., Guo, Q., Shi, J., Zhao, Y., Xiaokereti, J., & Tang, B. (2020). Diabetes mellitus is associated with severe infection and mortality in patients with COVID-19: A systematic review and meta-analysis. Archives of Medical Research, 51(7), 700–709.
5. Carey, I. M., Critchley, J. A., DeWilde, S., Harris, T., Hosking, F. J., & Cook, D. G. (2018). Risk of infection in type 1 and type 2 diabetes compared with the general population: A matched cohort study. Diabetes Care, 41(3), 513–521.
6. Janssen, A. W. M., Stienstra, R., Jaeger, M., van Gool, A. J., Joosten, L. A. B., Netea, M. G., Riksen, N. P., & Tack, C. J. (2021). Understanding the increased risk of infections in diabetes: Innate and adaptive immune responses in type 1 diabetes. Metabolism, 121, 154795.

7. Eibl, N., Spatz, M., Fischer, G. F., Mayr, W. R., Samstag, A., Wolf, H. M., Schernthaner, G., & Eibl, M. M. (2002). Impaired primary immune response in type-1 diabetes: Results from a controlled vaccination study. Clinical Immunology, 103(3), 249–259.
8. Handayani, N., Wahyuono, S., Hertiani, T., & Murwanti, R. (2018). Uji aktivitas fagositosis makrofag ekstrak etanol daun suji (Dracaena angustifolia (Medik) Roxb.) secara in vitro. Pharmaceutical and Medical Journal, 1(1). [In Indonesian].
9. Kapila, V., Wehrle, C. J., & Tuma, F. (2023). Physiology, spleen. In StatPearls. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK537307/
10. Jantan, I., Haque, M. A., Ilangkovan, M., & Arshad, L. (2019). An insight into the modulatory effects and mechanisms of action of Phyllanthus species and their bioactive metabolites on the immune system. Frontiers in Pharmacology, 10, 878.
11. Chakraborty, R., Biplab, D., Devanna, N., & Sen, S. (2012). Antiinflammatory, antinociceptive and antioxidant activities of Phyllanthus acidus L. extracts. Asian Pacific Journal of Tropical Biomedicine, 2(Suppl. 2), S953–S961.
12. Ibrahim, K. E., Al-Mutary, M. G., Bakhiet, A. O., & Khan, H. A. (2018). Histopathology of the liver, kidney, and spleen of mice exposed to gold nanoparticles. Molecules, 23(8), 1848.
13. Jafar, N., Edriss, H., & Nugent, K. (2016). The effect of short-term hyperglycemia on the innate immune system. The American Journal of the Medical Sciences, 351(2), 201–211.
14. Feng, X. J., Chen, W. X., Ni, X. Y., Little, P. J., Xu, S. W., Tang, L. Q., & Weng, J. P. (2021). Metformin, macrophage dysfunction and atherosclerosis. Frontiers in Immunology, 12, 682853.
15. Janeway, C., Paul, C., Travers, M., Walport, M., & Shlomchik, M. (2001). Immunobiology (5th ed.). Garland Science.
16. Luo, Z. F., Fang, X. L., Shu, G., Wang, S. B., Zhu, X. T., Gao, P., Chen, L. L., Chen, C. Y., Xi, Q. Y., Zhang, Y. L., & Jiang, Q. Y. (2011). Sorbic acid improves growth performance and regulates insulin-like growth factor system gene expression in swine. Journal of Animal Science, 89(8), 2356–2364.
17. Siddiqui, Z., Khan, M. I, Badruddeen, B, Akhtar, J., Ahmad, M. (2022). Multifunctional Role of Phyllanthus Acidus L. As a Therapeutic Agent for Management of Diabetes and Associated Complications: A Review. Biomedical and Pharmacology Journal,15(4), 1821-1831.
18. Oktaviona, E. E. P., Qomariyah, N., & Khaleyla, F. (2023). Hepatoprotective Activity of Phyllanthus acidus L. Leaf Extract in Type 2 Diabetes Mellitus Mice. LenteraBio : Berkala Ilmiah Biologi , 12(3), 381–388.
19. Chigurupati, S. (2020). Antioxidant and antidiabetic properties of Phyllanthus acidus (L.) Skeels ethanolic seed extract. International Food Research Journal, 27(4), 775-782.
20. Nemes, D., Kovács, R., Nagy, F., Tóth, Z., Herczegh, P., Borbás, A., Kelemen, V., Pfliegler, W. P., Rebenku, I., Hajdu, P. B., Fehér, P., Ujhelyi, Z., Fenyvesi, F., Váradi, J., Vecsernyés, V., & Bácskay, I. (2020). Comparative biocompatibility and antimicrobial studies of sorbic acid derivates. European Journal of Pharmaceutical Sciences, 143, 105162.
21. Spadaro, O., Camell, C. D., Bosurgi, L., Nguyen, K. Y., Youm, Y. H., Rothlin, C. V., & Dixit, V. D. (2017). IGF1 shapes macrophage activation in response to immunometabolic challenge. Cell Reports, 19(2), 225–234.
22. Luo, L., Cai, Y., Jiang, Y., Gong, Y., Cai, C., Lai, D., Jin, X., Guan,Z., & Qiu, Q. (2023). Pipecolic acid mitigates ferroptosis in diabetic retinopathy by regulating GPX4-YAP signaling. Biomedicine & Pharmacotherapy, 169, 115895.
23. Ngo, D. H., & Vo, T. S. (2019). An updated review on pharmaceutical properties of gamma-aminobutyric acid. Molecules, 24, 2678.
24. Richard, C., Lewis, E. D., Goruk, S., Wadge, E., Curtis, J. M., Jacobs, R. L., & Field, C. J. (2017). Feeding a mixture of choline forms to lactating dams improves the development of the immune system in Sprague-Dawley rat offspring. Nutrients, 9, 567.
25. Sikalidis, A. K. (2015). Amino acids and immune response: A role for cysteine, glutamine, phenylalanine, tryptophan and arginine in T-cell function and cancer? Pathology and Oncology Research, 21(1), 9–17.
26. Shivaprasad, H. N., Kharya, M. D., Rana, A. C., & Mohan, S. (2006). Preliminary immunomodulatory activities of aqueous extract of Terminalia chebula. Pharmaceutical Biology, 44, 32–34.