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Original Article

Inhibitory Effects of Lawsone Methyl Ether and Lawsone and their Synergistic Interactions with Acarbose against α-Glucosidase: *In silico* and *in vitro* Studies

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ABSTRACT

Background: Our previous research highlighted remarkable hypoglycemic and hypolipidemic potentials of lawsone methyl ether (LME or 2-methoxy-1,4-naphthoquinone) and lawsone (2-hydroxy-1,4-naphthoquinone) in diabetic rats via β-cell regeneration. This insighted us to explore their additional antidiabetic mechanisms against α-glucosidase using *in silico* and *in vitro* approaches. **Materials and Methods:** *In silico* molecular docking was performed via Autodock Vina, SwissADME, and Datawarrior software. However, an *in vitro* inhibitory assay was conducted against α-glucosidase. **Results:** *In silico* studies revealed promising binding conformations and interactions of LME and lawsone with the functional residues of the α-glucosidase protein, involving hydrogen bonding, Van der Waals, and pi-pi interactions, showing comparable binding energies of -5.4 and -5.6 kcal/mol, respectively. Additionally, LME and lawsone displayed favorable pharmacokinetic profiles, revealing no evident toxicity. *In vitro* α-glucosidase inhibitory assay indicated that LME (IC_{50} of 37.4 µg/mL) and lawsone (IC_{50} of 42.2 µg/mL) exhibited comparable inhibitory activities, while both of them possessed markedly higher activities than acarbose (IC_{50} of 440.6 µg/mL). Furthermore, study on synergistic effects among these naphthoquinones and acarbose illustrated that at ½IC_{50} of LME (18.7 µg/mL) and acarbose (220.3 µg/mL) exhibited a satisfactory synergistic effect against α-glucosidase, with a percentage inhibition of 88.7% and a fractional percentage inhibition index (FPI) of 2.0, while at ½IC_{50} of lawsone (21.1 µg/mL) and acarbose (220.3 µg/mL) produced an additive effect, with a percentage inhibition of 76.4% and a FPI of 1.7. **Conclusion:** Promising α-glucosidase inhibitory potentials of LME and lawsone underscore their additional mechanism alongside β-cell regeneration further supporting their outstanding antidiabetic capabilities.

Keywords: Diabetes mellitus, α-Glucosidase, Lawsone, Lawsone methyl ether, Molecular docking, Synergistic effect.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic condition marked by elevated blood glucose levels (hyperglycemia) and disruptions in the metabolism of carbohydrates, fats, and proteins. These disruptions result from deficiencies in insulin secretion, insulin sensitivity, or a combination of both factors. Insulin is a vital hormone produced by the pancreas; its primary role is to regulate blood sugar levels by promoting the uptake of glucose from the bloodstream into the body's cells. In type 1 DM, the pancreas is completely incapable of producing insulin. Conversely, in type 2 DM, the body is unable to efficiently use the insulin due to the development of insulin resistance.[1] DM is notably one of the most widespread metabolic diseases in the modern world. As urbanization increases and lifestyles become more sedentary, coupled with changes in dietary habits, the incidence of DM continues to rise. According to the International Diabetes Federation (IDF), approximately 463 million people were affected by DM in 2019. Tragically, of this number, 4.2 million individuals lost their lives due to diabetes-related complications. Furthermore, the economic burden of managing and treating the disease was staggering, with diabetes-related healthcare expenses reaching a total of USD 760 billion.[2]

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Postprandial hyperglycemia, primarily resulting from the excessive consumption of dietary carbohydrates, is the key clinical concern of type 2 DM. Therefore, slowing down the digestion and absorption of dietary carbohydrates might be a viable approach to manage these erratic and elevated postprandial glucose levels.[3] To achieve this, a range of oral hypoglycemic agents are clinically available, with α-glucosidase inhibitory agents seem to be the most effective ones. While miglitol and voglibose are the typical examples of α-glucosidase inhibitors, acarbose is the most commonly used drug of this group. Unfortunately, a significant drawback associated with acarbose is its potential to cause gastrointestinal side effects. Gastrointestinal complaints such as bloating, flatulence, diarrhea, and abdominal discomfort are reported in nearly half of the patients using this medication. The discomfort caused by gastrointestinal side effects can have a negative impact on patient compliance and adherence to the prescribed treatment regimen. Patients who experience these side effects might be more likely to skip doses or discontinue the medication altogether, which can compromise the effectiveness of their diabetes management.[4] Apart from this, acarbose also develops drug tolerance on long-term consumption. Moreover, it is always used in combination with other antidiabetic agents, which makes acarbose an expensive therapeutic choice.[5] The limitations associated with conventional antihyperglycemic drugs mentioned above highlight the need for the development of novel α-glucosidase inhibitors that offer improved efficacy and safety for effectively addressing the postprandial hyperglycemia. Innovative medications of this kind would surely overcome the limitations of existing treatments, enhancing the clinical outcomes and wellbeing of those patients suffering from chronic ailments like DM and related complications. Additionally, due to enhanced efficacy, non-toxicity, and minimal or no side effects, the World Health Organization (WHO) has also endorsed the utilization of traditional plants for the management of DM.[6] This drive for research led to the exploration of marvelous plant-derived α-glucosidase inhibitors such as resveratrol, berberine, curcumin, quercetin, and epigallocatechin gallate (EGCG), etc.[7] Of these plant-based comounds, plumbagin, shikonin, and rhinacanthin-C are particularly noteworthy. Because, they are naturally occurring 1,4-naphthoquinones with significant α-glucosidase inhibitory capabilities.[8-10]

Lawsone methyl ether (LME) and lawsone are also 1,4-naphthoquinones exhibiting striking structural similarities with shikonin, plumbagin, and rhinacanthin-C. However, both LME and lawsone can be naturally found in the leaves of *Impatiens balsamina* L.[11] In our previous *in vivo* research, we identified notable hypoglycemic, hypolipidemic, and pancreatic protective potentials of LME and lawsone in nicotinamide-streptozotocin induced diabetic rats. The primary mechanisms responsible for the above mentioned effects of LME and lawsone were the increased production and release of insulin, stemming from the regeneration of pancreatic β-cells.[12] Building upon these remarkable results from our previous work, we aimed to investigate the additional antidiabetic mechanisms of LME and lawsone against α-glucosidase using *in silico* as well as *in vitro* approaches. Nowadays, LME can be semi-synthesized from a commercially available lawsone with a high yield.[13] This characteristic enhances their potential for drug development, as the cost-effective production of LME via semi-synthesis or commercial availability of lawsone contrasts with the expenses involved in obtaining them from plants through extraction and purification procedures. Therefore, the current study involves a comparative α-glucosidase inhibitory analysis of LME and lawsone conducted via *in silico* as well as *in vitro* approaches. Additionally, the present study also assessed the synergistic interactions of LME and lawsone when combined with acarbose against α-glucosidase. It is noteworthy that this study is a two-pronged research approach to underscore the additional antidiabetic mechanisms of LME and lawsone, paving the way for the potential discovery of novel α-glucosidase inhibitors with improved efficacy and safety.

MATERIALS AND METHODS

Drugs and chemicals

α-Glucosidase from *Saccharomyces cerevisiae, p*-nitrophenyl-α-D-glucopyranoside (*p*NPG), acarbose, and lawsone were obtained from Sigma-Aldrich Chemical Co. (GmbH, Steinheim, Germany). LME was semi-synthesized and identified using a method previously described.[13] All the other chemicals and reagents were preferably of analytical grade.

Semi-synthesis of LME from lawsone

LME was semi-synthesized from commercially available lawsone using a very simple method, as outlined previously.[14] Nonetheless, a modification was implemented in the crystallization process, wherein ethanol was substituted for ethyl acetate.[13] The objective of this modification was to enhance the yield of LME while using a more eco-friendly solvent. The results of semi-synthesis revealed that the methylation of lawsone under acidic conditions produced yellow needle-like crystals of LME with an approximate yield of 72%. To confirm the identity of the semi-synthesized LME, it was compared with the standard LME using TLC chromatograms in three distinct solvent systems: hexane/ethyl acetate (6:4), hexane/chloroform (5:5), and hexane/methanol (7:3).

Molecular docking

Target protein and the naphthoquinones

The FASTA sequence of α-glucosidase (the target protein) was retrieved from the UniProt database, using maltose as a template. This sequence exhibited a 99% similarity to the modeled structure. The three-dimensional structure of the α-glucosidase macromolecule was modeled with the help of the MODELLER tool. The chemical structures of LME and lawsone, as depicted

in Figure 1, were drawn using the ChemDraw tool for the subsequent analysis.[15]

Molecular docking method

The chemical structures of the sample molecules: LME and lawsone, and the target protein: α-glucosidase in PDB (Protein Data Bank) format were imported to the Autodock Vina Software.[16] Heteroatoms, 3D protonation, water molecules, and the default ligand, which were attached to the target molecule, were removed. A number of polar hydrogens and Kollman charges were added to the molecular structures of ligands in order to prepare them for molecular docking analysis.[17] Grid box dimensions with centers (x=25.250, y=-1.167, z=19.667) and sizes (x=126, y=126, z=126) were generated using selective amino-acid residues. The active binding site containing active residues of LYS194, TYR197, GLU257, GLN260, PHE261, ASN264, VAL404, GLU405, ILE412, ARG413, TYR416, ASN417, TRP238, ASN414, ASN417, ALA418, and GLU421 was involved in the binding interactions with the selected ligand molecules. A credible scoring system was developed to represent the optimal binding energies and positions. Various molecular interactions, including hydrogen bonding, pi-bonding, and hydrophobic interactions, were identified when the docked complex was loaded into the Discovery Studio visualization tool.[18]

Determination of pharmacokinetic/ADMET profiles

SwissADME[19] and Datawarrior[20] software tools were used to determine the ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiles of LME and lawsone, and to predict their drug-likeness.[21]

In vitro α-glucosidase inhibitory assay

The *in vitro* α-glucosidase inhibition test was conducted following a previously set protocol.[22] Briefly, the solutions of α-glucosidase (0.1 U/mL), *p*NPG (0.375 mM), and sodium carbonate (0.2 mM) were individually prepared in 0.1 M potassium phosphate buffer (pH 6.8). Sample solutions were prepared in DMSO, ensuring a final concentration not exceeding 7%. In a 96-well plate, 20 µL of each sample was combined with an equivalent volume of α-glucosidase solution and then incubated at 37°C for 10 min. Afterward, 40 µL of the *p*NPG substrate solution was introduced and the mixture was further incubated for 40 min at the previously mentioned temperature. Following the incubation, the enzymatic reaction was halted by introducing 80 µL of sodium carbonate solution. The resulting reaction product, *p*-nitrophenol, was then quantified at 405 nm using the Varioskan™ LUX Multimode Microplate Reader (Thermo Scientific, MA, USA). Notably, the absorbance for both the control and blank experiments were determined by following the identical procedures. A solution of enzyme that had been deactivated by boiling served as the blank. For the control experiment, the sample solution was substituted with an equivalent concentration of DMSO dissolved in deionized water. Acarbose was used as a positive control. All the experiments were carried out in triplicate. In order to calculate the percentage of α-glucosidase inhibition, the following equation was used:

$$\alpha\text{-Glucosidase inhibition (\%)} = \left[\frac{(Ac - Ab) - (As - Ab)}{(Ac - Ab)}\right] \times 100$$

Where: A*b* represents the absorbance of the blank; A*c* represents the absorbance of the control; and A*s* represents the absorbance of the sample.

Determination of synergistic effect against α-glucosidase

A synergistic interaction between the naphthoquinones (LME and lawsone) and acarbose against α-glucosidase inhibitory activity was performed using a previously described method,[23] with a few modifications. The synergistic inhibitory activity was assessed using their IC_{50} values, by combining three concentrations (½IC_{50}, ¼IC_{50}, 1/8IC_{50}) of the naphthoquinones with that of the acarbose. This resulted in nine distinct combinations for each naphthoquinone and acarbose. The identical experimental protocol was followed as previously described for the α-glucosidase inhibitory assay. The fractional percentage inhibitory index (FPI) of the interactions was calculated using a formula given below:

$$\text{Fractional percentage inhibitory index} = \frac{\%\ \text{Inhibition}^{\,a}}{[\%\ \text{Inhibition}^{\,b} + \%\ \text{Inhibition}^{\,c}]}$$

Where, ^{a} % inhibition of acarbose combined with a naphthoquinone; ^{b} % Inhibition of acarbose; and ^{c} % Inhibition of naphthoquinone alone.

Two criteria were employed to evaluate the inhibitory interaction between each naphthoquinone combined with acarbose. Firstly, the combination needed to exhibit a percentage inhibition greater than 50%. Secondly, the interaction was classified as follows: synergistic when FPI ≥ 2, additive when 2 < FPI ≥ 0.5, and antagonist when FPI < 0.5.

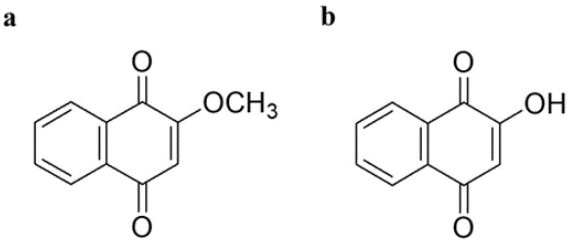

Figure 1: Chemical structures of lawsone methyl ether (a) and lawsone (b).

Statistical analysis

Statistical analyses were conducted using SPSS software, version 25 (SPSS Inc., Chicago, IL, USA). A one-way ANOVA, followed by Duncan's multiple comparison test was employed for the data analysis. The statistical significance was declared at $p < 0.05$. Following Duncan's multiple comparison test, all the samples under the same parameter (e.g., IC_{50}, % inhibition[a], or FPI) were compared not only with the standard but also with every other sample or the mixture within that parameter. The results were presented as the average of three repeated measurements (n=3) ± S.E.M.

RESULTS

In silico studies of LME and lawsone

Molecular docking

Table 1 provides an overview of the molecular docking outcomes for LME and lawsone with respect to α-glucosidase inhibition. LME exhibited the most favorable binding conformation with an energy of -5.4 kcal/mol (Figure 2a). This binding interaction involved the formation of a single hydrogen bond with the ASN264 residue, while Van Der Waals interactions were observed with LYS194, TYR197, GLU257, and GLN260 residues. Additionally, there were pi-pi T-shaped interactions found with the PHE261 residue (Figure 2b). On the other hand, lawsone showcased its most favorable binding conformation with an energy of -5.6 kcal/mol (Figure 3a), suggesting a strong propensity for hydrogen bonding. But given the predominantly hydrophobic nature of the target protein's active site, it only established a single hydrogen bond with the ARG413 residue. Also, Van Der Waals interactions were noted with GLU405, ILE412, ARG413, and ASN417 residues. Furthermore, the TYR416 and VAL404 residues were involved in alkyl and pi-alkyl interactions, respectively (Figure 3b).

Pharmacokinetic/ADMET profile estimation

Table 2 displays the *in silico* ADMET characteristics of LME and lawsone, covering various *in silico* aspects, including drug-likeness, water solubility, pharmacokinetics, medicinal chemistry, and

Table 1: Molecular docking results of LME and lawsone against α-glucosidase.

Compounds	Binding energies	Functional residues	Binding interactions
LME	-5.4 kcal/mol	LYS194, TYR197, GLU257, GLN260, PHE261, ASN264.	Van der Waals, hydrogen bonding, pi-pi T-shaped.
Lawsone	-5.6 kcal/mol	VAL404, GLU405, ILE412, ARG413, TYR416, ASN417.	Van der Waals, hydrogen bonding, pi-pi stacked, pi-alkyl.

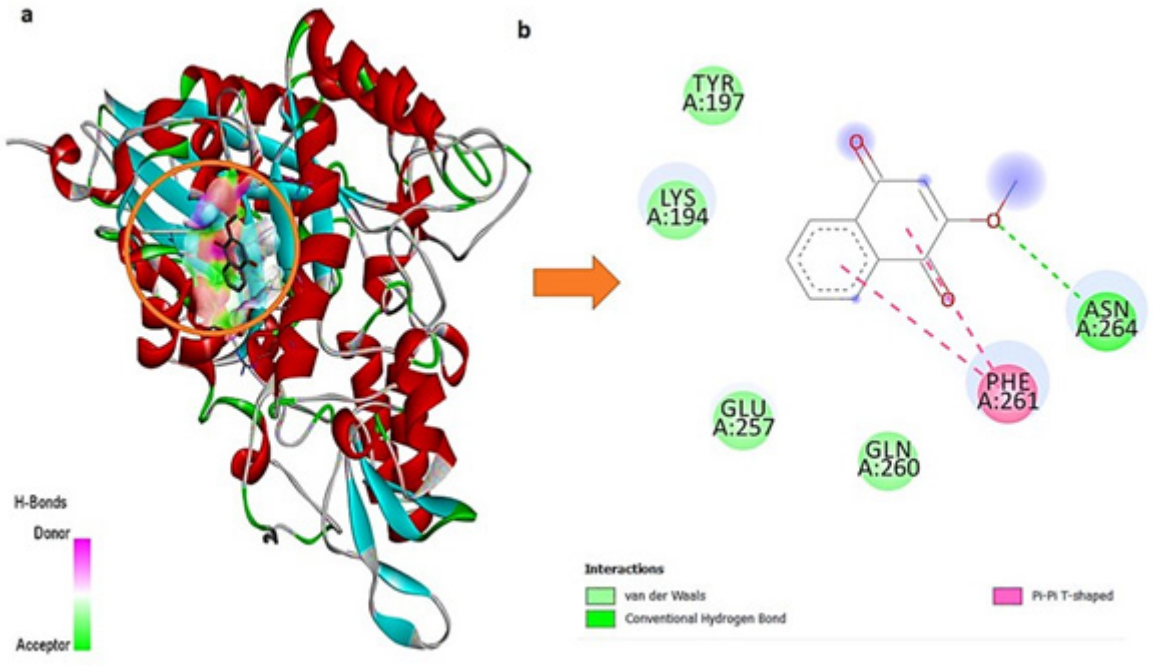

Figure 2: A graphical representation of the best bounded pose of lawsone methyl ether showing its hydrogen bonding capacity (a), and other binding interactions (b) with the active binding site residues of α-glucosidase.

estimated toxicity. According to the principles of drug-likeness theory,[24] both LME and lawsone met the required physicochemical standards of a suitable drug without any deviations. They displayed a balanced mix of hydrophilic and lipophilic qualities, contributing to enhanced gastrointestinal drug absorption (GI-DA) and permeability across the blood-brain barrier (BBB).[25] While both naphthoquinones strongly suppressed CYP1A2 activity, they were not substrates for P-glycoprotein (P-gp). Both LME and lawsone exhibited favorable skin penetration, with Log Kp values of -6.49 and -6.38 cm/s, respectively. In terms of medicinal chemistry, both PAINS and Brenk alerts indicated some negligible deviations.[21] The Brenk alert pointed out that the quinone-A group present in the structures of both the molecules might require optimization before advancing them to the subsequent stage of drug development. Furthermore, LME and lawsone displayed synthesis scores of 2.62 and 2.42, respectively, suggesting that they are more readily synthesizable. The toxicity estimations revealed that both LME and lawsone have favorable toxicity profiles. The analysis predicted them to be completely non-toxic regarding their tumorigenic, irritant, and reproductive effects (Table 2).

In vitro α-glucosidase inhibitory activities of LME and lawsone

Table 3 summarizes the *in vitro* inhibitory effects of LME, lawsone, and acarbose against α-glucosidase. To determine their IC_{50} values, LME and lawsone were tested at four distinct concentrations, ranging from 12.5 to 100 µg/mL. According

Table 2: Summary of ADMET profiles, *in silico* estimated for LME and lawsone.

Physicochemical parameters	LME	Lawsone
Molecular formula	$C_{11}H_8O_3$	$C_{10}H_6O_3$
Molecular weight	188.18 g/mol	174.15 g/mol
Number of rotatable bonds	1	0
Number of hydrogen bond acceptors	3	3
Number of hydrogen bond donors	0	1
Molar refractivity	50.14	45.81
Total polar surface area	43.37 Å^2	54.37 Å^2
Lipophilicity	1.65	1.4
Water solubility	-2.11	-2.13
Solubility class	Soluble	Soluble
Pharmacokinetics		
Gastrointestinal drug absorption (GI-DA)	High	High
Blood brain barrier (BBB) permeability	Yes	Yes
P-glycoprotein (P-gp) substrate	No	No
CYP1A2 inhibitor	Yes	Yes
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	No	No
Log Kp (skin permeation)	-6.49 cm/s	-6.38 cm/s
Medicinal chemistry		
PAINS alert	1 alert; quinone-A	1 alert; quinone-A
Brenk alert	0 alert	0 alert
Lead likeness rule (250 ≤ MW ≤ 350, Log P ≤ 3.5, RB ≤ 7)	No, 1 violation MW < 250	No, 1 violation MW < 250
Synthetic accessibility	2.62	2.42
Toxicity estimation		
Tumorigenic	No toxic effects	No toxic effects
Irritant	No toxic effects	No toxic effects
Reproductive	No toxic effects	No toxic effects

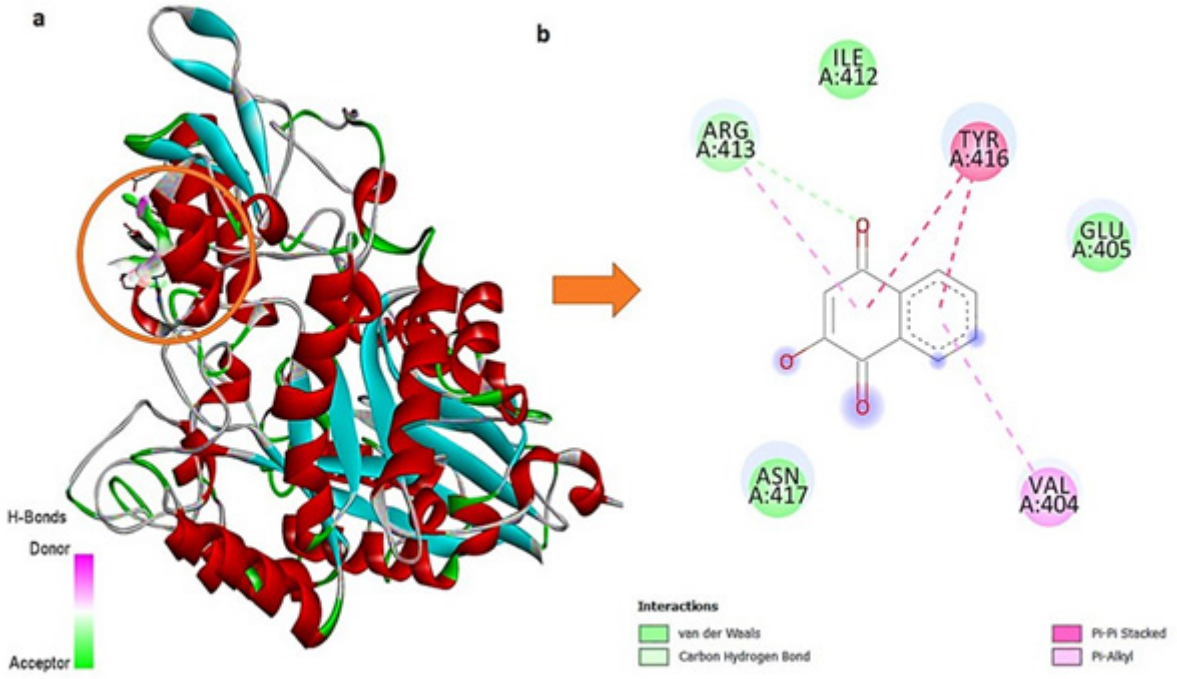

Figure 3: A graphical representation of the best bounded pose of lawsone showing its hydrogen bonding capacity (a), and other binding interactions (b) with the active binding site residues of α-glucosidase.

Table 3: α-Glucosidase inhibitory effects of LME and lawsone.

Compound	IC_{50} (μg/mL)
LME	37.4 ± 0.7[a]
Lawsone	42.2 ± 0.6[a]
Acarbose	440.6 ± 4.7[b]

Data are expressed as mean ± S.E.M. (n=3). Based on the Duncan's multiple range test, the values with different letters of the alphabet or superscripts ("a" and "b") indicate significant differences from one another at a significance level of $p < 0.05$. However, the values labeled with the same letters of the alphabet or superscripts indicate no significant differences.

to the results of *in vitro* assay, LME and lawsone demonstrated significantly similar ($p < 0.05$) inhibitory activities against α-glucosidase, exhibiting the IC_{50} values of 37.4 and 42.2 μg/mL, respectively. Nonetheless, both of the naphthoquinones showcased markedly higher inhibitory activities than that of the standard drug, acarbose (IC_{50} of 440.6 μg/mL).

α-Glucosidase inhibitory interactions of acarbose combined with LME and lawsone

The results of inhibitory interactions of acarbose combined with LME/lawsone are illustrated in Table 4. As per the FPI values, LME displayed synergistic effects when combined with acarbose at every dosage level. In contrast, the combination of lawsone and acarbose manifested an additive interaction. Notably, when LME was used at ½IC_{50} (18.7 μg/mL) and acarbose at ½IC_{50} (220.3 μg/mL), they together showed a pronounced synergistic effect, inhibiting α-glucosidase activity by 88.7% and yielding an FPI value of 2.0. In contrast, lawsone at ½IC_{50} (21.1 μg/mL), combined with acarbose ½IC_{50} (220.3 μg/mL), displayed the strongest additive effect on α-glucosidase inhibition, achieving a percentage inhibition of 76.4, with an FPI value of 1.7.

DISCUSSION

Molecular docking is a pivotal tool in the realm of computational biology and drug design, offering a glimpse into the intricate interplay of molecular interactions. It has recently become a widespread preliminary strategy for the discovery of novel drugs. This approach not only speeds up the early phases of drug development by identifying promising compounds but also paves the way for personalized medicine by tailoring drugs to individual genetic profiles.[26] Molecular docking provides an essential scoring system to highlight optimal binding positions, leading to the formation of the most favorable docked complex. This complex subsequently assists in discerning potential interactions between the protein and ligand. Recognizing these interactions is invaluable for understanding the mechanism by which a ligand binds to a protein's active site.[27] The constricted substrate binding site of α-glucosidase target protein is located close to the C-terminal, a confined region of beta strands of the catalytic domain and orienting the loop conformation towards the N-terminal of the beta-strand domain, thereby the active site being composed of residues from both the catalytic and beta-sheet domains.[28] According to the results of molecular docking study, both LME and lawsone displayed favorable binding conformations, suggesting that they fit well-posed within

Table 4: α-Glucosidase inhibitory interactions between the naphthoquinones and acarbose.

Compounds/Concentrations		% Inhibition [a]		% Inhibition[c]	FPI
		NQ	Acarbose		
LME/ ½IC_{50}	Acarbose/½ IC_{50}	23.2 ± 0.6^{f}	22.1± 0.4^{e}	88.7 ± 0.6^{f}	2.0
	Acarbose/¼ IC_{50}	23.2 ± 0.6^{f}	10.7 ± 0.1^{c}	68.9 ± 0.5^{e}	2.0
	Acarbose/⅛ IC_{50}	23.2 ± 0.6^{f}	5.8 ± 0.2^{ab}	57.8 ± 0.4^{d}	2.0
LME/ ¼ IC_{50}	Acarbose/½ IC_{50}	11.2 ± 0.1^{cd}	22.1 ± 0.4^{e}	66.7 ± 0.5^{e}	2.0
	Acarbose/¼ IC_{50}	11.2 ± 0.1^{cd}	10.7 ± 0.1^{c}	46.2 ± 0.9^{c}	2.1
	Acarbose/⅛ IC_{50}	11.2 ± 0.1^{cd}	5.8 ± 0.2^{ab}	34.6 ± 0.6^{b}	2.0
LME/ ⅛ IC_{50}	Acarbose/½ IC_{50}	7.2 ± 0.2^{b}	22.1 ± 0.4^{e}	57.2 ± 1.5^{d}	2.0
	Acarbose/¼ IC_{50}	7.2 ± 0.2^{b}	10.7 ± 0.1^{c}	35.2 ± 0.9^{b}	2.0
	Acarbose/⅛ IC_{50}	7.2 ± 0.2^{b}	5.8 ± 0.2^{ab}	25.2 ± 0.4^{a}	1.9
Lawsone/ ½IC_{50}	Acarbose/½ IC_{50}	23.8 ± 0.3^{f}	22.1 ± 0.4^{e}	76.4 ± 1.4^{f}	1.7
	Acarbose/¼ IC_{50}	23.8 ± 0.3^{f}	10.7 ± 0.1^{c}	61.7 ± 0.9^{e}	1.8
	Acarbose/⅛ IC_{50}	23.8 ± 0.3^{f}	5.8 ± 0.2^{ab}	54.0 1.3^{d}	1.8
Lawsone/ ¼IC_{50}	Acarbose/½ IC_{50}	12.3 ± 0.3^{d}	22.1 ± 0.4^{e}	61.1 ± 0.6^{e}	1.8
	Acarbose/¼ IC_{50}	12.3 ± 0.3^{d}	10.7 ± 0.1^{c}	43.2 ± 0.6^{c}	1.9
	Acarbose/⅛ IC_{50}	12.3 ± 0.3^{d}	5.8 ± 0.2^{ab}	33.8 ± 0.9^{b}	1.9
Lawsone/ ⅛IC_{50}	Acarbose/½ IC_{50}	5.8 ± 0.1^{a}	22.1 ± 0.4^{e}	50.8 ± 0.7^{d}	1.8
	Acarbose/¼ IC_{50}	5.8 ± 0.1^{a}	10.7 ± 0.1^{c}	29.5 ± 0.5^{b}	1.8
	Acarbose/⅛ IC_{50}	5.8 ± 0.1^{a}	5.8 ± 0.2^{ab}	21.5 ± 0.6^{a}	1.9

a % Inhibition of single compound; *c* % Inhibition of combined compounds; NQ=Naphthoquinone; FPI=Fractional percentage inhibitory index. The results were interpreted as synergistic effect when FPI ≥ 2.0, and additive effect when 2 < FPI ≥ 0.5. Data are expressed as mean ± S.E.M. (n=3). Based on the Duncan's multiple range test, the values with different letters of the alphabet or superscripts (a-f) indicate significant differences from one another at a significance level of $p < 0.05$. However, the values labeled with the same letters of the alphabet or superscripts indicate no significant differences.

the active site of α-glucosidase. This further implied that the sample molecules could establish stable binding connections with the amino acid pockets residing in the enzyme's active region, a crucial factor for effectively inhibiting its function. While molecular docking offers insightful predictions, it is imperative to substantiate these predictions with experimental evidence. Thus, conducting *in vitro* tests, and possibly *in vivo* studies, is crucial to affirm the inhibitory impact of LME and lawsone on α-glucosidase. Our *in silico* findings vividly underscored that beyond β-cell regeneration, inhibiting α-glucosidase is another pathway by which LME and lawsone exert their pronounced hypoglycemic, hypolipidemic, and pancreatic protective potentials.

Understanding the pharmacokinetic/ADMET profiles of potential drug candidates is crucial in the early stages of drug discovery. This ensures that only the most promising compounds move further along in the drug development pipeline, thereby saving time, effort, and resources.[17] For this purpose, molecular weight (MW), partition coefficient (Log P), hydrogen bond acceptor (HBA), hydrogen bond donor (HDB), total polar surface area (TPSA), molar refractivity (MR), and rotatable bond (RB) are important drug-like characteristics that are estimated for the given compound. According to the results of the present investigation, LME and lawsone have shown favorable pharmacokinetic profiles, suggesting effective absorption, distribution, metabolism, and excretion in the body. Moreover, they demonstrated no notable toxicities, with no evidence of causing cancer, skin irritation, or reproductive harm. The pharmacokinetic/ADMET properties play a pivotal role in assessing the viability of a compound for drug development. If LME and lawsone exhibit superior pharmacokinetic/ADMET profiles, it suggests they have a favorable absorption, distribution, metabolism, and excretion pattern without evident toxicities. This makes them promising candidates for further investigation as potential therapeutic agents. *In silico* studies are efficient and cost-effective for predicting potential biological activities of molecules. However, they indeed have limitations, and their outcomes can sometimes be speculative. Thus, while *in silico* analyses can give a preliminary insight into a molecule's potential, *in vivo* studies (using rodent models) offer more concrete evidence regarding efficacy and safety. Fortunately, the antihyperglycemic and antihyperlipidemic activities of LME and lawsone have already been substantiated via *in vivo* research. This prior evidence lends more weight to the *in silico* findings, suggesting that the predicted attributes of these compounds in computer models might well translate to tangible benefits in real biological systems.[12] The combination of *in silico* and *in vivo* data bolsters the credibility of the results and

suggests a promising avenue for further research. Nevertheless, it is essential to conduct additional preclinical and clinical studies to fully validate the efficacy and safety of LME and lawsone for managing diabetes and related metabolic conditions in human subjects.

In the current study, the IC_{50} values of LME and lawsone against α-glucosidase were found significantly comparable with a negligible difference, this suggested that both compounds had exhibited almost similar potencies in inhibiting the enzyme. This slight dissimilarity in the behavior of 1,4-naphthoquinones may be attributable to their different electrophilicity potentials. In the context of enzyme inhibitors, the electrophilicity of a molecule could influence its interaction with specific amino acid residues within the enzyme active site.[29] Which originates from various atoms or chemical groups attached to the C-2 of their quinone skeleton (Figure 1). Functional groups in molecules, like the methoxy (-OCH_3) and hydroxy (-OH) groups, can significantly affect the reactivity of the molecule. The presence of a methoxy group in LME as compared to the hydroxy group in lawsone seems to enhance LME's nucleophilicity. The enhanced nucleophilicity due to the methoxy group would mean LME has a stronger propensity for alkylation. In the context of enzyme inhibition, this implies that LME can form stronger or more favorable interactions with electrophilic sites within the enzyme's active site compared to lawsone. Notably, the comparable α-glucosidase inhibitory characteristics of LME and lawsone were consistent with their *in silico* binding energies against α-glucosidase. Promising potentials of LME and lawsone to regenerate pancreatic β-cells, as indicated in our prior study, offers a potentially transformative approach to diabetes management.[12] In contrast, the present study underscores the multifaceted therapeutic potential of LME and lawsone in diabetes management. Beyond their notable capacity to regenerate pancreatic β-cells and thereby address insulin deficiency, they also showcase potent inhibitory effects on α-glucosidase. This enzyme inhibition is pivotal in regulating postprandial hyperglycemia by slowing down carbohydrate digestion, which subsequently leads to a moderated rise in blood sugar levels post-meal. Thus, the dual mechanism-boosting endogenous insulin production and managing post-meal glucose surges-positions LME and lawsone as versatile and promising therapeutic agents. This synergy augments their significance in addressing not only the primary concerns of diabetes but also the broader spectrum of associated metabolic complications. Further research is warranted to explore their precise modes of action and clinical implications.

Recently, a plethora of studies have authenticated that naturally occurring 1,4-naphthoquinones demonstrate potent α-glucosidase inhibitory effects, aligning with earlier research findings on compounds like rhinacanthin-C from the *Rhinacanthus nasutus* L., shikonin from *Lithospermum erythrorhizon* L., and plumbagin from *Plumbago zeylanica* L. These results reinforce the potential therapeutic importance of such compounds as important antidiabetic moeities.[30] The findings from the present study contribute to the expanding body of research supporting the hypoglycemic properties of 1,4-naphthoquinones. The repeated observations across various studies underline the importance of these naturally occurring compounds in pharmaceutical research. Given their potential, 1,4-naphthoquinones may serve as promising therapeutic agents in regulating blood glucose and addressing associated metabolic disorders.

Chronic hyperglycemia and associated metabolic disturbances can have detrimental effects on various organs and systems leading to cardiovascular complications, retinopathy, nephropathy, and neuropathy.[31] LME and lawsone have demonstrated potent anti-inflammatory and antioxidant properties, as supported by previous studies.[32] Moreover, our prior *in vivo* experimentation on diabetic rats revealed promising results, showing that LME and lawsone exhibited remarkable hepatoprotective, nephroprotective, and pancreatic protective effects.[12] LME and lawsone, given their remarkable pharmacological effects, not only hold promise as potent anti-diabetic agents but also as protective agents against the long-term complications of diabetes. The potential of LME and lawsone to mitigate the oxidative stress and inflammation in diabetic patients aligns with the therapeutic benefits observed with rhinacanthin-C, as previously documented.[33] Thus, these compounds could play a dual role: helping regulate blood glucose levels and providing a protective shield against the damaging consequences of prolonged hyperglycemia.

Acarbose, when administered at clinical doses ranging from 100-300 mg/day, often results in suboptimal patient compliance due to its associated side effects. A significant proportion of patients, exceeding two-thirds, report experiencing gastrointestinal issues such as flatulence, cramps, diarrhea, and stomach distension. These side effects frequently lead clinicians to reduce the dose of acarbose, which subsequently diminishes its therapeutic efficacy. This presents a challenge, as the balance between managing hyperglycemia effectively and ensuring patient comfort becomes precarious.[34] It has been reported that acarbose is a competitive α-glucosidase inhibitor, while 1,4-naphthoquinones are the noncompetitive ones.[30] The distinct mechanisms of action between 1,4-naphthoquinones and acarbose insighted our interest, prompting an investigation into their potential synergistic inhibitory effects on α-glucosidase. Combining these compounds offered an enhanced therapeutic approach, allowing for lower doses of each and potentially reducing side effects while maximizing efficacy in inhibiting the enzyme. The observed synergistic interaction between LME and acarbose might be attributable to their distinct molecular structures and sizes. Given the differences in molecular bulk, combining the two could result in reduced steric hindrance, which in turn could facilitate a more efficient and tighter binding of LME to the α-glucosidase enzyme.

Furthermore, our research has revealed that the combinations of either LME or lawsone with acarbose displayed stronger inhibitory actions against α-glucosidase when compared to the combinations of acarbose with certain flavonoids such as cyanidin-3-galactoside and cyanidin-3-glucoside from *Moringa oleifera* L.[35] This suggested that LME and lawsone might have unique properties or interactions with acarbose that enhanced their combined inhibitory effects, emphasizing their potential as promising candidates for therapeutic applications in managing hyperglycemia.

CONCLUSION

In silico findings of the current study strongly supported the excellent drug-like properties and notable anti-diabetic potentials of LME and lawsone against α-glucosidase. A subsequent *in vitro* assay revealed strong inhibitory actions of LME and lawsone against α-glucosidase, further supporting the underlying mechanism behind their notable *in vivo* hypoglycemic effects. However, the synergistic and additive interactions of LME and lawsone with acarbose respectively, revealed a new paradigm in the treatment of diabetes. This novel approach allows for a reduction in the clinical dose of acarbose to one-fourth providing a completely new therapeutic strategy to effectively manage postprandial hyperglycemia using acarbose at safer and lower doses. Hence, the integrated use of *in silico, in vitro*, and *in vivo* analyses presents a comprehensive strategy that underscores the potential of LME and lawsone for pharmaceutical development. This convergence of evidence prompts further investigation to highlight their efficacy and safety in human clinical trials.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTION OF ALL AUTHORS

MK: Semi-Synthesis, Enzyme-Inhibition Assay, and writing the manuscript. **MAS and SB:** Molecular Docking Analysis and writing the Manuscript**. PP:** Conceptualization of the research and Methodology, Project Supervision, Administration, and writing the manuscript.

ABBREVIATIONS

ADMET: Absorption, distribution, metabolism, excretion, and toxicity; **ALA:** Alanine, **ANOVA:** Analysis of variance; **ARG:** Arginine; **ASN:** Asparagine; **BBB:** Blood brain barrier; **CYP:** Cytochrome P450; **DM:** Diabetes mellitus; **DMSO:** Dimethyl sulfoxide; **EGCG:** Epigallocatechin gallate; **FPI:** Fractional percentage inhibitory index; **GI-DA:** Gastrointestinal drug absorption; **GLN:** Glutamine; **GLU:** Glutamic acid; **HBA:** Hydrogen bond acceptor; **HBD:** Hydrogen bond donor; **IC_{50}:** Half maximal inhibitory concentration; **ILE:** Isoleucine; **LME:** Lawsone methyl ether; **LYS:** Lysine; **MR:** Molar refractivity; **MW:** Molecular weight; **NQ:** Naphthoquinone; **PHE:** Phenyl alanine; **pNPG:** p-Nitrophenyl-α-D-glucopyranoside; **RB:** Rotatable bond; **TPSA:** Total polar surface area; **TRP:** Tryptophan; **TYR:** Tyrosine; **VAL:** Valine; **WHO:** World Health Organization.

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