

Screening of Ethyl Acetate Fraction of *Ocimum gratissimum* Leaf Methanol Extract for Antioxidant Potentials and Alpha-Amylase and Alpha-Glucosidase Inhibitors

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a global health challenge characterised by impaired carbohydrate metabolism and oxidative stress, necessitating safer plant-based therapeutic alternatives. This study evaluated the antidiabetic and antioxidant potential of the ethyl acetate fraction of *Ocimum gratissimum* leaf extract (EAFOGL) using *in vitro* and *in silico* approaches. Enzyme inhibition assays revealed that EAFOGL moderately suppressed α -amylase and α -glucosidase activities, with IC_{50} values of 87.17 ± 5.20 and 49.84 ± 6.72 μ g/mL, respectively, relative to acarbose (52.12 ± 2.74 and 40.94 ± 3.44 μ g/mL). Antioxidant evaluations demonstrated radical scavenging and metal chelating activities with IC_{50} values of 90.32 ± 9.91 and 98.58 ± 13.29 μ g/mL, respectively, which were lower than standard antioxidants but still significant. LC-MS profiling identified five predominant compounds, namely lariciresinol, nitrendipine, eupatilin, 6,7-dimethoxy-2-(1-piperazinyl)-4-quinazolinamine, and N-succinoyl anthranilic acid. Molecular docking indicated strong binding affinities of these phytochemicals for α -amylase, α -glucosidase, and the Keap1 protein, with nitrendipine showing notable interactions with α -amylase (-6.91 kcal/mol) and Keap1 (-6.54 kcal/mol), and lariciresinol binding effectively to α -glucosidase (-6.98 kcal/mol). Key interactions involved hydrogen bonding and hydrophobic contact with catalytic residues such as His305 and Arg416. In conclusion, EAFOGL exhibited promising dual antidiabetic and antioxidant properties, supporting its potential use in mitigating hyperglycaemia and oxidative stress associated with T2DM. Further *in vivo* studies and mechanistic investigations are recommended to validate its therapeutic efficacy and establish its safety profile.

KEYWORDS: *Ocimum gratissimum*; Enzyme inhibition; Antioxidant; Molecular docking; Diabetes

1. INTRODUCTION

The increasing global rates of type 2 diabetes mellitus (T2DM) represent a significant public health issue. Characterised by persistent high blood sugar and insulin resistance, T2DM can result in complications such as heart, kidney, and nerve damage. A common treatment approach involves targeting digestive enzymes like α -amylase and α -glucosidase, which facilitate carbohydrate breakdown and glucose absorption, thereby reducing blood sugar spikes after meals.^{1,2} Oxidative stress also plays a critical role in T2DM by disrupting insulin signalling and damaging pancreatic β -cells through excessive reactive oxygen species (ROS).^{3,4} Consequently, there is an increasing demand for natural substances with antioxidant and enzyme-inhibiting properties.⁵ *Ocimum gratissimum* L., known as African basil, has traditional uses in managing diabetes and inflammation.⁶ Although crude extracts demonstrate potent bioactivity, investigating the mechanisms of its ethyl acetate fraction offers valuable research opportunities.⁷ While acarbose, a synthetic enzyme inhibitor, is effective clinically, it often causes gastrointestinal side effects, leading to growing interest in plant-based alternatives with fewer adverse effects.⁸ Redox-modulating capacity is evaluated using antioxidant tests like DPPH and metal chelation assays.^{9,10} Phytochemicals in complex extracts are identified via LC-MS, and molecular docking models illustrate how these compounds interact with targets such as α -amylase, α -glucosidase, and Keap1.¹² This study examines the antidiabetic and antioxidant effects of EAFOGL, identifies its phytochemicals through LC-MS, and investigates molecular interactions with key proteins using computer-aided docking analyses.

2. MATERIALS AND METHODS

2.1 Collection, Extraction, and Fractionation of Plants

Fresh leaves of *Ocimum gratissimum* from Bayero University, Nigeria, were authenticated (Voucher No. BUKHAN00306). The leaves were air-dried and ground into a powder, then extracted with 80% methanol (1:10 w/v) via maceration for 72 hours, followed by filtration. The filtrate was concentrated at 40 °C under reduced pressure. The crude extract was partitioned into *n*-hexane, chloroform, ethyl acetate, and *n*-butanol fractions. Based on bioactivity results, the EAFOGL was chosen for further

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2.2 Enzyme Inhibition Assays

The inhibitory activity of α -amylase was measured using the DNSA method.⁸ Briefly, 250 μ L of EAFOGL was mixed with phosphate buffer (pH 6.9) and α -amylase, then incubated before adding a starch solution. After incubation, the DNSA reagent was added, the mixture was boiled, and the absorbance was read at 540 nm. Acarbose served as the positive control. IC₅₀ values were calculated using nonlinear regression analysis.¹⁴ To assess α -glucosidase inhibition, 50 μ L of EAFOGL was incubated with α -glucosidase in phosphate buffer at 37 °C, then pNPG was added.¹⁵ The reaction was stopped with sodium carbonate, and absorbance was measured at 405 nm, with acarbose as the standard.^{16,17}

2.3 Antioxidant Assays

The DPPH assay consisted of mixing 0.1 mM DPPH with different concentrations of EAFOGL.¹⁸ After incubating in the dark for 30 minutes, absorbance was measured at 517 nm, using ascorbic acid as the standard. To evaluate metal chelation, FeCl₂ and the ferrozine reagent were used, and the absorbance was recorded at 562 nm, with EDTA serving as the reference standard.¹⁹

2.4 LC-MS Analysis

Phytochemical profiling was carried out via LC-MS using reverse-phase C18 chromatography in negative ion mode.¹¹ Mass spectra were matched with MassBank database entries to identify constituents based on m/z values and retention times.

2.5 Molecular Docking Studies

In silico docking of LC-MS-identified compounds was done against α -amylase (PDB: 1HNY), α -glucosidase (4J5T), and Keap1 (4L7B) using MOE 2019.01 software.^{20,21} Ligands were sketched with ChemDraw and optimised using MMFF94 in Avogadro.²² Docking interactions were visualised using Discovery Studio Visualizer (version 10).²³

2.6 Statistical Analysis

All experiments were performed in triplicate, and the results are shown as the mean \pm standard deviation (SD). Statistical analysis was conducted using a one-way ANOVA followed by Tukey's post hoc test with GraphPad Prism version 9.5.1. A significance threshold of $p < 0.05$ was set.

3. RESULTS AND DISCUSSION

3.1 Enzyme Inhibitory Activity of EAFOGL

The EAFOGL exhibited a dose-dependent inhibitory effect on both α -amylase and α -glucosidase enzymes. The recorded IC₅₀ values were 87.17 ± 5.20 μ g/mL for α -amylase and 49.84 ± 6.72 μ g/mL for α -glucosidase. In comparison, acarbose exhibited IC₅₀ values of 52.12 ± 2.74 μ g/mL for α -amylase and 40.94 ± 3.44 μ g/mL for α -glucosidase (**Figure 1**). Although EAFOGL was found to be less effective than acarbose, the results suggest a moderate level of enzyme inhibition, indicating that it possesses bioactive compounds with potential antidiabetic properties.¹⁵

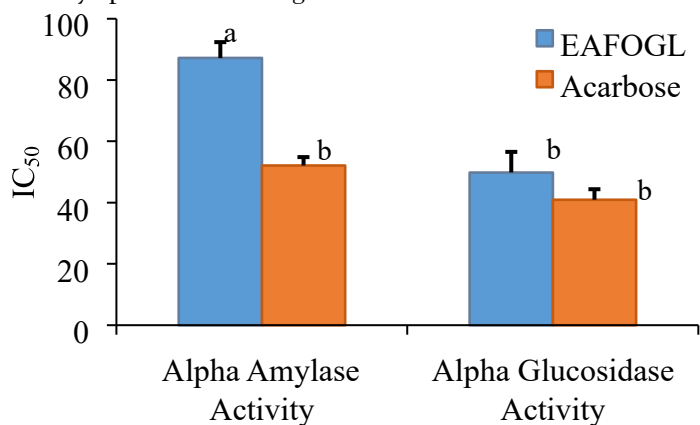


Figure 1: The inhibitory impact of EAFOGL on α -amylase and α -glucosidase.

Note: Bars marked with different letters indicate a statistically significant difference ($\alpha=0.05$); bars sharing the same letter do not show a significant difference.

3.2 Antioxidant Activity of EAFOGL

EAFOGL showed notable antioxidant activity in both assays. It had IC₅₀ values of $90.32 \pm 9.91 \mu\text{g/mL}$ for DPPH scavenging and $98.58 \pm 13.29 \mu\text{g/mL}$ for metal chelation, compared to ascorbic acid ($59.07 \pm 5.99 \mu\text{g/mL}$) and EDTA ($70.11 \pm 9.34 \mu\text{g/mL}$) (**Figure 2**). Though less potent than the standards, the results highlight EAFOGL's capacity to neutralise free radicals and chelate metal ions.^{9,10}

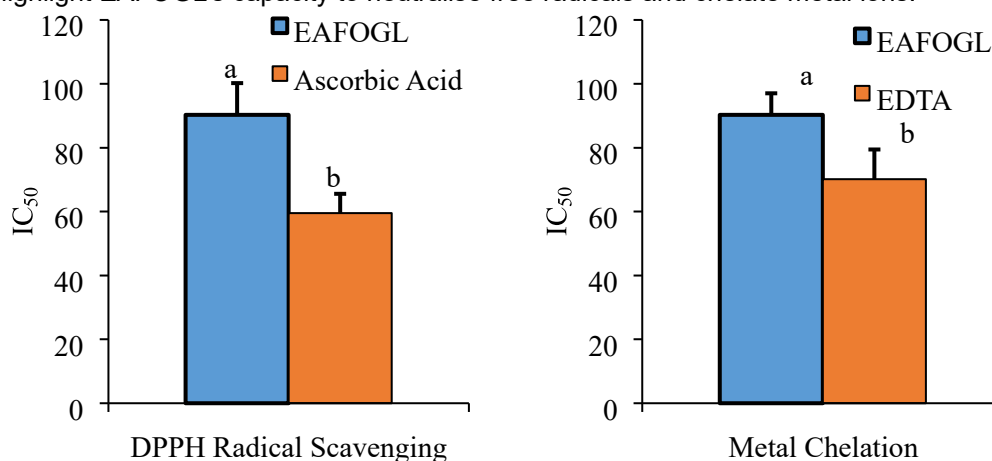


Figure 2: Scavenging Activity against DPPH Radicals and Metal Chelating Ability of EAFOGL

Note: Bars designated with different letters signify statistically significant differences ($\alpha = 0.05$), whereas bars sharing the same letter indicate no significant differences.

3.3 LC-MS Identification of Bioactive Compounds

LC-MS analysis of EAFOGL tentatively identified five compounds: lariciresinol, nitrendipine, eupatilin, 6,7-dimethoxy-2-(1-piperazinyl)-4-quinazolinamine, and N-succinoyl anthranilic acid (**Table 1**, **Figure 3**). Identification was based on m/z values and retention times matched against the MassBank database.¹¹

Table 1: Result of LC-MS analysis of OGEAF showing MZ (negative mode)

Retention time	Base peak (m/z)	Actual mass	Tentative compound
1.980	359.496	360.496	Lariciresinol
8.475	359.155	360.155	Nitrendipin
10.405	343.893	344.893	Euoatilin

2.605	288.335	289.335	6,7-Dimethoxy-2-(1-piperazinyl)-4-quinazolinamine
6.546	235.684	236.684	N-succinoyl anthranilic acid

NB: Compounds obtained from <https://massbank.eu/MassBank/Result.jsp>, using MZ in negative mode

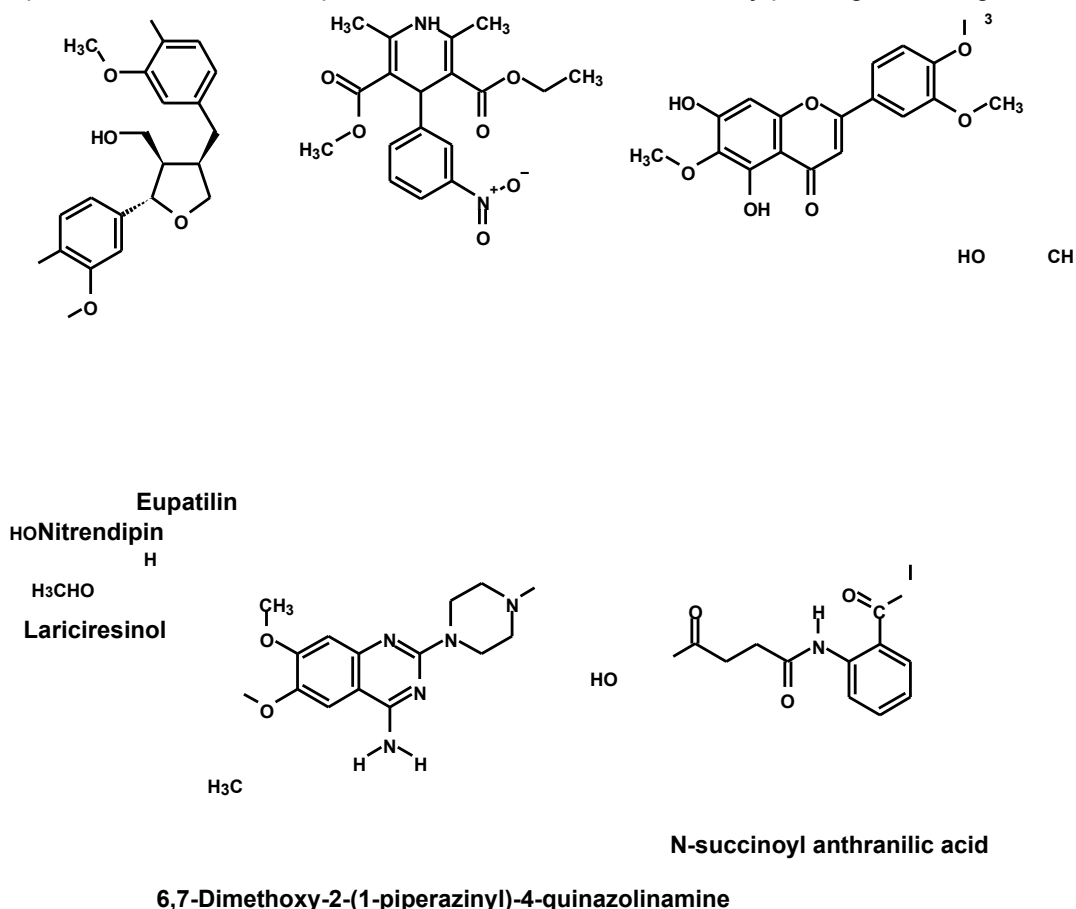


Figure 3: Structures of the Identified Compounds from LC-MS Analysis

3.4 Molecular Docking Analysis

In silico docking analysis demonstrated that nitrendipine and lariciresinol exhibited favourable binding affinities with α -amylase (-6.91 kcal/mol), α -glucosidase (-6.98 kcal/mol), and Keap1 (-6.54 kcal/mol) (**Table 2**). Although slightly weaker than acarbose (-8.63 and -6.53 kcal/mol), the results suggest potential interactions with key antidiabetic and antioxidant targets.^{12,16,19}

Ligands	α -Amylase	α -Glucosidase	Keap1
6,7-Dimethoxy-2-(1-piperazinyl)-4-quinazolinamine	-5.8232	-6.4801	-5.7186
Eupatilin	-6.4671	-6.40771	-6.2851
Lariciresinol	-6.7305	-6.9770	-5.9911
N-succinoyl anthranilic acid	-5.8070	-6.3553	-5.0126
Nitrendipine	-6.9058	-6.0953	-6.5437
Acarbose*	-8.6252	-6.5282	-
Bardoxolone methyl**	-	-	-6.5186

Table 2: Molecular docking scores of alpha-amylase, alpha-glucosidase, and Keap1 with the ligands

* Standard control ligand for alpha amylase and alpha-glucosidase proteins **
Standard control ligand for Keap1 protein

The docking uncovered important interactions, including hydrogen bonds, π - π stacking, salt bridges, and hydrophobic interactions. Notably, nitrendipine bound to α -amylase via His305, Arg195, and Glu233, residues also targeted by acarbose.¹⁶

4. CONCLUSION

EAFOWL demonstrated moderate α -glucosidase and α -amylase inhibition, alongside significant antioxidant activity. LC-MS and molecular docking identified compounds, particularly lariciresinol, nitrendipine, and eupatilin, as likely contributors. The findings suggest EAFOWL may exert dual antidiabetic and antioxidant effects through enzyme inhibition and Nrf2 pathway activation, warranting further pharmacological validation.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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