Antioxidant and *In Vitro* Antidiabetic Properties of *Lansium domesticum* Leaves Extracted with Solvents of Varying Polarity

Jiro Hasegawa Situmorang[1], Ana Yulyana[2], Ririn Astyka[3], Hafid Syahputra[4], and Muhammad Fauzan Lubis[3*]

[1]*Center for Biomedical Research, National Research and Innovation Agency (BRIN), KST Soekarno, Jl. Raya Bogor No. 970, Cibinong 16915, Indonesia*

[2]*Department of Pharmacy, Faculty of Pharmacy, Universitas Pancasila, Jl. Srengseng Sawah, Jakarta Selatan 12640, Indonesia*

[3]*Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Jl. Tri Dharma, Padang Bulan, Medan 20155, Indonesia*

[4]*Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Jl. Palembang Prabumulih Km. 32, Indralaya 30862, Indonesia*

* ***Corresponding author:***

tel: +62-81264353744
email: fauzan.lubis@usu.ac.id

Received: August 26, 2025
Accepted: November 20, 2025

DOI: 10.22146/ijc.110695

Abstract: *Conventional therapies for diabetes mellitus, such as oral hypoglycemic agents, are often limited by side effects and incomplete glycemic control, highlighting the need for safer alternatives. Exploring natural remedies, such as* Lansium domesticum*, is compelling, as this plant has been traditionally used for diabetes therapy. This study aimed to investigate how solvent polarity and affinity influence phytochemical content, antioxidant activity, and enzyme inhibitory potential of* L. domesticum *leaf extracts. Extraction was conducted using methanol, ethanol, and acetone (50%, 75%, 100%), and distilled water. Total phenolic content (TPC) and total flavonoid content (TFC) were quantified, and antioxidant activities were assessed via total antioxidant activity, DPPH, and FRAP assays. Antidiabetic activity was evaluated* in vitro *through α-glucosidase and α-amylase inhibition assays. The results indicated that 100% ethanol extract exhibited the highest TPC and TFC, correlating strongly with superior antioxidant and enzyme inhibitory activities. The extract demonstrated the most potent inhibition of α-glucosidase and α-amylase, with IC_{50} values of 70.64 and 105.13 µg/mL, respectively. Pearson correlation analysis revealed strong negative correlations between phytochemical contents and IC_{50} values. Overall, ethanol proved to be the most effective solvent for extracting bioactive compounds from* L. domesticum *leaves, underscoring its potential as natural antioxidant and antidiabetic agent.*

Keywords: Lansium domesticum*; antioxidant; antidiabetic; solvent polarity*

■ INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both [1]. The global incidence of diabetes continues to rise, creating an urgent need for more effective and safe therapeutic strategies [2]. Although oral hypoglycemic agents such as metformin and acarbose are widely prescribed, their long-term use is often limited by gastrointestinal side effects, incomplete glycemic control, and safety concerns [3]. These limitations have sparked growing interest in natural products as alternative or complementary therapies, as many medicinal plants offer bioactive compounds with promising antidiabetic and antioxidant properties.

Excessive production of reactive oxygen species (ROS) leads to oxidative stress, a phenomenon that plays a central role in the pathogenesis of diabetes complications. Oxidative stress exacerbates insulin

resistance, impairs pancreatic β-cell function, and contributes to chronic complications such as cardiovascular disease, nephropathy, and neuropathy [4]. Therefore, plant-derived antioxidants are of great interest, as they can both neutralize free radicals and inhibit carbohydrate-digesting enzymes such as α-glucosidase and α-amylase, offering a dual therapeutic benefit [5-6]. Within this context, *Lansium domesticum*, a plant traditionally used in Southeast Asia for treating fever, malaria, and gastrointestinal disorders, is considered. Recent studies also reported that its fruit and bark extracts show antimicrobial, anticancer, and nephroprotective effects [7]. However, the pharmacological potential of its leaves remains underexplored. Investigating the leaves in particular is compelling, as they are traditionally used in diabetes management and may provide novel sources of antidiabetic and antioxidant compounds.

The therapeutic potential of *L. domesticum* leaves, like many other medicinal plants, is primarily attributed to their phytochemical composition [8]. Among these, total phenolic content (TPC) and total flavonoid content (TFC) are considered key contributors to bioactivity. Both phenolics and flavonoids possess remarkable antioxidant properties, functioning through the neutralization of free radicals and the chelation of metal ions involved in oxidative reactions [9]. In addition, these molecules can also inhibit carbohydrate-degrading enzymes, thereby reducing glucose absorption and improving the management of postprandial glycemia [10-11]. Thus, evaluating the relationship between TPC and TFC and their effects on antioxidant and antidiabetic activities is crucial for assessing the functional potential of *L. domesticum* leaf extracts.

Since phenolics and flavonoids are the major contributors to antioxidant and antidiabetic activities, their extraction efficiency strongly depends on the choice of the solvent. Solvent polarity plays a crucial role in determining the solubility of phytochemicals, thereby influencing both yield and bioefficacy of the extracts [12]. Previous studies have indicated that the polarity of the extraction solvent influences bioactivity, with more polar solvents enhancing the inhibition of α-amylase, α-glucosidase, and free radicals [13-14]. Recent advances have underscored the therapeutic potential of plant-derived phenolic compounds in managing oxidative stress-related disorders, including diabetes. Phenolic-rich extracts of *Silybum marianum* demonstrated potent antioxidant and antimicrobial activities, highlighting the importance of solvent polarity in maximizing phenolic recovery and biological efficacy [15]. Similarly, *Sargassum cristae* folium exhibited a notable TPC, which was related to its antioxidant activity [16]. However, comparative studies investigating how different solvents influence the antioxidant and antidiabetic properties of *L. domesticum* leaf extract remain scarce, highlighting the need for further exploration.

To address this gap, the present work aimed to optimize the extraction of *L. domesticum* leaves using solvent polarity, including methanol, ethanol, acetone (at concentrations of 100, 75, and 50%), and water. The antioxidant capacity of the extracts was determined through total antioxidant capacity, DPPH radical scavenging, and FRAP assays. *In vitro* antidiabetic potential was evaluated based on the inhibition of α-glucosidase and α-amylase enzymes. By identifying the most effective solvent system for maximizing the therapeutic properties of *L. domesticum* leaves, this work provides insights that may support their future application in functional foods and phytopharmaceutical development.

■ EXPERIMENTAL SECTION

Materials

Unless otherwise specified, all chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany) and Sigma Aldrich (St. Louis, MO, USA). Reference standards, including gallic acid, quercetin, and ascorbic acid (≥ 98% purity), were used. Additionally, all kits for α-glucosidase and α-amylase inhibition tests were provided by Elabscience (Houston, TX, USA).

Instrumentation

The main instruments employed were as follows: mechanical grinder (HR-2200, Philips, Netherlands), rotary evaporator (EYELA N-1001, Tokyo, Japan), UV-vis

spectrophotometer (Shimadzu UV-1800, Kyoto, Japan), microplate reader (Spectrostar Nano, BMG Labtech, Germany), and Thermo Scientific Q Exactive Orbitrap MS (Thermo Fisher Scientific, Waltham, MA, USA).

Procedure

Plant sample collection and preparation

Fresh leaves of *L. domesticum* were collected from a cultivated area in Deli Serdang, North Sumatra, Indonesia, in July 2024. Taxonomic verification and authentication of the plant material were performed by a botanist affiliated with the Herbarium of Universitas Sumatera Utara, and a voucher specimen (No. 3005/MEDA/2024) was deposited for future reference. Freshly collected leaves were rinsed carefully under running tap water and subsequently with distilled water to eliminate any adhering dust or impurities. The cleaned samples were shade-dried at ambient temperature for 7–10 days until a stable weight was obtained. After drying, the leaves were pulverized with a mechanical grinder and sieved through a 60-mesh screen. The resulting powder was stored in airtight containers at room temperature, shielded from moisture and light, until it was used for extraction.

Extraction procedure

The extraction of bioactive constituents from *L. domesticum* leaf powder was performed using a maceration method. Fifty grams of dried powder were immersed in 500 mL of different solvents, which are methanol, ethanol, acetone (100, 75, and 50% (v/v), respectively), and distilled water, placed separately in Erlenmeyer flasks. The mixtures were maintained at 25 ± 2 °C with occasional agitation for 72 h. Following maceration, the solutions were filtered through Whatman No. 1 paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at 40 °C. Residual solvent was removed by drying the extracts in a silica gel desiccator until a constant weight was achieved. Thereby, the yield (%) of each extract was calculated before it was stored at 4 °C in amber vials for subsequent analysis [17].

Determination of total phenolic content

The Folin–Ciocalteu colorimetric method, with slight modification from Sumaiyah et al. [18] and Nasution et al. [19], was employed to determine the TPC of the extracts. A volume of 200 µL of extract (1 mg/mL) was mixed with 1 mL of 10% Folin–Ciocalteu reagent and incubated for 5 min at room temperature in the dark. Subsequently, 800 µL of 7.5% Na_2CO_3 was added, and the mixture was further incubated in the dark at 25 °C for 30 min. The absorbance was read at 765 nm. The results were calculated against a gallic acid calibration curve and expressed as mg GAE per g extract.

Determination of total flavonoid content

The aluminum chloride assay was employed to quantify TFC following Astyka et al. [20] with slight adjustments. In this method, 500 µL of extract (1 mg/mL) was mixed with 300 µL of 5% sodium nitrite ($NaNO_2$). After 5 min, 300 µL of 10% $AlCl_3$ was added, and after an additional 6 min, 2 mL of 1 M NaOH was introduced. The solution volume was brought to 5 mL with distilled water, vortexed thoroughly, and its absorbance was determined at 510 nm. TFC was expressed as mg QE per g extract using quercetin as the calibration standard.

Determination of total antioxidant activity

The phosphomolybdenum method, with modifications based on Mansoori et al. [21], was employed to measure total antioxidant activity (TAA). A 0.2 mL aliquot of extract solution (25–400 µg/mL in water) was combined with 1.8 mL of reagent containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The reaction mixtures were incubated at 90 °C in capped tubes for 90 min and then cooled to room temperature. Absorbance was recorded at 695 nm. Ascorbic acid (5–60 µg/mL) was used to construct the calibration curve, and the results were expressed as milligrams of AAE per gram of extract.

DPPH radical scavenging assay

The free radical quenching ability of the extracts was measured using the DPPH method described by Lubis et al. [22], with slight modifications. Briefly, 1 mL of the extract solutions (25–400 µg/mL) was mixed with 1 mL of 0.1 mM DPPH in methanol. The mixtures were incubated at ambient temperature in the dark for 30 min. Absorbance was determined at 517 nm. A methanol solution was used as the blank, while DPPH without extract served as the control. Radical scavenging

activity was expressed as percentage inhibition calculated using Eq. (1);

$$\text{Inhibition}(\%) = \frac{A0 - A1}{A0} \times 100\% \quad (1)$$

where A_0 is the absorbance of the control solution, and A_1 is the absorbance of the extract solution.

Ferring reducing antioxidant power assay

The reducing capacity of the extracts was assessed using the FRAP assay, as described by Kiss et al. [23]. Briefly, 200 µL of extract (concentration range: 25–400 µg/mL) was mixed with 500 µL phosphate buffer (0.2 M, pH 6.6) and 500 µL potassium ferricyanide solution (1%). The mixture was incubated at 50 °C for 20 min, followed by the addition of 500 µL trichloroacetic acid (10%). After centrifugation at 3,000 rpm for 10 min, 500 µL of the supernatant was combined with 500 µL distilled water and 100 µL of 0.1% $FeCl_3$. The absorbance was then read at 700 nm. Higher absorbance indicated stronger reducing activity.

In vitro *antidiabetic activity*

α-Glucosidase and α-amylase inhibitory activities were determined using reagent kits from Elabscience (Houston, TX, USA) following the manufacturer's guidelines with minor adjustments. Extracts dissolved in water were prepared at concentrations ranging from 25 to 500 µg/mL. For α-glucosidase inhibition, 50 µL of extract was combined with the enzyme solution and incubated at 37 °C. The substrate (*p*-nitrophenyl-α-D-glucopyranoside) was then added, and after incubation, the reaction was stopped by the addition of the stop solution. The absorbance was read at 405 nm using a 96-well microplate reader. For α-amylase inhibition, 50 µL of extract was incubated with the enzyme solution at 37 °C, followed by the addition of soluble starch as substrate. After incubation, the kit color reagent was added, and absorbance was recorded at 540 nm. Acarbose served as the positive control. The percentage of inhibition was calculated using Eq. (2) [24-25].

$$\text{Inhibition}(\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\% \quad (2)$$

In this equation, $A_{control}$ represents the absorbance of the control solution lacking extract, and A_{sample} indicates the absorbance obtained with the extract. IC_{50} values were determined from the inhibition curves plotted using different extract concentrations.

Phytochemical analysis using liquid chromatography high-resolution mass spectrometry

Liquid chromatography-high-resolution mass spectrometry (LC-HRMS) was employed to identify the secondary metabolites of the most effective extract. The analysis was equipped with an electrospray ionization (ESI) source, operated in negative ion mode. Chromatographic separation was achieved on a C_{18} reversed-phase column (2.1 × 100 mm, 1.7 µm) at 40 °C. The mobile phases consisted of (A) 0.1% formic acid in water and (B) acetonitrile, with a gradient elution was 0–1 min, 5%, B; 1–10 min, linear increase to 40% B; 10–18 min, linear increases to 95% B; held at 95% for 2 min; returned to 5% B over 1 min and equilibrated for 3 min. The flow rate was set at 0.3 mL/min, and the injection volume was 5 µL. The MS conditions included a scan range of *m/z* 100–1000, a resolution of 70,000, a sheath gas flow rate of 35 arb, an auxiliary gas flow rate of 10 arb, a spray voltage of −3.0 kV, and a capillary temperature of 320 °C. For MS/MS fragmentation, a normalized collision energy of 30 eV was applied in data-dependent acquisition mode. Compound identification was based on comparison of accurate mass-to-charge ratios (*m/z*) in the $[M–H]^-$ ion form, retention times, and, where available, fragmentation patterns, with entries in the METLIN and HMDB spectral databases [26].

Statistical analysis

All experimental procedures were conducted in triplicate, and data were expressed as mean ± SD. IC_{50} values for antioxidant (DPPH) and enzyme inhibition (α-glucosidase, α-amylase) assays were estimated by nonlinear regression analysis in GraphPad Prism (version 9.0). Group differences were analyzed using one-way ANOVA with Tukey's post hoc test, considering $p < 0.05$ as statistically significant. Pearson correlation was also applied to examine the relationship between TPC, TFC, and observed antioxidant/antidiabetic effects. Correlation coefficients and statistical significance were obtained using SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA).

■ RESULTS AND DISCUSSION

Effect of Different Solvents on Yield and Phytochemical Content

The isolation of phytochemicals from plant materials typically involves several preparatory steps, such as milling, grinding, and homogenization, with extraction being the most critical for recovering bioactive compounds [27]. The efficiency of extraction is governed by multiple parameters, including the chemical nature of the phytochemicals, the particle size of the sample, the extraction method, solvent polarity, pH, temperature, extraction time, and the composition of the plant matrix [28]. Among these, under consistent time and temperature, the type of solvent and sample composition are considered the most influential factors. In the present study, leaf extracts of *L. domesticum* were obtained using water and various concentrations (50, 75, and 100%) of aqueous methanol, ethanol, and acetone. Extraction yields ranged from 13.57 ± 1.35% with pure acetone to a maximum of 33.46 ± 2.05% with 50% aqueous ethanol (Table 1). This result suggests that solvent mixtures with intermediate polarity, such as 50% aqueous ethanol, are more effective at extracting a broader range of phytochemicals compared to either pure solvents or water alone. Comparable yields were observed with 50% aqueous acetone (32.56 ± 1.75%) and 75% aqueous methanol (31.56 ± 1.03%), further supporting the advantage of aqueous-organic combinations. On the other hand, water as a single solvent achieved a moderate yield (21.50 ± 1.50%). In comparison, the lowest yield was obtained with 100% acetone, highlighting the limitations of pure organic solvents in extracting a diverse range of phytochemical constituents. The high extraction yields of aqueous solvent mixtures may not only be attributed to the presence of phenolic compounds but also to the co-extraction of other soluble compounds such as proteins and carbohydrates, which tend to have higher solubility in water and ethanol compared to methanol and acetone. The synergistic use of water and organic solvents enhances the solubilization of both polar and semi-polar constituents, leading to improved extraction efficiency. These findings are consistent with previous studies on medicinal plants, which also reported higher yields when using aqueous organic solvent systems [29-31].

The TPC of *L. domesticum* leaf extracts was found to vary considerably across different solvent systems (Table 1). The highest TPC value was observed in the

Table 1. Extraction yield, TPC, and TFC of *L. domesticum* leaf extracts obtained using various solvents

Samples	Parameters		
	Extraction yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
Water	21.50 ± 1.50	5.35 ± 0.36	4.67 ± 0.12
Methanol			
100% methanol	28.56 ± 1.60	21.64 ± 2.56	10.34 ± 0.67
75% aqueous methanol	31.56 ± 1.03	25.12 ± 1.78	20.46 ± 1.35
50% aqueous methanol	29.46 ± 1.27	10.45 ± 1.21	6.35 ± 0.57
Ethanol			
100% ethanol	18.46 ± 1.67	33.46 ± 2.67	21.35 ± 1.86
75% aqueous ethanol	27.45 ± 1.46	22.34 ± 1.67	12.57 ± 1.07
50% aqueous ethanol	33.46 ± 2.05	21.46 ± 1.45	11.05 ± 1.02
Acetone			
100% acetone	13.57 ± 1.35	32.67 ± 2.78	26.44 ± 2.46
75% acetone	26.68 ± 1.12	30.35 ± 1.64	24.67 ± 1.50
50% acetone	32.56 ± 1.75	27.89 ± 2.12	20.85 ± 1.47

All data are given as mean ± standard deviation (n = 3). Total phenolic content (TPC) was quantified as mg GAE/g extract, whereas total flavonoid content (TFC) was determined as mg QE/g extract

extract obtained with 100% ethanol (33.46 ± 2.67 mg GAE/g extract), but it was not significantly different from the extracts obtained with 100% acetone (32.67 ± 2.78 mg GAE/g) and 75% acetone (30.35 ± 1.64 mg GAE/g). These results suggest that pure or highly concentrated organic solvents, particularly ethanol and acetone, are more effective in extracting phenolic compounds, possibly due to their ability to disrupt cell membranes and enhance the solubility of medium to low-polarity phenolics [32]. Interestingly, while 50% aqueous ethanol showed the highest extraction yield, its TPC (21.46 ± 1.45 mg GAE/g) was significantly lower than that of the pure ethanol extract. This indicates that a higher extraction yield does not always correspond to a higher phenolic content, as the co-extraction of non-phenolic components (e.g., carbohydrates, proteins) in aqueous mixtures may dilute the relative concentration of phenolics in the final extract [33]. A similar trend was observed for aqueous methanol and acetone extracts, where an increased water content improved yield but did not necessarily enhance phenolic purity. The TPC results also reflect the influence of solvent polarity. While water is highly polar, it yielded the lowest TPC (5.35 ± 0.36 mg GAE/g), underscoring its limited efficiency in extracting phenolics, many of which are only moderately polar. In contrast, the presence of organic solvents with intermediate polarity enhances the solubility and mass transfer of phenolics from the plant matrix into the solvent. These findings align with prior reports stating that the polarity of the solvent must match the polarity of the target phenolics to optimize their extraction [34-36].

Subsequently, the TFC exhibited considerable variation depending on the solvent system used for extraction (Table 1). The highest TFC was observed in the extract prepared with 100% acetone (26.44 ± 2.46 mg QE/g extract), followed by 75% acetone (24.67 ± 1.50 mg QE/g) and 100% ethanol (21.35 ± 1.86 mg QE/g). These findings suggest that flavonoids, particularly those with lower polarity, such as aglycones, are more effectively extracted using pure or highly concentrated organic solvents [37]. The high affinity of acetone for non-polar to moderately polar compounds may explain its superior performance in extracting flavonoid constituents. In contrast, aqueous solvent mixtures such as 50% ethanol and 50% methanol yielded moderate TFC values (11.05 ± 1.02 and 6.35 ± 0.57 mg QE/g, respectively), despite producing the highest overall extraction yields. This indicates that maximum extraction yield does not necessarily correlate with high flavonoid content, as aqueous mixtures may co-extract other polar compounds that do not contribute to TFC, thereby lowering the relative concentration of flavonoids in the extract [38]. Water as a sole solvent resulted in the lowest TFC (4.67 ± 0.12 mg QE/g), highlighting its inefficiency in dissolving flavonoid compounds, which often require organic solvents for effective extraction. These results are consistent with the behavior of flavonoids in other plant species, where their solubility and extraction efficiency are strongly dependent on the solvent's polarity, hydrogen-bonding capacity, and ability to penetrate the cell wall [39-40]. Overall, the findings suggest that solvent affinity also plays a significant role in determining extraction efficiency. Ethanol, with its intermediate polarity and amphiphilic properties, can dissolve both hydrophilic and lipophilic compounds, which explains its higher recovery of phenolics and flavonoids compared to water. This dual solubility profile enhances ethanol's ability to yield extracts with stronger antioxidant and enzyme inhibitory activities. Nevertheless, this reinforces the importance of solvent optimization tailored to the specific class of phytochemicals being targeted.

Solvents Effect on Antioxidant Activity

The antioxidant capacity of *L. domesticum* leaf extracts was comprehensively evaluated using three complementary assays: TAA, DPPH radical scavenging, and FRAP. Across all assays, both extract concentration and solvent type had a pronounced effect on the antioxidant performance of the extracts. TAA results revealed an apparent concentration-dependent increase in antioxidant activity for all solvent systems tested (25–400 μg/mL, Fig. 1). Notably, at 400 μg/mL, extracts obtained using 100% ethanol (98.21 ± 0.47 μg AAE/mL), 100% acetone (93.12 ± 0.47 μg AAE/mL), and 100% methanol (90.82 ± 0.45 μg AAE/mL) exhibited the highest

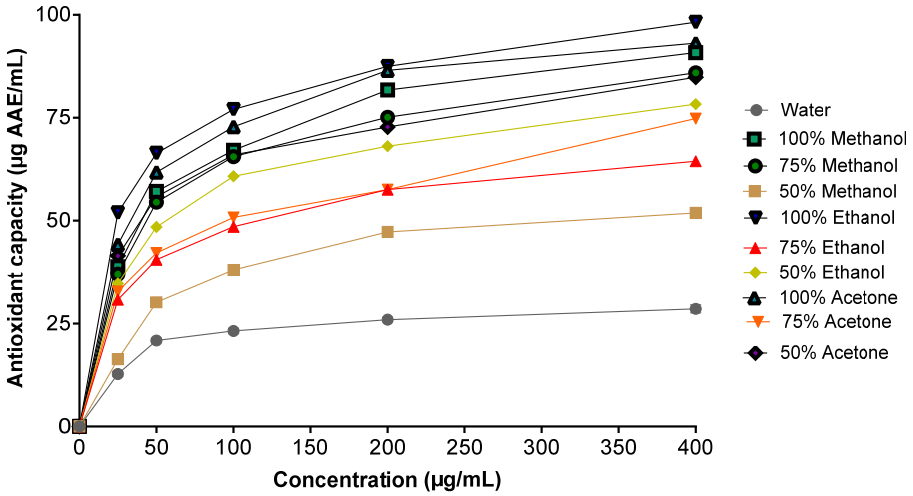

Fig 1. Total antioxidant activity of *L. domesticum* leaf extracts using solvents of varying polarity across different concentrations

antioxidant performance. These findings highlight the superior ability of pure organic solvents to extract redox-active compounds from plant matrices. The enhanced TAA values in these extracts likely reflect the efficient solubilization of phenolics and flavonoids, known to contribute through both hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms [41]. In contrast, water demonstrated the lowest antioxidant activity, with a TAA of only 28.56 ± 1.00 µg AAE/mL, indicating its limited ability to extract less polar antioxidant constituents. Although water is widely regarded as a green solvent, its polarity and poor membrane-disrupting capacity may hinder the release of active compounds [42]. Statistical analysis using Tukey's HSD test confirmed significant differences ($p < 0.05$) in TAA among solvent systems. Extracts obtained with 100% ethanol, 100% acetone, and 100% methanol showed significantly higher activity than those extracted with water, 50% methanol, and 50% ethanol, underscoring the influence of solvent polarity, dielectric constant, and hydrogen-bonding capability on extraction efficiency. The TAA trends were strongly aligned with both TPC and TFC data. However, some discrepancies, such as the slightly lower TAA in 100% methanol despite its high TPC, suggest that antioxidant activity also depends on compound structure, reactivity, and synergism rather than concentration alone [43].

The DPPH assay supported the TAA findings, showing a similar solvent- and concentration-dependent response (Fig. 2). At 400 µg/mL, the highest radical scavenging was recorded in 100% ethanol (95.56 ± 0.99%), followed by 100% acetone (90.46 ± 1.00%), 50% acetone (86.22 ± 1.45%), and 100% methanol (86.34 ± 1.18%). These results confirm the effectiveness of pure or high-content organic solvents in solubilizing compounds that can neutralize free radicals via HAT mechanisms [44]. Conversely, water (51.50 ± 1.06%) and 50% methanol (60.09 ± 0.55%) extracts exhibited the weakest activity. The low performance of water may be attributed to its limited capacity to dissolve less polar antioxidant molecules, such as flavonoid aglycones and specific phenolic acids [45]. Although one-way ANOVA could not be applied due to low intra-group variance, Tukey's HSD analysis revealed that 100% ethanol and 100% acetone extracts were significantly more active ($p < 0.05$) than water and most aqueous solvents. These statistical outcomes strengthen the conclusion that solvent polarity is critical in optimizing free radical scavenging capacity. To complement the dose-dependent antioxidant activity observed in the DPPH assay, IC_{50} values were calculated to quantify the potency of each solvent extract (Table 2). The IC_{50} represents the concentration of extract required to achieve 50% inhibition in a given assay. Lower IC_{50} values indicate higher biological activity [46]. Among

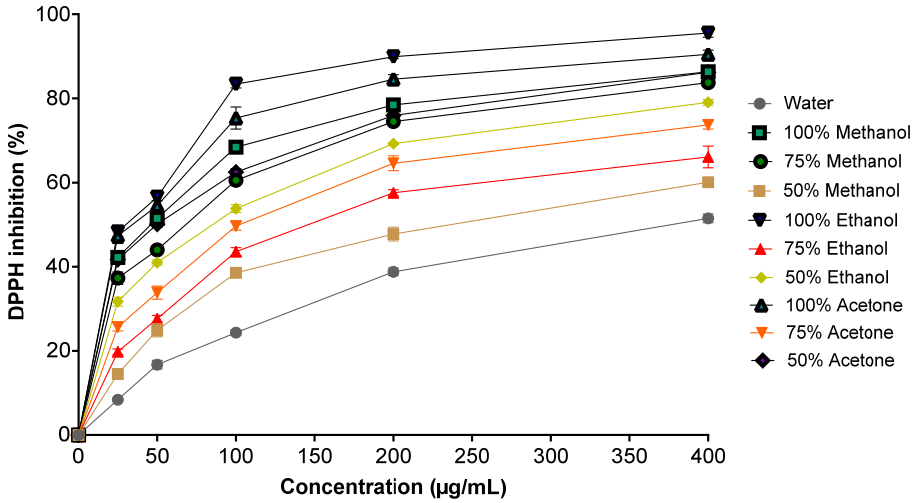

Fig 2. DPPH radical scavenging activity of *L. domesticum* leaf extracts at various concentrations

Table 2. IC_{50} values of *L. domesticum* leaf extracts for antioxidant and *in vitro* antidiabetic activities

Extracts	IC_{50} (µg/mL)			Activity levels
	DPPH radical scavenging	α-glucosidase inhibition	α-amylase inhibition	
Water	220.00	706.37	720.45	Weak
Methanol				
100% methanol	66.30	70.64	105.13	Strong
75% aqueous methanol	108.20	118.19	180.55	Moderate
50% aqueous methanol	95.60	695.32	680.32	Strong
Ethanol				
100% ethanol	42.30	53.32	55.06	Strong
75% aqueous ethanol	97.41	604.68	610.34	Strong
50% aqueous ethanol	115.40	123.45	254.75	Moderate
Acetone				
100% acetone	58.33	64.23	65.67	Strong
75% acetone	103.40	494.56	480.54	Moderate
50% acetone	122.90	220.67	273.83	Moderate

Lower IC_{50} values indicate stronger biological activity. Extracts were prepared using various solvents (water, methanol, ethanol, and acetone) at concentrations of 50%, 75%, and 100%. Each value represents the mean of three replicates. Activity levels are created based on the IC_{50} value of DPPH radical scavenging activity

the tested extracts, 100% ethanol showed the strongest DPPH radical scavenging activity, with an IC_{50} of 42.30 µg/mL, followed by 100% acetone (58.33 µg/mL) and 100% methanol (66.30 µg/mL). Water extract, on the other hand, exhibited the weakest scavenging activity, with an IC_{50} of 220.0 µg/mL, indicating a limited ability to neutralize free radicals. These results are consistent with the DPPH inhibition data, reinforcing that organic solvents are more effective in extracting compounds with radical-scavenging properties. In addition, a similar report revealed that the extraction of ginger using ethanol exhibited the best antioxidant activity [47]. The result is not different from other reports that illustrated the 70% ethanol-enhanced antioxidant properties of several samples against DPPH radical scavenging activity [48]. Therefore, the use of organic solvents, such as ethanol, is more effective in providing an extract with the highest DPPH radical scavenging activity.

In the FRAP assay, antioxidant potential was reflected in absorbance increases at 593 nm, indicative

of ferric-to-ferrous ion reduction (Fig. 3). Extracts from 100% ethanol (0.9667 ± 0.01), 100% acetone (0.7667 ± 0.01), and 100% methanol (0.7300 ± 0.01) showed the highest absorbance, consistent with their performance in TAA and DPPH. These solvents are evidently more effective in extracting electron-donating compounds responsible for reducing power via SET mechanisms [49]. In contrast, water (0.1967 ± 0.00) and 50% methanol (0.4033 ± 0.40) showed the lowest absorbance, reflecting limited extraction of redox-active metabolites. Although ANOVA was again inapplicable due to homogeneous values within groups, Tukey HSD analysis confirmed that 100% ethanol and 100% acetone differed significantly ($p < 0.05$) from water and aqueous solvents. The absence of significant differences between 100% ethanol, methanol, and acetone suggests that these solvents have comparable efficiency in extracting antioxidant constituents. Therefore, the FRAP results further support the solvent trends observed in TAA and DPPH, reinforcing that solvent polarity not only modulates extract yield but also affects antioxidant efficacy. Although no conversion to µmol Fe^{2+} was performed, absorbance values alone provide a reliable comparative index of reducing capacity. This result is similar to that of a previous study, which found that ethanol is the best solvent for the resulting *Allium cepa* extract, yielding the highest FRAP antioxidant activity [50]. On the other hand, the *Vigna mungo* extraction using ethanol demonstrated the highest antioxidant properties compared to methanol and acetone [51]. Moreover, the application of ethanol to *Tamarindus indica* extract yielded a similar result [52].

Solvents' Effect on Antidiabetic Enzyme Inhibitor Activity

The antidiabetic potential of *L. domesticum* leaf extracts was evaluated through their inhibitory activity against two key carbohydrate-hydrolyzing enzymes, including α-glucosidase and α-amylase. The results demonstrated a clear concentration-dependent inhibition pattern across all solvent systems, with significant differences in efficacy based on solvent polarity, as shown in Fig. 4 and 5. Fig. 4 demonstrates the effect of extracts at 500 µg/mL, that the most potent α-glucosidase inhibition was observed in the extract obtained with 100% ethanol (95.31 ± 0.88%), followed by 100% acetone (83.97 ± 0.45%), 100% methanol (78.74 ± 0.72%), and 75% methanol (70.55 ± 0.26%). These findings highlight the superior performance of pure organic solvents in extracting bioactive compounds that can disrupt α-glucosidase activity, an enzyme involved in the final step of carbohydrate digestion [53]. Conversely, the water extract exhibited the weakest inhibitory activity (23.08 ± 0.64%), emphasizing water's limited ability to solubilize non-polar or moderately polar enzyme inhibitors [54]. Moderate inhibition was also observed in aqueous-organic solvent mixtures, particularly 50% ethanol (68.54%) and 50% acetone (66.40%), suggesting

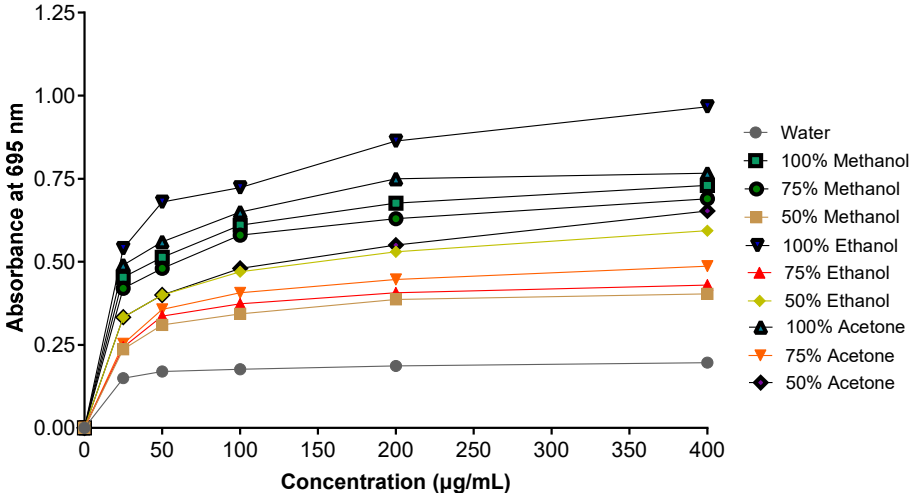

Fig 3. Ferric reducing antioxidant power (FRAP) of *L. domesticum* leaf extracts at various concentrations

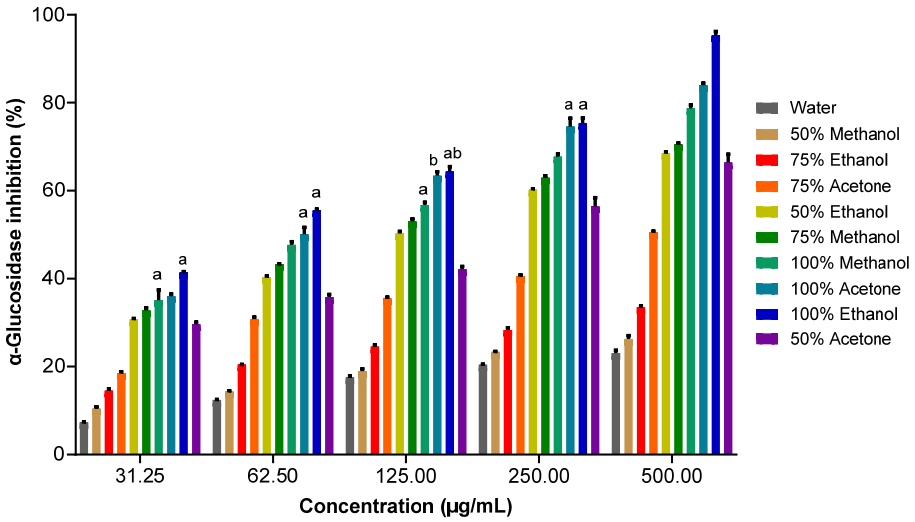

Fig 4. Inhibitory activity of *L. domesticum* leaf extracts against α-glucosidase enzyme at various concentrations. Bars with the same superscript letter are not significantly different ($p > 0.05$), while bars without superscript letters indicate significantly different ($p < 0.05$)

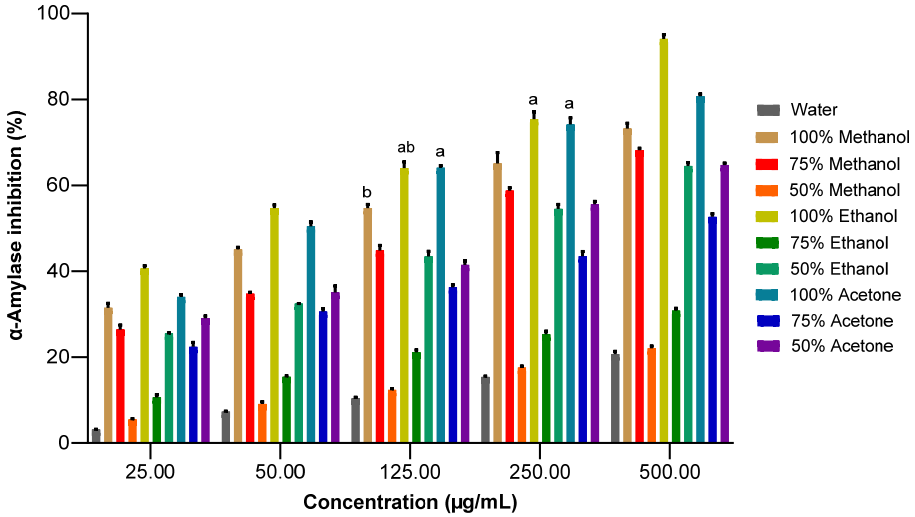

Fig 5. Inhibitory activity of *L. domesticum* leaf extracts against α-amylase enzyme at various concentrations. Bars with the same superscript letter are not significantly different ($p > 0.05$), while bars without superscript letters indicate significantly different ($p < 0.05$)

that mixed-polarity solvents enhance the co-extraction of both hydrophilic and lipophilic constituents. These trends were further confirmed by IC_{50} values (Table 2), where 100% methanol showed the lowest IC_{50} (70.64 µg/mL), followed by 75% methanol (118.19 µg/mL), while water had the highest IC_{50} (706.37 µg/mL). This inverse relationship between IC_{50} and inhibitory potency reinforces the role of solvent polarity in maximizing the yield of α-glucosidase inhibitors, likely flavonoids and phenolic acids, which exhibit competitive or mixed-mode inhibition [50].

A similar pattern was observed in the α-amylase inhibition assay (Fig. 5). At 500 µg/mL, the highest inhibition was recorded in 100% ethanol extract (94.07 ± 0.99%), followed by 100% acetone (80.74 ± 0.52%), 100% methanol (73.24 ± 1.20%), and 75% methanol (68.18 ± 0.46%). Once again, the water extract displayed the weakest inhibition (20.71 ± 0.61%).

These results corroborate the notion that highly organic solvents are more effective in extracting enzyme-inhibitory compounds [55]. Aqueous-organic solvents exhibited intermediate activity, particularly 50% ethanol (64.50 ± 0.83%), which is consistent with their ability to extract a broader range of phytochemicals. The IC_{50} values for α-amylase inhibition support this trend (Table 2). Furthermore, 100% methanol had the lowest IC_{50} (105.13 μg/mL), while 75% methanol (180.55 μg/mL) and water (720.45 μg/mL) were less effective. These results suggest that similar classes of bioactive compounds may inhibit both α-glucosidase and α-amylase, possibly through shared mechanisms such as substrate binding interference or active site occupancy [56-58].

Taken together, the data clearly demonstrate that solvent polarity is a critical determinant in the extraction of antidiabetic compounds. Pure ethanol and methanol consistently yielded extracts with the highest inhibitory activity across both enzyme assays, as well as in antioxidant evaluations (TAA, DPPH, and FRAP). A previous study reported similar results, showing that the methanol extract of *Quercus coccifera* has the strongest activity in inhibiting α-glucosidase and α-amylase enzymes compared to *n*-hexane and water extracts [59]. Additionally, the methanol extract of *Halimeda tuna* has better α-glucosidase inhibition activity than its water extract [60]. Additionally, the methanol extract of *Moringa oleifera* demonstrated the highest inhibition activity against α-glucosidase and α-amylase compared to the *n*-hexane and ethyl acetate extracts [61]. These solvents likely promote the extraction of phenolic and flavonoid-rich compounds with multifunctional bioactivity. The dual inhibitory effects observed suggest that *L. domesticum* leaves possess promising therapeutic potential for managing postprandial hyperglycemia. Beyond plant-based metabolites, recent studies have also explored metal-organic complexes such as Fe(III)-arginine, which demonstrated significant glucose-lowering effects in alloxan-induced diabetic mice through antioxidant and β-cell protective mechanisms [62]. The findings collectively emphasize the importance of exploring diverse bioactive sources as antioxidants for diabetes management, thereby supporting the rationale of investigating the solvent-dependent phytochemical profile and bioactivity of *L. domesticum* leaf extracts. Future research should focus on the isolation, identification, and mechanistic evaluation of the active compounds within these high-performing extracts, with a particular emphasis on ethanol and methanol-based systems for their broad-spectrum bioactivity.

Correlation Between Phytochemical Content and Biological Activities

To explore the linear associations between phytochemical composition and bioactivities, Pearson correlation analysis was conducted for TPC, TFC, DPPH, FRAP, and IC_{50} values of α-amylase and α-glucosidase. The outcomes are summarized in Table 3. TPC demonstrated a powerful positive correlation with TFC (r = 0.929), suggesting that flavonoids are major

Table 3. Relationships (Pearson correlation coefficients) between phytochemical levels (TPC, TFC), antioxidant activities (DPPH, FRAP), and enzyme inhibition (α-amylase, α-glucosidase) in *L. domesticum* leaf extracts

Assay	Pearson correlation (r)					
	TPC	TFC	DPPH	FRAP	α-Amylase inhibition	α-Glucosidase inhibition
TPC		0.929	−0.710	0.796	−0.745	−0.657
TFC	0.929		−0.529	0.646	−0.617	−0.557
DPPH	−0.710	−0.529		−0.808	0.665	0.595
FRAP	0.796	0.646	−0.808		−0.940	−0.903
α-Amylase inhibition	−0.745	−0.617	0.665	−0.940		0.988
α-Glucosidase inhibition	−0.697	−0.557	0.595	−0.903	0.988	

Direct relationships are represented by positive values, while inverse relationships are defined by negative values. Strong negative associations between IC_{50} and TPC/TFC indicate that increased phytochemical content is associated with improved biological performance. Significant results ($p < 0.05$) are emphasized in bold type

contributors to total phenolics. A strong positive correlation was also found between TPC and FRAP (r = 0.796), confirming the role of phenolics in antioxidant reducing power. By contrast, TPC showed strong negative correlations with DPPH IC_{50} (r = −0.710), α-amylase IC_{50} (r = −0.745), and α-glucosidase IC_{50} (r = −0.657). Although these correlations are negative, this reflects a favorable biological relationship: as TPC increases, the IC_{50} values of antioxidant and enzyme inhibitory activities decrease, indicating stronger bioactivity. Thus, extracts with elevated phenolic concentrations tended to show stronger antioxidant and antidiabetic activities. Similarly, TFC was positively linked with FRAP (r = 0.646) and negatively linked with DPPH (r = −0.529), α-amylase (r = −0.617), and α-glucosidase (r = −0.557) IC_{50} values, confirming the functional role of flavonoids in enhancing both antioxidant and enzyme inhibitory activities. Notably, FRAP exhibited a robust negative correlation with the IC_{50} values of α-amylase (r = −0.940) and α-glucosidase (r = −0.903), reinforcing the idea that the antioxidant power of the extract is closely linked to its capacity to inhibit carbohydrate-hydrolyzing enzymes. A near-perfect positive correlation was observed between α-amylase and α-glucosidase IC_{50} values (r = 0.988), suggesting a consistent inhibitory trend across both enzymes. Overall, the negative correlations between phytochemical content and IC_{50} values of antioxidant and antidiabetic activities indicate that the higher the concentration of bioactive compounds, the stronger the biological activity. These outcomes reinforce the notion that phenolic and flavonoid compounds are key contributors to the extracts' antioxidant activity and their effectiveness in enzyme inhibition.

To support the biological activities of *L. domesticum* extracts, this study confirmed the secondary metabolites contained in the ethanolic extract of *L. domesticum* leaf, as presented in Table 4. Table 4 revealed the presence of diverse secondary metabolites encompassing phenolic acids, flavonoids, coumarins, sterols, xanthones, and triterpenoids. A total of thirteen compounds were successfully annotated based on accurate mass, molecular formula, and diagnostic fragmentation patterns, indicating the chemical complexity of the extract. These metabolites exhibited both polar and nonpolar characteristics, as reflected in their retention times, which ranged from 2.13 to 18.49 min. This variation corresponded to phenolic acids eluting earlier and triterpenoids or sterols appearing

Table 4. LC-HRMS data of compounds identified in the ethanol extract of *L. domesticum*

RT (min)	Compound	Ion type	Formula	Molecular mass (Da)	*m/z* (observed)	Ionization mode
5.92	Apigenin-*O*-glucuronide	$[M-H]^-$	$C_{21}H_{18}O_{12}$	462.36	461.07	Negative
11.36	11,12-Dihydroxy-2-methoxypicrasa-2,13(21)-diene-1,16-dione	$[M+H]^+$	$C_{23}H_{30}O_5$	386.49	387.26	Positive
12.17	1,4a-dimethyl-9-oxo-7(propan-2-yl)1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1 carboxylic acid	$[M+H]^+$	$C_{20}H_{28}O_3$	316.44	317.21	Positive
5.25	Vanillic acid	$[M-H]^-$	$C_8H_8O_4$	168.15	166.06	Negative
6.61	Genistein 4'-*O*-glucuronide	$[M-H]^-$	$C_{21}H_{18}O_{11}$	446.36	445.13	Negative
12.73	4β-Methylzymosterol-4-carbaldehyde	$[M+H]^+$	$C_{29}H_{46}O_2$	426.67	427.36	Positive
7.61	Kaempferol	$[M-H]^-$	$C_{15}H_{10}O_6$	286.24	285.13	Negative
18.49	(22*R*,23*R*,24*S*)-22,23-Dihydroxyergost-4-en-3-one	$[M+H]^+$	$C_{28}H_{46}O_3$	430.67	431.35	Positive
14.30	2-Hydroxy-3-{[(2*E*)-3-(4-hydroxyphenyl)-2-propenoyl]oxy}lup-20(29)-en-28-oic acid	$[M-H]^-$	$C_{39}H_{50}O_6$	598.82	597.36	Negative
14.11	1,3,7-Trihydroxy-6-methoxy-4,5-diisoprenylxanthone	$[M-H]^-$	$C_{24}H_{26}O_6$	410.46	409.16	Negative
2.13	Gentisic acid	$[M-H]_-$	$C_7H_6O_4$	154.12	153.15	Negative
8.36	Apigenin	$[M-H]^-$	$C_{15}H_{10}O_5$	270.24	269.04	Negative
9.50	Scrophulein	$[M-H]^-$	$C_{11}H_8O_5$	220.18	219.03	Negative

at later retention times. Among the polar constituents, gentisic acid and vanillic acid were identified as predominant phenolic acids. Both are known for their strong antioxidant capacity, primarily through hydrogen-donating and radical-scavenging mechanisms, which significantly contribute to the DPPH and FRAP activities of the ethanolic extract [63-64].

Flavonoids represented the primary class of bioactive compounds, with apigenin, kaempferol, and genistein 4'-*O*-glucuronide being the key identified flavones and isoflavones. The presence of these compounds supports the extract's high antioxidant, α-glucosidase, and α-amylase inhibitory activity, as these flavonoids are widely recognized for their ability to modulate carbohydrate metabolism and oxidative stress pathways [65-67]. In addition, nonpolar metabolites were dominated by triterpenoids and sterols, including (22*R*,23*R*,24*S*)-22,23-dihydroxyergost-4-en-3-one, 4β-methylzymosterol-4-carbaldehyde, and 11,12-dihydroxy-2-methoxypicrasa-2,13(21)-diene-1,16-dione. These compounds are typical of limonoid and ergostane-type skeletons, which have been reported in *L. domesticum* and other members of the Meliaceae family [68]. Their structural features, such as hydroxyl and carbonyl substitutions, contribute to anti-inflammatory and membrane-stabilizing activities, complementing the antioxidant effects of phenolic constituents [69].

Therefore, 2-hydroxy-3-{[(2*E*)-3-(4-hydroxyphenyl)-2-propenoyl]oxy}lup-20(29)-en-28-oic acid was detected as a phenolic-triterpenoid hybrid. This simultaneous presence of triterpenoid and *p*-coumaroyl groups suggests potential synergistic antioxidant and α-glucosidase inhibitory mechanisms. Scrophulein, a coumarin derivative, was also identified, contributing to the diversity of oxygenated phenolic compounds in the extract. Furthermore, the identification of these compounds aligns with previous reports describing the occurrence of limonoids, flavonoids, and phenolic acids in *L. domesticum*. Overall, the chemical profile highlights the presence of multifunctional metabolites with strong antioxidant potential and relevant biological properties that support the observed *in vitro* antidiabetic and radical-scavenging activities. These findings provide valuable insights into the phytochemical basis of *L. domesticum* leaf bioactivity and validate its traditional use as an antidiabetic agent. Moreover, the identified bioactive compounds and demonstrated inhibitory effects suggest that *L. domesticum* leaf extract has potential as an oral

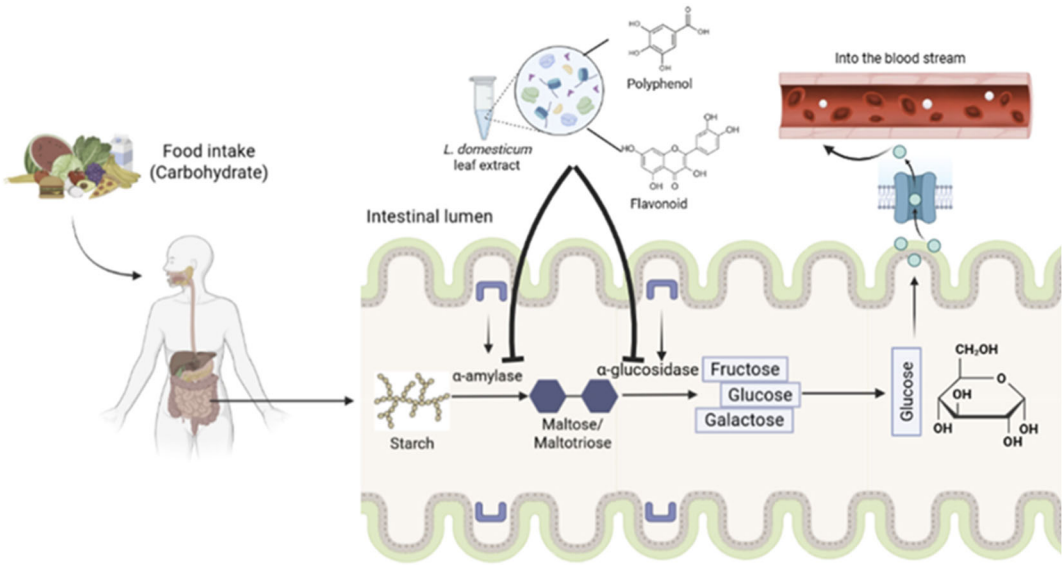

Fig 6. Proposed mechanism of *L. domesticum* leaf extract in inhibiting postprandial glucose absorption through the suppression of α-amylase and α-glucosidase activities in the intestinal lumen. Polyphenols and flavonoids contained in the extract interfere with carbohydrate hydrolysis, delaying glucose uptake into the bloodstream and thereby reducing hyperglycemia

antidiabetic treatment, either through direct administration (e.g., oral gavage) or incorporation into functional food formulations, as illustrated in Fig. 6.

■ CONCLUSION

This investigation revealed that the type of solvent has a significant impact on both the phytochemical composition and biological activities of *L. domesticum* extracts. Pure organic solvents, especially ethanol and acetone, yielded superior levels of phenolic and flavonoid compounds, which were linked to pronounced antioxidant and antidiabetic enzyme inhibitory effects. The inverse correlation between phytochemical content and IC_{50} values emphasizes the therapeutic significance of *L. domesticum* leaves as a natural strategy to combat oxidative stress and regulate postprandial glucose levels. These outcomes provide valuable scientific support for the potential application of *L. domesticum* in nutraceuticals and phytopharmaceuticals.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support provided by Universitas Sumatera Utara through the research grant scheme *Hibah Penelitian Universitas Sumatera Utara Tahun Anggaran 2024* (Contract No. 71/UN5.4.10.S/PPM/KP-TALENTA/RB1/2024). The authors also thank the Faculty of Pharmacy, Universitas Sumatera Utara, for providing laboratory facilities and technical support during the study.

■ CONFLICT OF INTEREST

The authors have no conflict of interest.

■ AUTHOR CONTRIBUTIONS

Conceptualization, methodology, investigation, and supervision: Jiro Hasegawa Situmorang and Muhammad Fauzan Lubis; Analysis: Ririn Astyka, Muhammad Fauzan Lubis, and Ana Yulyana; Writing draft preparation: Hafid Syahputra, Muhammad Fauzan Lubis, Ririn Astyka; Review and editing: Jiro Hasegawa Situmorang and Ana Yulyana. All authors have read and approved this article for publication.

■ REFERENCES

[1] Mohan, S., and Egan, A.M., 2024, Diagnosis and treatment of hyperglycemia in pregnancy: Type 2 diabetes mellitus and gestational diabetes, *Endocrinol. Metab. Clin. North Am.*, 53 (3), 335–347.

[2] Husna, F., Marisa, M., Suryawati, S., Suyatna, F.D., Husnah, H., Hakim, R.W., Aulia, M.I., and Dasopang, E.A., 2025, Traditional remedies from Aceh for diabetes mellitus treatment: Patterns of use in rural-urban areas in Aceh, *Clin. Epidemiol. Global Health*, 34, 102079.

[3] Yang, Y., Chen, Z., Zhao, X., Xie, H., Du, L., Gao, H., and Xie, C., 2022, Mechanisms of kaempferol in the treatment of diabetes: A comprehensive and latest review, *Front. Endocrinol.*, 13, 990299.

[4] Tangvarasittichai, S., 2015, Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus, *World J. Diabetes*, 6 (3), 456–480.

[5] Gong, L., Feng, D., Wang, T., Ren, Y., Liu, Y., and Wang, J., 2020, Inhibitors of α-amylase and α-glucosidase: Potential linkage for whole cereal foods on prevention of hyperglycemia, *Food Sci. Nutr.*, 8 (12), 6320–6337.

[6] Chokki, M., Cudălbeanu, M., Zongo, C., Dah-Nouvlessounon, D., Ghinea, I.O., Furdui, B., Raclea, R., Savadogo, A., Baba-Moussa, L., Avamescu, S.M., Dinica, R.M., and Baba-Moussa, F., 2020, Exploring antioxidant and enzymes (A-amylase and B-glucosidase) inhibitory activity of *Morinda lucida* and *Momordica charantia* leaves from Benin, *Foods*, 9 (4), 434.

[7] Abdallah, H.M., Mohamed, G.A., and Ibrahim, S.R.M., 2022, *Lansium domesticum*—A fruit with multi-benefits: Traditional uses, phytochemicals, nutritional value, and bioactivities, *Nutrients*, 14 (7), 1531.

[8] Febriani, H., Lubis, M.F., Sumaiyah, S., Hasibuan, P.A.Z., Syahputra, R.A., Astyka, R., and Juwita, N.A., 2025, Optimization of microwave-assisted extraction to obtain a polyphenol-rich crude extract from duku (*Lansium domesticum* Corr.) leaf and the correlation with antioxidant and cytotoxic activities, *Kuwait J. Sci.*, 52 (1), 100315.

[9] Asem, N., Abdul Gapar, N.A., Abd Hapit, N.H., and Omar, E.A., 2020, Correlation between total

phenolic and flavonoid contents with antioxidant activity of Malaysian stingless bee propolis extract, *J. Apic. Res.*, 59 (4), 437–442.

[10] Dudoit, A., Benbouguerra, N., Richard, T., Hornedo-Ortega, R., Valls-Onayet, J., Coussot, G., and Saucier, C., 2020, α-Glucosidase inhibitory activity of Tannat grape phenolic extracts in relation to their ripening stages, *Biomolecules*, 10 (8), 1088.

[11] Olennikov, D.N., Chirikova, N.K., Kashchenko, N.I., Nikolaev, V.M., Kim, S.W., and Vennos, C., 2018, Bioactive phenolics of the genus *Artemisia* (Asteraceae): HPLC-DAD-ESI-TQ-MS/MS profile of the Siberian species and their inhibitory potential against α-amylase and α-glucosidase, *Front. Pharmacol.*, 9, 756.

[12] Mokrani, A., and Madani, K., 2016, Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (*Prunus persica* L.) fruit, *Sep. Purif. Technol.*, 162, 68–76.

[13] Venkatachalam, R., Kalimuthu, K., Chinnadurai, V., Saravanan, M., Radhakrishnan, R., Shanmuganathan, R., and Pugazhendhi, A., 2020, Various solvent effects on phytochemical constituent profiles, analysis of antioxidant and antidiabetic activities of *Hopea parviflora*, *Process Biochem.*, 89, 227–232.

[14] Atanu, F.O., Ikeojukwu, A., Owolabi, P.A., and Avwioroko, O.J., 2022, Evaluation of chemical composition, *in vitro* antioxidant, and antidiabetic activities of solvent extracts of *Irvingia gabonensis* leaves, *Heliyon*, 8 (7), e09922.

[15] Al-Hamdany, I.Y.A., Mohammed, M.J., and Al-Taei, S.M.S., 2025, Exploring the antimicrobial and antioxidant properties of *Silybum marianum* tissue-cultured phenolic extracts, *J. Multidiscip. Appl. Nat. Sci.*, 5 (2), 429–445.

[16] Lestari, S., Husni, A., Nurjanah, N., and Firdaus, M., 2025, Chemical characteristic, antioxidant activity, and consumer acceptance level of kombucha from *Sargassum cristaefolium* seaweed tea, *J. Multidiscip. Appl. Nat. Sci.*, 5 (3), 949–968.

[17] Hasibuan, P.A.Z., Keliat, J.M., and Lubis, M.F., 2024, Combination of cisplatin and ethyl acetate extract of *Vernonia amygdalina* Delile induces cell cycle arrest and apoptosis on PANC-1 cells via PI3K/mTOR, *J. Pharm. Pharmacogn. Res.*, 12 (5), 870–880.

[18] Sumaiyah, S., Murwanti, R., Illian, D.N., Lubis, M.F., and Tampubolon, K., 2024, New insights of response surface methodology approach in optimizing total phenolic content of *Zanthoxylum acanthopodium* DC. fruit extracted using microwave-assisted extraction and the impact to antioxidant activity, *Indones. J. Chem.*, 24 (6), 1743–1759.

[19] Nasution, H.M., Yulyana, A., Utama, R.F., Bangar, R.I., Kaban, V.E., Daulay, W., Astyka, R., and Lubis, M.F., 2025, Synergistic mechanism of *Phyllanthus emblica* extract and tetracycline against multidrug-resistant *Acinetobacter baumannii*, *Narra J*, 5 (1), e1939.

[20] Astyka, R., Hasibuan, P.A.Z., Sumaiyah, S., Juwita, N.A. and Lubis, M.F., 2024, Optimization of microwave-assisted extraction of total flavonoid content from red betel leaf (*Piper crocatum* Ruiz and Pav) and its correlation with antioxidant and antibacterial activities using response surface methodology, *J. Appl. Pharm. Sci.*, 14 (8), 150–159.

[21] Mansoori, A., Singh, N., Dubey, S.K., Thakur, T.K., Alkan, N., Das, S.N., and Kumar, A., 2020, Phytochemical characterization and assessment of crude extracts from *Latana camara* L. for antioxidant and antimicrobial activity, *Front. Agron.*, 2, 582268.

[22] Lubis, M.F., Syahputra, H., Illian, D.N. and Kaban, V.E., 2022, Antioxidant activity and nephroprotective effect of *Lansium parasiticum* leaves in doxorubicin-induced rats, *J. Res. Pharm.*, 26 (3), 565–573.

[23] Kiss, A., Papp, V.A., Pál, A., Prokisch, J., Mirani, S., Toth, B.E., and Alshaal, T., 2025, Comparative study on antioxidant capacity of diverse food matrices: Applicability, suitability, and inter-correlation of multiple assays to assess polyphenol and antioxidant status, *Antioxidant*, 14 (3), 317.

[24] Yulyana, A., Amin, C., Simanjuntak, P., Abdillah, S., Rohman, A., and Mugiyanto, E., 2023, Assessing

the antimetabolite activity of anthocyanins in Cantigi fruits from two conservation sites in Indonesia, *Indones. J. Pharm.*, 34 (3), 450–459.

[25] Yulyana, A., Chaidir, C., Simanjuntak, P., Sulastri, L., and Abdillah, S., 2023, The water fraction of Cantigi (*Vaccinium variegatum* Bl. Miq.) fruits demonstrate the highest antimetabolic syndrome properties on enzyme assay, *Pharmacia*, 70 (3), 587–594.

[26] Suryani, M., Yulyana, A., Sumaiyah, S., Fitri, K., Lubis, L.D., Daulay, W., Surbakti, C., Astyka, R., and Lubis, M.F., 2025, Microwave-assisted extraction enhances the antioxidant and anti-diabetic activities of polyphenol-rich *Phyllanthus emblica* fruit extract, *Discover Food*, 5 (1), 244.

[27] Al-Mansoub, M.A., Asmawi, M.Z., and Murugaiyah, V., 2014, Effect of extraction solvents and plant parts used on the antihyperlipidemic and antioxidant effects of *Garcinia atroviridis*: A comparative study, *J. Sci. Food Agric.*, 94 (8), 1552–1558.

[28] Dong, J., Zhou, K., Ge, X., Xu, N., Wang, X., He, Q., Zhang, C., Chu, J., and Li, Q., 2022, Effects of extraction technique on the content and antioxidant activity of flavonoids from *Gossypium hirsutum* Linn. flowers, *Molecules*, 27 (17), 5627.

[29] Sepahpour, S., Selamat, J., Abdul Manap, M.Y., Khatib, A., and Abdull Razis, A.F., 2018, Comparative analysis of chemical composition, antioxidant activity and quantitative characterization of some phenolic compounds in selected herbs and spices in different solvent extraction systems, *Molecules*, 23 (2), 402.

[30] Chatepa, L.E.C., Mwamatope, B., Chikowe, I., and Masamba, K.G., 2024, Effects of solvent extraction on the phytoconstituents and *in vitro* antioxidant activity properties of leaf extracts of the two selected medicinal plants from Malawi, *BMC Complementary Med. Ther.*, 24 (1), 317.

[31] Ghasemzadeh, A., Jaafar, H.Z.E., Juraimi, A.S., and Tayebi-Meigooni, A., 2015, Comparative evaluation of different extraction techniques and solvents for the assay of phytochemicals and antioxidant activity of Hashemi rice bran, *Molecules*, 20 (6), 10822–10838.

[32] Gil-Martín, E., Forbes-Hernández, T., Romero, A., Cianciosi, D., Giampieri, F., and Battino, M., 2022, Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products, *Food Chem.*, 378, 131918.

[33] Méndez, D.A., Fabra, M.J., Odriozola-Serrano, I., Martín-Belloso, O., Salvia-Trujillo, L., López-Rubio, A., and Martínez-Abad, A., 2022, Influence of the extraction conditions on the carbohydrate and phenolic composition of functional pectin from persimmon waste streams, *Food Hydrocolloids*, 123, 107066.

[34] El Mannoubi, I., 2023, Impact of different solvents on extraction yield, phenolic composition, *in vitro* antioxidant and antibacterial activities of deseeded *Opuntia stricta* fruit, *J. Umm Al-Qura Univ. Appl. Sci.*, 9 (2), 176–184.

[35] Kaplan, M., Yilmaz, M.M., Say, R., Köprü, S., and Karaman, K., 2020, Bioactive properties of hydroalcoholic extract from *Origanum onites* L. as affected by glycerol incorporation, *Saudi J. Biol. Sci.*, 27 (8), 1938–1946.

[36] Santana, Á.L., and Macedo, G.A., 2019, Effects of hydroalcoholic and enzyme-assisted extraction processes on the recovery of catechins and methylxanthines from crude and waste seeds of guarana (*Paullinia cupana*), *Food Chem.*, 281, 222–230.

[37] Chaves, J.O., de Souza, M.C., da Silva, L.C., Lachos-Perez, D., Torres-Mayanga, P.C., Machado, A.P.F., Forster-Carneiro, T., Vázquez-Espinosa, M., González-de-Peredo, A.V., Barbero, G.F., and Rostagno, M.A., 2020, Extraction of flavonoids from natural sources using modern techniques, *Front. Chem.*, 8, 507887.

[38] Liu, X., Liu, Y., Shan, C., Yang, X., Zhang, Q., Xu, N., Xu, L., and Song, W., 2022, Effects of five extraction methods on total content, composition, and stability of flavonoids in jujube, *Food Chem.: X*, 14, 100287.

[39] Dirar, A.I., Alsaadi, D.H.M., Wada, M., Mohamed, M.A., Watanabe, T., and Devkota, H.P., 2019, Effects of extraction solvents on total phenolic and

flavonoid contents and biological activities of extracts from Sudanese medicinal plants, *S. Afr. J. Bot.*, 120, 261–267.

[40] Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S., and Ju, Y.H., 2014, Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*, *J. Food Drug Anal.*, 22 (3), 296–302.

[41] Ojha, S., Raj, A., Roy, A., and Roy, S., 2018, Extraction of total phenolics, flavonoids and tannins from *Paederia foetida* L. leaves and their relation with antioxidant activity, *Pharmacogn. J.*, 10 (3), 541–547.

[42] Bakhouche, K., Dhaouadi, Z., Jaidane, N., and Hammoutène, D., 2015, Comparative antioxidant potency and solvent polarity effects on HAT mechanisms of tocopherols, *Comput. Theor. Chem.*, 1060, 58–65.

[43] Herrera-Pool, E., Ramos-Díaz, A.L., Lizardi-Jiménez, M.A., Pech-Cohuo, S., Ayora-Talavera, T., Cuevas-Bernardino, J.C., García-Cruz, U., and Pacheco, N., 2021, Effect of solvent polarity on the Ultrasound Assisted extraction and antioxidant activity of phenolic compounds from habanero pepper leaves (*Capsicum chinense*) and its identification by UPLC-PDA-ESI-MS/MS, *Ultrason. Sonochem.*, 76, 105658.

[44] Abolmaesoomi, M., Abdul Aziz, A., Mat Junit, S., and Mohd Ali, J., 2019, *Ficus deltoidea*: Effects of solvent polarity on antioxidant and anti-proliferative activities in breast and colon cancer cells, *Eur. J. Integr. Med.*, 28, 57–67.

[45] Ye, F., Liang, Q., Li, H., and Zhao, G., 2015, Solvent effects on phenolic content, composition, and antioxidant activity of extracts from florets of sunflower (*Helianthus annuus* L.), *Ind. Crops Prod.*, 76, 574–581.

[46] Lubis, M.F., Sumaiyah, S., Lubis, L.D., Fitri, K., and Astyka, R., 2024, Application of Box-Behnken design for optimization of *Vernonia amygdalina* stem bark extract in relation to its antioxidant and anti-colon cancer activity, *Arabian J. Chem.*, 17 (4), 105702.

[47] Akullo, J.O., Kiage-Mokua, B.N., Nakimbugwe, D., Ng'ang'a, J., and Kinyuru, J., 2023, Phytochemical profile and antioxidant activity of various solvent extracts of two varieties of ginger and garlic, *Heliyon*, 9 (8), e18806.

[48] Mohammed, E.A., Abdalla, I.G., Alfawaz, M.A., Mohammed, M.A., Al Maiman, S.A., Osman, M.A., Yagoub, A.E.A., and Hassan, A.B., 2022, Effects of extraction solvents on the total phenolic content, total flavonoid content, and antioxidant activity in the aerial part of root vegetables, *Agriculture*, 12 (11), 1820.

[49] Adebiyi, O.E., Olayemi, F.O., Ning-Hua, T., and Guang-Zhi, Z., 2017, *In vitro antioxidant* activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of *Grewia carpinifolia*, *Beni-Suef Univ. J. Basic Appl. Sci.*, 6 (1), 10–14.

[50] Maser, W.H., Maiyah, N., Nagarajan, M., Kingwasharapong, P., Senphan, T., Ali, A.M.M., and Bavisetty, S.C.B., 2023, Effect of different extraction solvents on the yield and enzyme inhibition (α-amylase, α-glucosidase, and lipase) activity of some vegetables, *Biodiversitas*, 24 (6), 3320–3331.

[51] Hossain, M.A., Arafat, M.Y., Alam, M., and Hossain, M.M., 2021, Effect of solvent types on the antioxidant activity and total flavonoids of some Bangladeshi legumes, *Food Res.*, 5 (4), 329–335.

[52] Yeasmen, N., and Islam, M.N., 2015, Ethanol as a solvent and hot extraction technique preserved the antioxidant properties of tamarind (*Tamarindus indica*) seed, *J. Adv. Vet. Anim. Res.*, 2 (3), 332–337.

[53] Lin, Y.T., Lin, H.R., Yang, C.S., Liaw, C.C., Sung, P.J., Kuo, Y.H., Cheng, M.J., and Chen, J.J., 2022, Antioxidant and anti-α-glucosidase activities of various solvent extracts and major bioactive components from the fruits of *Crataegus pinnatifida*, *Antioxidants*, 11 (2), 320.

[54] Ervina, M., Diva, J., Caroline, C., and Soewandi, A., 2023, The solvents influence in the continuous extraction to antioxidant and α-glucosidase inhibition of *Cinnamomum burmannii* bark, *Food Res.*, 7 (4), 258–264.

[55] Li, C.W., Chu, Y.C., Huang, C.Y., Fu, S.L., and Chen, J.J., 2020, Evaluation of antioxidant and anti-

α-glucosidase activities of various solvent extracts and major bioactive components from the seeds of *Myristica fragrans*, *Molecules*, 25 (21), 5198.

[56] Saltos, M.B.V., Puente, B.F.N., Faraone, I., Milella, L., De Tommasi, N., and Braca, A., 2015, Inhibitors of α-amylase and α-glucosidase from *Andromachia igniaria* Humb. & Bonpl, *Phytochem. Lett.*, 14, 45–50.

[57] Khan, S.A., Al Kiyumi, A.R., Al Sheidi, M.S., Al Khusaibi, T.S., Al Shehhi, N.M., and Alam, T., 2016, *In vitro* inhibitory effects on α-glucosidase and α-amylase level and antioxidant potential of seeds of *Phoenix dactylifera* L., *Asian Pac. J. Trop. Biomed.*, 6 (4), 322–329.

[58] Wibowo, S., Wardhani, S.K., Hidayati, L., Wijayanti, N., Matsuo, K., Costa, J., Nugraha, Y., Siregar, J.E., and Nuringtyas, T.R., 2024, Investigation of α-glucosidase and α-amylase inhibition for antidiabetic potential of agarwood (*Aquilaria malaccensis*) leaves extract, *Biocatal. Agric. Biotechnol.*, 58, 103152.

[59] Jaber, S.A., 2023, *In vitro* alpha-amylase and alpha-glucosidase inhibitory activity and *in vivo* antidiabetic activity of *Quercus coccifera* (Oak tree) leaves extracts, *Saudi J. Biol. Sci.*, 30 (7), 103688.

[60] Gazali, M., Jolanda, O., Husni, A., Nurjanah, N., Abd Majid, F.A., Zuriat, Z., and Syafitri, R., 2023, *In vitro* α-amylase and α-glucosidase inhibitory activity of green seaweed *Halimeda tuna* extract from coast of Lhok Bubon, Aceh, *Plants*, 12 (2), 393.

[61] Magaji, U.F., Sacan, O., and Yanardag, R., 2020, Alpha amylase, alpha glucosidase and glycation inhibitory activity of *Moringa oleifera* extracts, *S. Afr. J. Bot.*, 128, 225–230.

[62] Ambarwati, Y., Nurhasanah, N., Karima, N., and Purnomo, H., 2025, Antidiabetic activity test of Fe(III) complex compound with arginine ligand in male mice (*Mus musculus* L.), *J. Multidiscip. Appl. Nat. Sci.*, 5 (1), 141–157.

[63] Mardani-Ghahfarokhi, A., and Farhoosh, R., 2020, Antioxidant activity and mechanism of inhibitory action of gentisic and α-resorcylic acids, *Sci. Rep.*, 10 (1), 19487.

[64] Magiera, A., Kołodziejczyk-Czepas, J., and Olszewska, M.A., 2025, Antioxidant and anti-inflammatory effects of vanillic acid in human plasma, human neutrophils, and non-cellular models *in vitro*, *Molecules*, 30 (3), 467.

[65] Liu, H., Huang, P., Wang, X., Ma, Y., Tong, J., Li, J., and Ding, H., 2024, Apigenin analogs as α-glucosidase inhibitors with antidiabetic activity, *Bioorg. Chem.*, 143, 107059.

[66] Hendra, R., Army, M.K., Frimayanti, N., Teruna, H.Y., Abdulah, R., and Nugraha, A.S., 2024, α-Glucosidase and α-amylase inhibitory activity of flavonols from *Stenochlaena palustris* (Burm.f.) Bedd, *Saudi Pharm. J.*, 32 (2), 101940.

[67] Jia, J., Dou, B., Gao, M., Zhang, C., Liu, Y., and Zhang, N., 2024, Effect of genistein on starch digestion *in vitro* and its mechanism of action, *Foods*, 13 (17), 2809.

[68] Fan, W., Fan, L., Wang, Z., and Yang, L., 2021, Limonoids from the genus *Melia* (Meliaceae): Phytochemistry, synthesis, bioactivities, pharmacokinetics, and toxicology, *Front. Pharmacol.*, 12, 795565.

[69] Hilmayanti, E., Nurlelasari, N., Supratman, U., Kabayama, K., Shimoyama, A., and Fukase, K., 2022, Limonoids with anti-inflammatory activity: A review, *Phytochemistry*, 204, 113469.