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Antidiabetic and Pharmacokinetic Properties of *Shorea macrophylla* Fruits' Extracts in Borneo

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ABSTRACT

The global rise in diabetes prevalence has intensified the search for effective and safer natural antidiabetic agents. *Shorea macrophylla* fruits, known for their lipogenesis effects, present a promising avenue. This study explores the antidiabetic properties of *S. macrophylla* fruits' crude extracts through *in vitro* assays for α-amylase and advanced glycation end-products (AGEs) inhibition, alongside molecular docking for inhibitor prediction and *in silico* pharmacokinetic evaluation. While all extracts exhibited mild inhibitory effects on α-amylase, they are significantly less effective than acarbose. Methanolic (MeOH) extract demonstrated the strongest inhibitory effects on AGEs, surpassing other extracts at 100 µg/ml. However, it exhibits no significant differences compared to Aminoguanidine (AG), suggesting its potential to become an alternative antiglycation source. Molecular docking revealed that five compounds, methyl stearate, methyl palmitate, methyl arachidate, methyl oleate, and methyl linoleate, had higher binding energies than acarbose for Human pancreatic alpha-amylase (HPA) (PDB ID: 5E0F). However, their binding energies with the receptor for advanced glycation end-products (RAGE) (PDB ID: 3O3U) were lower than AG (-3.515 kcal/mol), ranging from -5.760 to -6.510 kcal/mol with amino acid residue ARG-66 consistently involved in hydrogen bonding interactions. Analysis of pharmacokinetic properties confirmed that these compounds adhere to Lipinski's Rule of Five, indicating their drug-like properties despite generally poor solubility and potential skin irritation. In summary, *S. macrophylla* fruits' crude extracts, particularly the MeOH extract, show promise as antiglycation agents, necessitating further *in vivo* studies to validate these findings for drug development.

Keywords: α-amylase, antidiabetic, molecular docking, pharmacokinetic, *Shorea macrophylla*

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INTRODUCTION

Diabetes has a substantial impact on socioeconomic development and community health globally. Based on the International Diabetes Federation (IDF), the number of individuals (aged 20–79) worldwide with diabetes was 536.6 million in 2021. Additionally, it is projected that by 2030 and 2045, the number will rise to 643 million and 783 million, respectively (Magliano & Boyko, 2021). Malaysia has the greatest prevalence of diabetes in the Western Pacific, costing the country about 600 million USD annually (Ganasegeran, 2021). For instance, there was a total of 4.5 million adults in Malaysia who had diabetes in 2021, with a prevalence of 20%, posing a serious risk to public health.

Though synthetic drugs like insulin, insulin sensitisers, insulin secretagogues, and reactive oxygen species (ROS) inhibitors have been used to treat diabetes, concerns have been raised about their adverse effects (Alam et al., 2022). These include hallucinations, memory loss, drowsiness (Arora et al., 2021), renal failure, and weight gain (Blahova et al., 2021). Despite the potential of traditional medicinal plants, the antidiabetic and pharmacokinetic properties of *Shorea macrophylla* remain largely unexplored. Thus, exploring *S. macrophylla* fruits' crude extracts could offer the possibility of discovering a safer and more effective antidiabetic agent.

Addressing postprandial hyperglycemia (PPHG) is crucial for diabetes treatment. By lowering PPHG, compounds that hinder the natural carbohydrases α-glucosidase and α-amylase can be effective treatments for diabetes (Lankatillake et al., 2021). According to Luo et al. (2019), enzyme inhibitors are common targets in drug discovery for metabolic diseases, including diabetes. Similar research on medicinal plants for diabetes treatment had been performed in a variety of *in vitro* approaches, such as α-amylase, α-glucosidase, β-glucosidase (Bouyahya et al., 2021), and advanced glycation end products (AGEs) (Nur Akmal et al., 2021). Consequently, screening plant extracts for enzyme inhibition is a typical method for finding antidiabetic agents (Rajan et al., 2020). *Shorea macrophylla*, a tropical plant from Kalimantan and Borneo Malaysia, is known for its high fat content. Chew (2023) noted that methanolic and diethyl ether extracts of *S. macrophylla* fruits promote lipogenesis, that linked to obesity and Type 2 diabetes (T2DM). Thus, it is hypothesised that *S. macrophylla* fruit extracts could assist in T2DM management. This study aims to examine the antidiabetic properties of *S. macrophylla* fruits' crude extracts and evaluate the pharmacokinetic properties through *in vitro* and *in silico* approaches respectively.

MATERIALS AND METHODS

In Vitro Inhibition of α-amylase

A total of four different *S. macrophylla* fruit crude extracts (hexane, dichloromethane, ethyl acetate, and methanol) were obtained from the Animal Biotechnology Lab at Universiti Malaysia Sarawak. This assay was carried out according to Wickrmaratne et al. (2016)

with slight modifications. Different concentrations of test extracts ranging from 62.5 to 1000 µg/ml, α-amylase enzyme (2 U /mL) (Sigma-Aldrich, US), and 1 % soluble starch (Sigma-Aldrich, US) were dissolved in 0.02 M sodium phosphate buffer containing 6 mM NaCl (pH 6.9). Exactly 200 µL of α-amylase was added to each test tube containing 200 µL of sample or acarbose at various concentrations. The mixture was incubated at 37°C for 15 minutes before adding 200 µL of starch. After vortexing and further incubation at 37°C for another 15 minutes, 200 µL of 3,5-Dinitrosalicylic acid (DNSA) colour reagent was added. The reaction mixture was boiled (85-90°C) for 5 minutes, cooled to room temperature, and diluted. Acarbose served as a positive control, and a negative control with 100% enzyme activity was prepared without the sample. A blank using only DNSA was also prepared. Absorbance readings were read at 540 nm using a microplate reader (Infinite M200 PRO / TECAN, Switzerland). Triplicates were performed. Raw data were adjusted by subtracting the absorbance of the blank. Results were expressed as the percentage inhibition of α-amylase, calculated by: ([Absorbance reading of negative control-Absorbance reading of sample]/[Absorbance reading of negative control]) ×100.

In Vitro Inhibition of AGEs

This assay was carried out by referring to the experiment by Sekhon-Loodu and Rupasinghe (2019) with minor modifications. The incubation mixtures were dissolved in 0.2 M sodium phosphate buffer (pH 7.4) containing sodium azide (0.02% w/v) to make up a final volume of 600 µL, consisting of 200 µL of each BSA (5 mg/ml), D-glucose (36 mg/ml), and sample extracts (ranging from 5–100 µg/ml) or aminoguanidine (AG), a known inhibitor for AGEs. Negative control was performed when the samples were omitted. The mixtures were then subjected to incubation at 37◦C for a week. After a week, the fluorescence readings were obtained utilising a microplate reader (Infinite M200 PRO/, TECAN, Switzerland) at 360 nm and 420 nm of the excitation and emission wavelengths (Starowicz & Zielinski, 2019). Triplicates were done. The final result was expressed in terms of the percentage of inhibition of AGEs (%), calculated by using the equation: Percentage Inhibition of AGEs (%) = 1- ([Fluorescence of the test sample]/ [Fluorescence of control]) ×100%.

Molecular Docking Analysis

The natural compounds shared across all four extracts, previously identified by Chew (2023) via Gas chromatography-mass spectrometry (GC-MS) analysis, were selected as ligands to be docked with diabetes-related proteins. The chosen proteins were Human pancreatic alpha-amylase in complex with mini-montbretin A (HPA) (PDB ID: 5E0F) and the Crystal Structure of Human Receptor for Advanced Glycation End-products (RAGE) (PDB ID: 3O3U). Molecular docking was performed using UCSF Chimera v1.17.3 and AutoDock Vina. Protein structures were obtained from the Protein Data Bank (PDB), and the canonical

SMILES of the selected ligands were retrieved from the PubChem database before being checked for their most stable molecular geometry by performing energy minimisation using Gaussian 16. For HPA (PDB ID: 5E0F), the grid box was centred at coordinates -7.20, 5.69, -23.42 (Ahmed et al., 2023) with dimensions of 30, 27, 24.75 (Belaiba et al., 2020). For RAGE (PDB ID: 3O3U), the grid box was centred at coordinates 19.898, 17.096, and 69.161 (Tambe et al., 2022) with dimensions of 42, 42, and 42.

In Silico Pharmacokinetics Analysis

The pharmacokinetic properties of the compounds consistently found in various crude extracts of *S. macrophylla* fruit were analysed using two freely accessible web-based ADMET predictor platforms: SwissADME and ADMETlab2.0. The results were summarised and interpreted based on their physicochemical features, pharmacokinetic properties, toxicity, and drug-likeness.

Statistical Analysis

All the *in vitro* antidiabetic assays were conducted in triplicates, and the resulting data were presented as mean ± standard deviation (n=3). Statistical analysis was performed using Microsoft Excel for Microsoft 365 MSO (Version 2403 Build 16.0.17425.20176) 64-bit. One-way analysis of variance (ANOVA) and Tukey tests were used to evaluate significant differences (p<0.05) between the extracts and their respective control.

RESULTS AND DISCUSSION

In Vitro Inhibition of α-amylase Assay

Shorea macrophylla, locally known as engkabang, is well known for its high-fat content. A study by Chew (2023) revealed that the methanolic and diethyl ether crude extracts of *S. macrophylla* fruits stimulate lipogenesis, a process closely linked to Type 2 Diabetes Mellitus (T2DM) (Imamura et al., 2020). Excess lipogenesis leads to increased adiposity and ectopic fat deposition, further contributing to insulin resistance and dysregulated glucose homeostasis, the hallmarks of T2DM (Ahmed et al., 2021). Despite this, specific compounds within these extracts may offer therapeutic potential for managing T2DM by regulating glucose uptake. Therefore, *in vitro* antidiabetic assays targeting the enzyme α-amylase and advanced glycation end-products (AGEs) were conducted to elucidate their potential underlying antidiabetic mechanisms. In this study, the inhibitory effects of four distinct crude extracts of *S. macrophylla* fruit, namely hexane (Hex), ethyl acetate (EA), dichloromethane (DCM), and methanolic (MeOH), were examined on α-amylase activity. A range of concentrations from 62.5 µg/ml to 1000 µg/ml was applied to each extract. Acarbose, a known inhibitor, was used for comparison at the same concentrations.

Figure 1 demonstrates that all samples (Hex, EA, DCM, MeOH) exhibited mild inhibition of α-amylase compared to acarbose. The color of the 3,5-dinitrosalicylic acid (DNSA) shift from yellow to orange indicated the presence of reducing sugars, with intensity corresponding to their concentration (Jain et al., 2020). The orange colouration in *S. macrophylla* extracts suggests the partial formation of reducing sugars, indicating intermediate α-amylase inhibition. Figure 2 shows that the inhibitory effects of both acarbose and *S. macrophylla* fruit's crude extracts increase with rising concentrations, indicating a dose-dependent response. Acarbose achieved 60.86% inhibition at 62.5 µg/ml, 77.45% at 125 µg/ml, and plateaued at 83.9% at 250 µg/ml, double the inhibition of the other extracts at the same concentration. At the highest tested concentration (1000 µg/ml), the inhibition effects were 42.40% (EA), 43.68% (DCM), 48.24% (Hex), and 48.42% (MeOH). Among the extracts, MeOH exhibited the strongest inhibitory effects on α-amylase. However, statistical analysis revealed that their efficacy is significantly lower (p<0.01) than that of acarbose (refer to Table 1).

Though the inhibitory effects of *S. macrophylla* fruits' crude extracts were significantly less effective (p<0.01) than acarbose (as shown in Table 1), the similarly mild inhibition effects across all the extracts can be discussed based on their compounds analysed through Gas chromatography-mass spectrometry (GC-MS). According to the GC-MS list revealed by Chew (2023), five main compounds appeared consistently across the four extracts. The compounds were hexadecanoate <methyl-> (methyl palmitate), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (methyl linoleate) 9-octadecenoic acid, methyl ester, (E) (methyl oleate), methyl stearate, and eicosanoate <methyl-> (methyl arachidate).

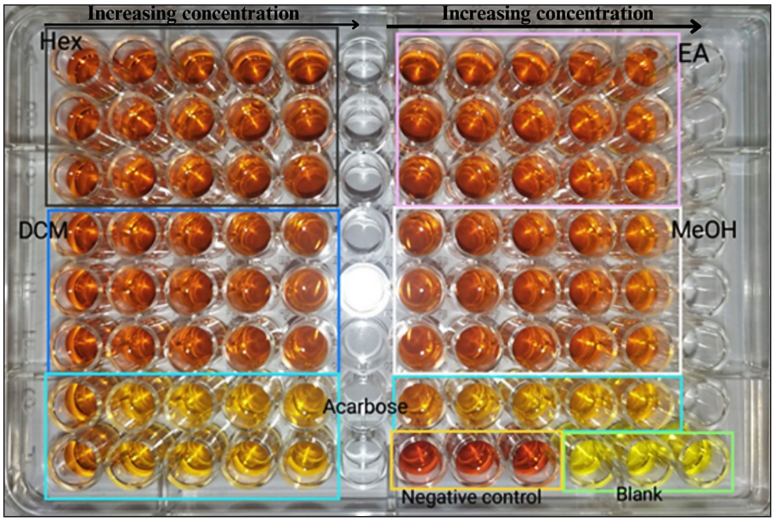

Figure 1. Result of the *in vitro* inhibition of α-amylase assay. The microplate is filled with samples and acarbose in increasing concentration (62.5-1000 µg/ml) from left to right except for the negative control and blank

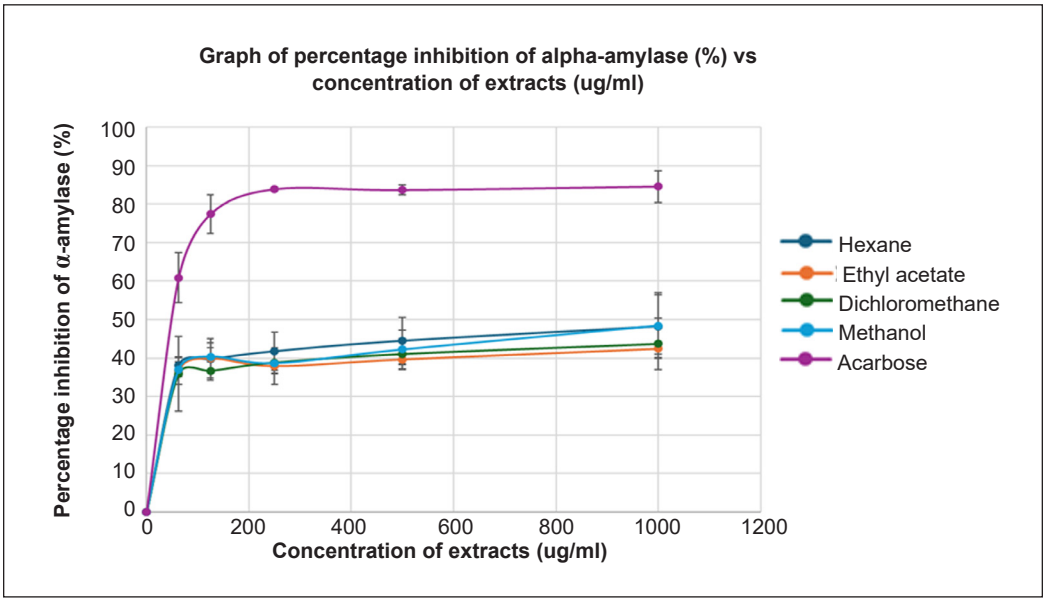

Figure 2. The inhibitory effects of α-amylase by different extracts at various concentrations. All the findings were displayed as mean ± SD (n = 3)

Table 1
Percentage of inhibition of alpha-amylase (%) by different Shorea macrophylla *extracts*

Extracts	Percentage of inhibition of alpha-amylase (%)				
	62.5 (ug/ml)	**125 (ug/ml)**	**250 (ug/ml)**	**500 (ug/ml)**	**1000 (ug/ml)**
Hex	38.12±2.02^{b}	39.68±2.93^{b}	41.76±4.90^{b}	44.49±6.11^{b}	48.24±8.15^{b}
EA	36.70±3.62^{b}	39.92±5.19^{b}	37.90±4.76^{b}	39.64±1.35^{b}	42.40±1.44^{b}
DCM	35.90±9.65^{b}	36.66±2.40^{b}	38.83±2.85^{b}	41.03±4.00^{b}	43.68±6.74^{b}
MeOH	37.07±1.88^{b}	40.34±3.70^{b}	38.65±2.82^{b}	42.21±4.96^{b}	48.42±8.58^{b}
Acarbose	60.86±6.52^{a}	77.45±5.05^{a}	83.9±0.53^{a}	83.69±1.35^{a}	84.59±4.13^{a}

Note. Statistical analysis of differences between each type of extract and acarbose (control). Data with different letters indicates the pair has a significant difference (p<0.01)

Hexadecanoic acid, or palmitic acid, along with its esters, has been recognised by Sivagurunathan and Xavier (2014) for their anti-androgenic properties. Moreover, palmitic acid and 9,12-Octadecadienoic acid (Z, Z) have demonstrated hypocholesterolemic effects, thereby reducing HDL cholesterol levels. Given the paramount importance of maintaining optimal cholesterol levels to mitigate cardiovascular risks (Kim et al., 2022), particularly in T2DM patients prone to dyslipidemia (Ahmmed et al., 2021), the regulation of cholesterol levels is important in the management of T2DM and associated cardiovascular complications. However, no study directly reveals that methyl palmitate and methyl linoleate show inhibition effects on α-amylase.

On the other side, research conducted by Ryan et al. (2000) suggests that consumption of oleic acid and stearic acid can reduce blood glucose levels. Findings by Paulraj et al. (2014) also concluded that the ethanolic extract of *Passiflora foetida* L. (bitter gourd) exhibited inhibitory effects on both α-amylase and α-glucosidase activity. Their GC-MS analysis reveals the presence of palmitic acid, methyl linoleate, oleic acid, stearic acid, and linolenic acid. Given this evidence, four of the five main compounds in *S. macrophylla* fruit, that are methyl palmitate, methyl linoleate, methyl oleate, and methyl stearate may contribute to the antidiabetic properties observed. Those compounds share the chemical structures and functional groups with their parent acids, which have been proven to have antidiabetic properties.

However, adding a methyl group (-CH3), which distinguishes them from their parent acid, may alter some chemical properties. Nevertheless, it is noted that before GC-MS analysis, Chew (2023) conducted derivatisation steps for their samples rich in fatty acids. It is to increase the volatility of the fatty acids for Gas Chromatography- mass spectrometry (GCMS) data collection. Consequently, the fatty acid methyl esters (FAMEs) detected (methyl stearate, methyl palmitate, methyl arachidate, methyl oleate, and methyl linoleate) could predominantly represent their respective parent acids, such as stearic acid, palmitic acid, arachidic acid, oleic acid, and linoleic acid. Therefore, further research is required to validate the antidiabetic potential of these fatty acids, particularly their impact on α-amylase.

In Vitro Inhibition of AGEs

Advanced glycation end-products (AGEs) constitute a diverse group of compounds formed from various mechanisms and precursors, both endogenously and exogenously. Typically, they are formed through non-enzymatic condensation (Twarda-Clapa et al., 2022). Accumulating in tissues and organs, AGEs disrupt normal cellular processes and trigger related inflammatory pathways, thereby promoting ageing-related conditions and chronic diseases. These include diabetes complications and cardiovascular diseases (Khan et al., 2020). Thus, inhibitors of AGEs can help manage diabetes complications.

Based on the results in Figure 3, the Hexane (Hex) extract showed the least inhibitory effect, with a slight increase in inhibition from 15.84% at 5 µg/ml to 18.49% at 100 µg/ml. Conversely, methanolic (MeOH) extract demonstrated the highest efficacy in inhibiting AGEs, steadily increasing from 5 µg/ml to 100 µg/ml, reaching 41.11% inhibition at the highest concentration, over twice that of other extracts. Notably, MeOH's 41.11% inhibition at 100 µg/ml surpassed the reference drug's 34.53% at the same concentration. Statistical analysis (Table 2) revealed that Hex, Ethyl acetate (EA), and Dichloromethane (DCM) were significantly less effective ($p<0.05$) compared to Aminoguanidine (AG), making them less desirable. In contrast, MeOH's inhibition effects on AGEs were not significantly different from AG, indicating its potency *in vitro*.

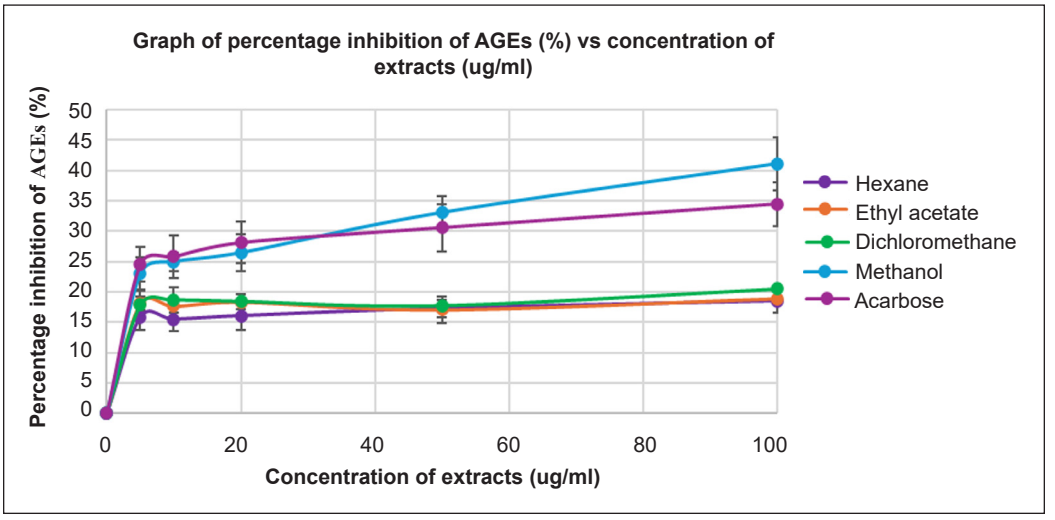

Figure 3. Comparison of AGEs inhibition by different extracts. The data presented are in the form of mean ± SD (n = 3)

Table 2
Percentage inhibition of AGEs (%) by different Shorea macrophylla *extracts*

Extracts	Percentage Inhibition of AGEs (%)				
	5 (ug/ml)	10 (ug/ml)	20 (ug/ml)	50 (ug/ml)	100 (ug/ml)
Hex	15.84±2.04^{b**}	15.49±1.90^{b**}	16.05±2.35^{b**}	17.29±1.45^{b**}	18.49±1.97^{b**}
EA	18.08±1.22^{b*}	17.62±1.11^{b**}	18.32±1.22^{b**}	17.05±2.13^{b**}	18.89±0.34^{b**}
DCM	17.88±2.40^{b*}	18.68±2.15^{b*}	18.47±0.25^{b**}	17.77±0.27^{b**}	20.49±0.47^{b**}
MeOH	23.01±2.77^{a}	25.00±1.54^{a}	26.47±3.07^{a}	33.07±2.65^{a}	41.11±4.38^{a}
AG	24.58±2.85^{a}	25.83±3.57^{a}	28.14±3.44^{a}	30.62±3.91^{a}	34.53±3.61^{a}

Note. Statistical analysis of differences between the extracts and AG (control). Different letters denote the presence of significant differences with *= p<0.05, **= p<0.01

The comparable inhibitory effects of MeOH extract with AG suggest that MeOH can be an alternative to AGE inhibitors. Furthermore, the inhibitory effects imply that the effectiveness of the MeOH extract may be attributed to the compounds with an antiglycation effect. Thus, MeOH extract's primary components (more than 1% GC-MS abundance) were discussed. It included the five compounds mentioned previously (methyl stearate, methyl palmitate, methyl arachidate, methyl oleate, and methyl linoleate) (Chew, 2023).

A study by Sowmiya et al. (2021) identified the potential of methyl linoleate in anti-cancer and anti-inflammatory responses, positing it as a probable contributor to AGEs inhibition in the current investigation. Furthermore, several investigations have underscored these compounds' anti-cancer, antioxidant, and anti-inflammatory attributes. For example, both antioxidant and anti-inflammatory properties have been ascribed to

palmitic acid (Odu et al., 2023; Paulraj et al., 2014; Sivagurunathan & Xavier, 2014). Similarly, methyl oleate has exhibited antioxidant and anti-cancer activities (Yu et al., 2005). However, no study has been exploring the antiglycation properties of methyl stearate and methyl arachidate.

There is a correlation between these properties (anti-cancer, antioxidant, and anti-inflammatory) and the inhibition of AGEs. Certain anti-cancer agents may possess anti-glycation properties due to the shared signalling pathways, such as the nuclear factor (NF)-κB pathway, implicated in both cancer and diabetes (Dariya & Nagaraju, 2020). Additionally, chronic inflammation has been closely associated with AGE formation (Salazar et al., 2021), underscoring the potential of compounds endowed with anti-inflammatory properties to attenuate inflammatory responses, thus potentially reducing AGE formation. Moreover, compounds exhibiting antioxidants help neutralise reactive oxygen species (ROS) (Parcheta et al., 2021), reducing oxidative stress and inhibiting AGEs formation. Given that the previously mentioned compounds either exhibit indirect antiglycation properties or are the parent acid with those identified in this study (such as methyl palmitate), their potential contribution to AGE inhibition is suggested. It is also noteworthy that other compounds in relatively low abundances within the undiscovered crude extracts might also contribute to AGE inhibition. Hence, further investigation is required to examine the antiglycation properties of these compounds.

Molecular Docking with Human Pancreatic Alpha-amylase (PDB ID: 5E0F)

Enzyme α-amylase plays a key role in breaking down starch into sugars. Inhibiting its activity can help control blood sugar levels in diabetic patients (Chigurupati et al., 2022). Generally, more negative binding energies indicate stronger binding affinities between the ligands and the protein. The docking results, summarised in Table 3, indicate that methyl oleate exhibited the lowest binding energy (-5.431 kcal/mol) and highest affinity to Human Pancreatic alpha-amylase (HPA), followed by methyl linoleate (-5.126 kcal/mol), methyl arachidate (-5.103 kcal/mol), methyl stearate (-5.099 kcal/mol), and methyl palmitate (-4.821 kcal/mol). Figure 4 presents the optimal positions for molecular interactions between these ligands and HPA. Notably, acarbose showed significantly lower binding energy (-8.116 kcal/mol) and stronger affinity compared to all the ligands.

Studies on HPA's X-ray crystal structure and enzyme kinetics have revealed three critical amino acid residues in its active site: ASP-197, GLU-233, and ASP-300. These residues are essential for starch hydrolysis, with ASP-197 serving as a catalytic nucleophile, GLU-233 as an acid-base catalyst, and ASP-300 optimising substrate orientation (Kikiowo et al., 2020). In the current docking study with HPA (PDB id: 5E0F), methyl palmitate, methyl arachidate, and methyl oleate formed H-bonds with THR-163 at bond distances of 2.632 Å, 2.463 Å, and 2.399 Å, respectively (refer to Figure 5).

Table 3
Molecular docking result of the FAMEs with HPA (PDB ID: 5E0F)

Compounds/Proteins	Pubchem CID	Binding energy (kcal mol^{-1})	Number of hydrogen bonds	Best hydrogen bonding position
Methyl Stearate Canonical SMILES CCCCCCCCCCCCCC CCCC(=O)OC	8201	-5.099	2	HIS 201 (2.240) TYR 151 (2.387)
Methyl Palmitate Canonical SMILES: CCCCCCCCCCCCC CCC(=O)OC	8181	-4.821	1	THR 163 (2.632)
Methyl Arachidate Canonical SMILES: CCCCCCCCCCCCC CCCCCCC(=O)OC	14259	-5.103	1	THR 163 (2.463)
Methyl Oleate Canonical SMILES: CCCCCCCCC=CCC CCCCCC(=O)OC	5364509	-5.431	1	THR 163 (2.399)
Methyl Linoleate Canonical SMILES: CCCCCC=CCC=CC CCCCCCC(=O)OC	5284421	-5.126	1	HIS 299 (2.168)
Acarbose Canonical SMILES: CC1C(C(C(C(O1)O C2C(OC(C(C2O)O) OC3C(OC(C(C3O)O) O)CO)CO)O)O)NC4 C=C(C(C(C4O)O)O)CO	41774	-8.116	4	ALA 106 (2.412) HIS 305 (2.380) GLU 233 (2.238) LYS 200 (2.711)

Methyl linoleate, on the other hand, interacted with HIS-299 at a bond distance of 2.168 Å. The same amino acid residues THR-163 and HIS-299 at the active site of 5E0F were also identified in the study by Ghannay et al. (2020). Only methyl stearate formed two H-bonds in this study, interacting with HIS-201 (2.240 Å) and TYR-151 (2.387 Å). These amino acid residues (THR-163, HIS-201, TYR-151) were the same as those revealed in Mohamed and Ibrahim's (2022) study. However, all FAMEs had higher predicted binding energies than acarbose, indicating their relatively weaker binding affinities towards HPA, according to Table 3. The docking scores of acarbose in this study closely resembled those reported by Chigurupati et al. (2021) (-8.10 kcal/mol), with four same interacting residues (LYS-200, GLU-233, ASP-300, and HIS-305) observed, except for ASP-300.

In short, the docking scores for the compounds with HPA in this study ranged from -4.821 kcal/mol to -5.431 kcal/mol. A comparison with a similar investigation conducted by Ahmed et al. (2023) reveals that these scores are relatively lower. It indicated that

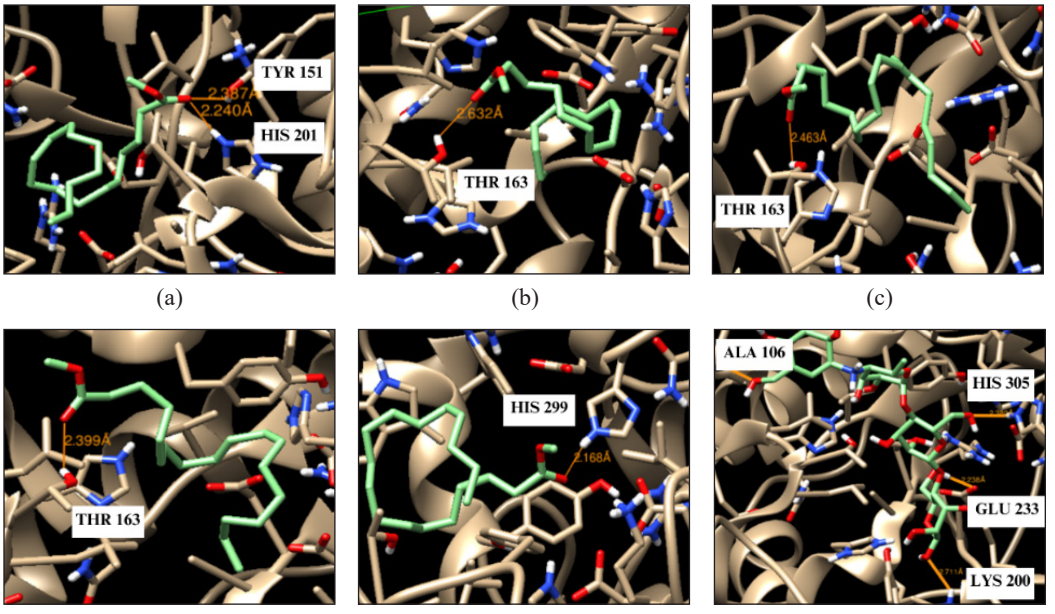

Figure 4. Compilation of top binding poses for the FAMEs and acarbose docked to HPA (PDB ID: 5E0F): (a) Methyl stearate; (b) Methyl palmitate; (c) Methyl arachidate; (d) Methyl oleate; (e) Methyl linoleate; and (f) Acarbose

the five selected compounds are predicted to have a higher binding affinity, potentially offering greater efficacy in managing blood glucose levels. However, in contrast to studies conducted by Belaiba et al. (2023) and Aroua et al. (2023), the binding scores obtained herein are higher, implying weaker interactions with HPA compared to those compounds. Hence, additional investigation and analysis are needed to enhance the potential of these compounds for improved efficacy.

Molecular Docking with RAGE (PDB ID: 3O3U)

The receptor for advanced glycation end-products (RAGE) is a transmembrane receptor resembling immunoglobulins and has multiple isoforms. It binds various endogenous extracellular ligands and intracellular effectors, initiating signalling cascades that produce reactive oxygen species (ROS), inflammatory responses, cell proliferation, or apoptosis and upregulates RAGE (Bongarzone et al., 2017). Extensive research has established correlations between RAGE activity and various pathological conditions, including diabetes (Ramasamy et al., 2011). Given its involvement in numerous pathological states, RAGE has become an appealing target for therapeutic strategy.

The docking scores for the ligands with RAGE were summarised in Table 4. Methyl linoleate among the ligands posed the lowest binding energy (-6.510 kcal/mol) and highest

Table 4
Molecular docking result of the FAMEs with RAGE (PDB ID: 3O3U)

Compounds/Proteins	Puchem CID	Binding Energy (kcal mol−1)	Number of Hydrogen Bonds	Best Hydrogen Bonding position
Methyl Stearate Canonical SMILES CCCCCCCCCCCCCCCCC CC(=O)OC	8201	-5.760	1	ARG 66 (2.264)
Methyl Palmitate Canonical SMILES: CCCCCCCCCCCCCCCC (=O)OC	8181	-6.210	3	ARG 66 (2.351) ARG 66 (2.209) ARG 66 (2.547)
Methyl Arachidate Canonical SMILES: CCCCCCCCCCCCCCCCC CCC(=O)OC	14259	-6.194	1	ARG 66 (1.989)
Methyl Oleate Canonical SMILES: CCCCCCCCC=CCCCCC CCC(=O)OC	5364509	-6.191	2	ARG 66 (2.176) ARG 66 (2.241)
Methyl Linoleate Canonical SMILES: CCCCCC=CCC=CCCCCC CCC(=O)OC	5284421	-6.510	2	ARG 66 (2.348) ARG 66 (2.295)
Aminoguanidine Canonical SMILES: C(=NN)(N)N	2146	-3.515	3	LEU 151 (2.151) ALA 206 (2.195) ILE 348 (2.134)

affinity to RAGE, followed by methyl palmitate (-6.210 kcal/mol), methyl arachidate (-6.194 kcal/mol), methyl oleate (-6.191 kcal/mol) and methyl stearate (-5.760 kcal/mol). The best position for molecular interactions between these ligands with RAGE is presented in Figure 5. The binding energy of Aminoguanidine (AG) for RAGE was higher (-3.638 kcal/mol) compared to all the compounds. It indicates that the tested ligands have a stronger affinity with RAGE than AG, emphasising their possibility and efficacy as a therapeutic agent in managing diabetes complications.

In docking simulations with RAGE, all five compounds engaged in hydrogen bonding (H-bond) interactions with ARG-66 of the 3O3U protein, displaying varying numbers and bond distances (Table 4). Notably, these interactions consistently involved the nitrogen atom of arginine and the oxygen atom of the ligand. Concordant with the findings of Abbas et al. (2014), ARG-66 emerged as a pivotal amino acid residue in the binding interactions of 3O3U, alongside LYS-15, ARG-66, MET-330, GLU-44, GLU-45, GLU-54, GLU-111, GLU-153, ASP-14, and ASP-65. All the FAMEs tested formed H-bonds with ARG-66, indicating that this residue is a crucial interaction site on the 3O3U protein.

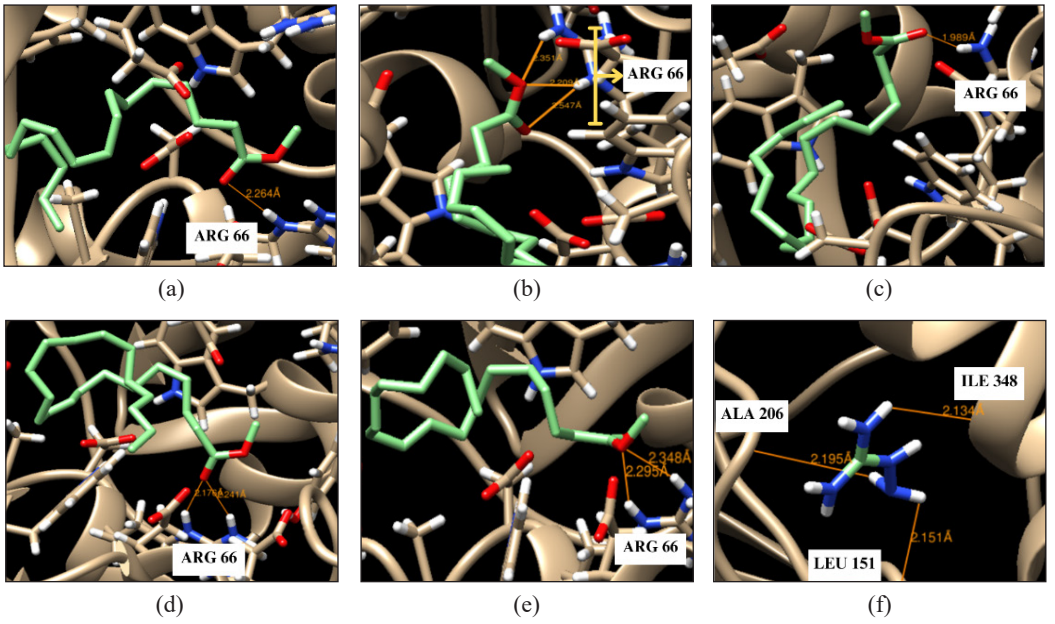

Figure 5. Best binding poses of selected FAMEs when interacting with RAGE (PDB ID: 3O3U): (a) Methyl stearate; (b) Methyl palmitate; (c) Methyl arachidate; (d) Methyl oleate; (e) Methyl linoleate; and (f) Aminoguanidine

Besides, the involvement of ARG-66 across different ligands suggests a conserved binding mechanism. Due to specific location and properties, this residue might be part of a highly conserved binding pocket or active site that interacts favourably with these ligands. According to Sokalingam et al. (2012), arginine is known for forming strong H-bonds and electrostatic interactions due to its positively charged guanidinium group. This could explain why it frequently forms H-bonds with various ligands. In short, the binding energies of the compounds in this study, ranging from -5.760 kcal/mol to -6.510 kcal/mol, show notable affinity towards RAGE. However, the binding energy is generally higher compared to those reported in the Tambe et al. (2022) study.

Overall, the docking results with 5E0F and 3O3U demonstrated that the five FAMEs exhibited considerable affinity towards the active sites of HPA and RAGE. Both methyl oleate (-5.431 kcal/mol) and methyl linoleate (-6.510 kcal/mol) showed the lowest binding affinities for HPA and RAGE respectively. This suggests their strongest inhibitory potential among other compounds, making them a promising candidate for further study.

Evaluation of the *In Silico* Pharmacokinetic Properties

In extending to the observed biological activities demonstrated by *S. macrophylla* fruit's crude extracts in previous *in vitro* and molecular docking studies, the five FAMEs were subjected to comprehensive *in silico* analysis. Utilising SwissADME and ADMETlab2.0,

this evaluation encompassed medicinal chemistry, physicochemical properties, and pharmacokinetic (ADME) profiles. According to Sadeghi et al. (2021), an imperative criterion in selecting a chemical for therapeutic candidacy is its absence of toxicity. Thus, the toxicity of these FAMEs was also predicted using ADMETlab2.0. The aim was to determine their suitability as potential antidiabetic drug candidates.

The analysis of physicochemical properties showed in Table 5 predicts that all tested FAMEs exhibited consistent molecular structures, characterised by two H-bond acceptors and no H-bond donors. Their shared topological polar surface area (TPSA) value of 26.30 Å indicates uniform polarity, influencing interactions with biological targets. However, solubility varied among the FAMEs. Good water solubility is crucial for optimal absorption and oral bioavailability (Agrawal et al., 2024). Using the estimated solubility (ESOL) model, all compounds were classified as moderately soluble, except methyl arachidate, which was poorly soluble. Further refinement with Log S (SILICOS-IT) confirmed methyl linoleate as moderately soluble, while others were downgraded to poor solubility. This highlights the importance of multiple predictive models. Besides, lipophilicity, which is crucial for drug absorption across cell membranes, was calculated using five models (XLOGP3, WLOGP, MLOGP, SILICOS-IT, and iLOGP), and summarised into consensus prediction of the logarithm of the partition coefficient (P) of a compound between n-octanol and water (consensus Log Po/w) (Daina et al., 2017). Methyl arachidate showed the highest lipophilicity (6.96), indicating a strong affinity for

Table 5
The physicochemical properties of the FAMEs

Compounds	Methyl Stearate	Methyl Palmitate	Methyl Arachidate	Methyl Oleate	Methyl Linoleate
Formula	$C_{19}H_{38}O_2$	$C_{17}H_{34}O_2$	$C_{21}H_{42}O_2$	$C_{19}H_{36}O_2$	$C_{19}H_{34}O_2$
MW	298.5	270.45	326.56	296.49	294.47
nHA	2	2	2	2	2
nHD	0	0	0	0	0
TPSA	26.30	26.30	26.30	26.30	26.30
Log S (ESOL)	-5.83	-5.18	-6.47	-5.32	-4.97
ESOL class	Moderately soluble	Moderately soluble	Poorly soluble	Moderately soluble	Moderately soluble
Log S (SILICOS-IT)	-6.81	-6.01	-7.61	-6.09	-5.37
SILICOS-IT class	Poorly soluble	Poorly soluble	Poorly soluble	Poorly soluble	Moderately soluble
Consensus Log $P_{o/w}$	6.24	5.54	6.96	5.95	5.69

Note. MW: Molecular Weight, nHA: Number of H-bond acceptors, nHD: number of H-bond donors, TPSA: Topological polar surface area, Log S: solubility, ESOL: estimated solubility, Consensus Log $P_{o/w}$: consensus prediction of the logarithm of the partition coefficient (P) of a compound between n-octanol and water, key measures of lipophilicity

lipid environments, while methyl palmitate had the lowest lipophilicity (5.54), suggesting a preference for water solubility.

When developing antidiabetic drugs, the focus is on targeting organs involved in glucose metabolism, such as the liver, muscles, adipose tissue, and pancreas. Therefore, pharmacological properties should also prioritise factors such as GI absorption, distribution to target organs, metabolic stability, and excretion. Based on Table 6, all compounds, except methyl arachidate, were predicted to have high GI absorption, indicating their potential for intestinal absorption following oral administration (Al-Ghamdi et al., 2021). Besides, P-glycoprotein (P-gp) is critical in drug absorption and elimination (Pirzada et al., 2024). The selected FAMEs in this study are not considered P-gp substrates, and this is significant because P-gp inducers and inhibitors can alter absorption and excretion processes, potentially affecting drug efficacy or toxicity (Yamazaki et al., 2019). All tested FAMEs except methyl palmitate were non-blood-brain-barrier (BBB) permeants, aligning with the common traits of antidiabetic drugs, which typically do not need to cross the BBB. However, the BBB permeation of methyl palmitate suggests potential for central nervous system (CNS) targeting, offering opportunities for neurological therapeutic interventions. Notably, all compounds are cytochrome P450 1A2 enzyme inhibitors (CYP1A2), while only methyl linoleate is CYP2C9 inhibitor. None of the tested FAMEs inhibit CYP2C19, CYP2D6, or CYP3A4.

Regarding skin permeability, the current study found that the compounds have low skin permeability overall (Alade et al., 2023), indicating their unsuitability for transdermal

Table 6
The pharmacokinetic properties (ADME) of selected compounds predicted using SwissADME

Compounds	Methyl Stearate	Methyl Palmitate	Methyl Arachidate	Methyl Oleate	Methyl Linoleate
GI absorption	High	High	Low	High	High
Pgp-substrate	No	No	No	No	No
BBB penetration	No	Yes	No	No	No
CYP 1A2 inhibitor	Yes	Yes	Yes	Yes	Yes
CYP 2C19 inhibitor	No	No	No	No	No
CYP 2C9 inhibitor	No	No	No	No	Yes
CYP 2D6 inhibitor	No	No	No	No	No
CYP 3A4 inhibitor	No	No	No	No	No
Log Kp	-2.190 cm/s	-2.710cm/s	-1.690 cm/s	-2.820 cm/s	-3.250 cm/s
CL (mL/min/kg)	4.767	4.995	4.655	5.771	7.742
Half-life ($T_{1/2}$)	0.201	0.281	0.143	0.261	0.343

Note. GI absorption: gastrointestinal absorption, P-gp substrate: P-glycoprotein substrate, BBB penetration: blood-brain barrier penetration, CYP inhibitors: cytochrome P450 enzymes inhibitors, Log Kp: skin permeation, CL: Clearance rates

drug products. Clearance rates (CL) and half-life ($T_{1/2}$) highlighted pharmacokinetic differences, with methyl oleate (5.771 mL/min/kg) and methyl linoleate (7.742 mL/min/kg) showing moderate CL, while the others showed low CL. ADMETlab2.0 predictions indicated that methyl linoleate had the highest probability (0.343) of having a long $T_{1/2}$. A high CL indicates rapid elimination, while a long $T_{1/2}$ suggests prolonged drug exposure, potentially reducing dosing frequency (Adepu & Ramakrishna, 2021). Since the probabilities of having a long half-life for all tested FAMEs ranged from 0.143 to 0.343, which were relatively low, this suggests that frequent dosing may be necessary to maintain therapeutic levels.

The *in silico* pharmacokinetic assessment of the compounds in Table 7 revealed several key characteristics. No Pan Assay Interference Compounds (PAINS) or Brenk violations were identified across the tested compounds, indicating a favourable medicinal chemistry profile, as Brenk signals denote hazardous, reactive, and metabolically unstable fragments (Ononamadu & Ibrahim, 2021). The absence of PAINS alerts suggests a lack of structural features commonly associated with false positives in computational assays (Ahmad et al., 2023). However, all FAMEs displayed two lead-likeness criteria violations, indicating potential issues with pharmacokinetic behaviour and drug-likeness. All FAMEs exhibited a very low probability of human hepatotoxicity (H-HT) and Ames test for mutagenicity (AMES), suggesting minimal toxicity risk and reduced likelihood of liver damage, enhancing their suitability for drug development (Flores-Holguín et al., 2021). Nonetheless, high skin sensitisation scores across all compounds highlight the importance of early skin irritation assessment. Carcinogenicity probabilities ranged from 0.042 to 0.467, with methyl linoleate having the highest risk. Despite these variations, all tested FAMEs adhered to the Lipinski Rule of Five, suggesting drug-like properties (Rao & Hariprasad, 2021).

Table 7
The medicinal chemistry, toxicity, and drug-likeness of the selected compounds

Compounds	Methyl stearate	Methyl palmitate	Methyl arachidate	Methyl oleate	Methyl linoleate
PAINS	0	0	0	0	0
Brenk	0	0	0	0	0
Lead-likeness # violations	2	2	2	2	2
H-HT	Low (0.025)	Low (0.026)	Low (0.023)	Low (0.018)	Low (0.011)
AMES Toxicity	Low (0.006)	Low (0.006)	Low (0.006)	Low (0.005)	Low (0.016)
Skin sensitisation	High (0.96)	High (0.955)	High (0.964)	High (0.97)	High (0.975)
Carcinogenicity	Low (0.05)	Low (0.063)	Low (0.042)	Low (0.113)	Moderate (0.467)
Lipinski #violations	Accepted	Accepted	Accepted	Accepted	Accepted

Note. PAINS = Pan Assay Interference Compounds, Brenk = Brenk's Rule, AMES toxicity = Ames test for mutagenicity, H-HT = Human hepatotoxicity

Linking the *in silico* molecular docking results with pharmacokinetic predictions, methyl oleate and methyl linoleate are the two insightful antidiabetic drug candidates. Both of them comply with Lipinski's Rule of Five, indicating good potential for oral administration due to favourable gastrointestinal absorption. However, these two compounds are less suitable for transdermal use, as a high risk of skin sensitisation is predicted. With moderate renal clearance, they can avoid overly fast or slow elimination issues. Nevertheless, formulation adjustments are recommended to improve the solubility of methyl oleate, while methyl linoleate needs modification to address its predicted carcinogenicity risk.

CONCLUSION

The study revealed that all crude extracts of *S. macrophylla* fruits (Hex, EA, DCM, and MeOH) exhibited varied inhibitory effects on both α-amylase and AGE formation *in vitro*. While their effects on α-amylase were mild and significantly less effective ($p<0.01$) than acarbose, the MeOH extract showed the strongest effect on AGE formation, doubling the efficacy of other extracts at 100 µg/ml. However, no significant differences were found between the MeOH extract and the control AG, suggesting its potential as a natural alternative with similar therapeutic benefits. Molecular docking and *in silico* pharmacokinetic analyses indicated considerable affinity of the five compounds towards the active sites of HPA and RAGE. Although these compounds had lower affinity for HPA compared to acarbose, their affinities for RAGE were higher than AG, indicating a stronger interaction. Furthermore, ADMET analysis suggested drug-like properties for all tested FAMEs, with concerns regarding poor solubility and skin irritation, emphasising the need for compound modification before drug development. In short, MeOH crude extract of *S. macrophylla* fruits has the potential to act as an antiglycation agent. Further research is needed to validate whether individual compounds or interactions between multiple compounds are responsible for the observed antiglycation effects.

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