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# Glucose oxidase as a model enzyme for antidiabetic activity evaluation of medicinal plants: *In vitro* and *in silico* evidence

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**ABSTRACT:** Diabetes mellitus is a major public health problem in the world. In Africa, more than 80% of patients use plants for their treatment. However, the methods of validation of endogenous knowledge usually used are costly. The alternative method developed in this study aims at creating hyperglycemia *in vitro* and exploiting the metabolic pathway involving glucose oxidase for UV-visible spectrophotometric screening of medicinal plants' antidiabetic activity. The evolution of glucose oxidation as a function of drug concentration is followed by UV-visible spectrophotometry. The formation of the stable complex between the enzyme and the inhibitor is studied using molecular docking. Drugs used (Gliben) and plant extracts exhibited an *in vitro* hypoglycemic effect by reducing exponentially, *in vitro*, the level of free glucose. The results also showed that *L. multiflora* is more active than *V. amygdalina* ($IC_{50}$: 1.36 ± 0.09 mg/mL Vs $IC_{50}$: 3.00 ± 0.54 mg/mL). Gliben (0.5 mg/mL) and *L. multiflora* (2 mg/mL) reduced both the rate of oxidation of glucose by glucose oxidase (catalytic power $\mathbf{V}_{max}$: 0.84 ± 0.11 mg*$mL^{-1}$*$min^{-1}$ for Gliben and 1.72 ± 0.13 mg*$mL^{-1}$*$min^{-1}$ for *L. multiflora*); and the affinity of this enzyme for its substrate-glucose ($\mathbf{K_M}$: 15.11 ± 2.72 mg*$mL^{-1}$for Gliben and 9.17 ± 1.56 mg*$mL^{-1}$for *L. multiflora*) when these results are compared to enzyme catalysis in the absence of inhibitor ($\mathbf{V}_{max}$: 2.86 ± 0.44 mg*$mL^{-1}$*$min^{-1}$; $\mathbf{K_M}$: 8.07 ± 1.96 mg*$mL^{-1}$). The binding of GOX (1GAL) to selected phytocompounds derived from *L. multiflora* was confirmed by molecular docking. The most stable complexes were obtained for four compounds; **8** (**-10.1±0.0** Kcal/mol), **6** (**-9.5±0.1** Kcal/mol), **3** (**-8.3±0.0** Kcal/mol) and 9 (**-8.2±0.1** Kcal/mol). Among these, compounds **8** and **6** formed complexes with the enzyme stabilized by hydrogen bonds, the compound **8** forms 5 hydrogen bonds (**ASN514, ASP424, ARG95**, **TYP68**, **LEU65**) while compound **6** forms 2 hydrogen bonds (ASN514 and SER422). However, no H-bonding interaction occurs in the complex that involves ligands **9** and **3** despite their high binding energy (-8.2±0.1 Kcal/mol and -8.3±0.0 Kcal/mol respectively). Glucose oxidase can serve as a marker enzyme for *in vitro* antidiabetic activity evaluation of medicinal plants.

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## 1. INTRODUCTION

Glucose oxidase ($\beta$-D-glucose: oxygen-1-oxidoreductase or GOX) is an enzyme that catalyzes the oxidation of $\beta$-D-glucopyranose to gluconic acid using molecular oxygen as an electron acceptor with simultaneous production of hydrogen peroxide (or hydrogen peroxide) as shown in the metabolic pathway below (Figure 1):

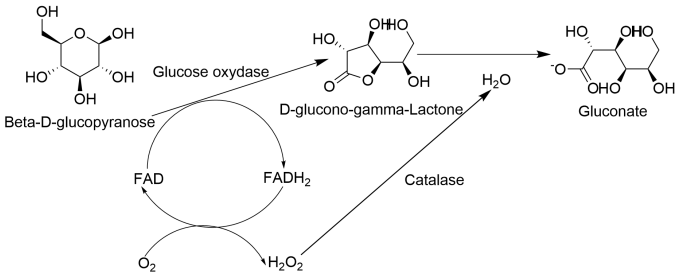


**Figure 1.** Metabolic pathway of the oxidation of $\beta$-D-glucopyranose

Glucose oxidase (GOX) has a molecular weight between 130 and 175 kDa and specifically catalyzes the reaction involving the anomeric beta form of glucose. Its optimum pH is 5.5 and its isoelectric point (pI) is 4.2. The metabolic pathway involving this enzyme can be replicated *in vitro* and exploited as a biochemical technique for screening plants with anti-hyperglycemic properties (anti-diabetic plants) (Khang et al., 2011). In this case, it is sufficient to add to the reaction medium a peroxidase that catalyzes the reduction of hydrogen peroxide ($H_2O_2$) to water. The reaction is highlighted by the addition of a colorless chromogenic (secondary substrate) which is oxidized to a colored product whose intensity is proportional to the concentration of glucose in solution. Two chromogenic substrates are generally used. These are ABTS (2, 2'-Azino-di-(3-ethylbenzthiazolin-sulfonate) which can be oxidized by $H_2O_2$ to a bluish-green product absorbing at 420 nm; and O-dianisidine which can be oxidized to quinone-imine, a dye which absorbs at 500 nm (Mgbeje et al., 2016). For this purpose, hyper-glycemia *in vitro* (glucose $\geq$ 100 mg/dL) can be detected using UV-visible spectrophotometry. Thus, any plant extract which can reduce the intensity of coloration of the reaction medium and thus the concentration of free glucose in solution would be displayed anti-hyperglycemic or hypoglycemic properties (Khang et al., 2011). UV-visible spectrometry can thus be used as the best means of studying the interaction of natural substances with GOX and glucose.

In the case of glucose oxidase inhibition (decrease of the affinity of the enzyme for the glucose substrate and/or the speed of the reaction involving the enzyme), such natural substances that interact with GOX and glucose are called Glucophages, as they allow experimental reduction of the concentration of free glucose (signal intensity) *in vitro* (hypoglycemic effect). Accordingly, in the absence of an inhibitor or when the extract is not active, the intensity of the signal (coloration) is strong. By the other hand, in the presence of an active extract (inhibitor), the signal intensity is low, probably due to the sequestration of free glucose in the form of a ternary glucose oxidase-glucose-inhibitor complex. The hypoglycemic effect *in vitro* is thus expressed as the result of inhibition of the oxidation of glucose to gluconic acid by glucose oxidase. In other words, any GOX inhibitor is a potential anti-hyperglycemic or hypoglycemic agent. It has been reported in the literature that natural substances are generally mixed inhibitors of key enzymes of carbohydrate metabolism and have the property of decreasing their catalytic power and reaction speed. Two biochemical mechanisms could explain this behavior. First, the free enzyme would attach to glucose (substrate) and the enzyme-substrate complex (ES) formed would then bind the inhibitor to give ESI. In a second step, the GOX enzyme would attach to the inhibitor and the binary ES complex would bind the glucose substrate to form the ternary ESI complex (Mgbeje et al., 2016). The present study aimed to use glucose oxidase as a marker enzyme for the *in vitro* detection of the anti-diabetic activity of two medicinal plants used in traditional medicine for the management of diabetes. UV-visible spectrophotometry, used in this study to monitor the activity of GOX, is based on electronic delocalization (transition) which leads to absorption bands characterized by wavelength and intensity. The absorption of light in the visible region of the spectrum by the chromogenic substrate can be modified in the presence of GOX inhibitor. Studying the absorption spectrum of the chromogenic substrate (ABTS or O-dianividin) is a very easy and reliable way to detect the action of GOX inhibitors and therefore to identify compounds/extracts with anti-hyperglycemic or hypoglycemic properties (Sushil & Talambedu, 2012).

## 2. MATERIAL AND METHODS

### 2.1. *In vitro* study

#### *2.1.1 Plant material and reagents*

The reactants consist of a glucose solution 17 mmol/L (3 g/L), extracts of Lippia multiflora and Vernonia amygdalina, and the enzymatic reagent consisting of the mixture glucose oxidase 15 KUI/L, peroxidase 1.5 KUI/L, and phenol 77 mmol/L in 0.1 M Potassium phosphate buffer: 1.36 g $KH_2PO_4$; 2.28 g $K_2HPO_4$*$3H_2O$; 100 mL distilled water (Merck pa) at pH 7.0.

#### *2.1.2 Free glucose level evaluation*

Glucose is oxidized by oxygen to gluconic acid, producing hydrogen peroxide $H_2O_2$. The latter is dosed using colorimetric method. In the presence of peroxidase, the peroxide oxidizes phenol to quinone-imine whose absorbance is measured at 505 nm. Briefly, ten $\mu$L of a glucose solution (3 g/L) are mixed with other 10 $\mu$L of plant extract at different concentrations. The blank consists of distilled water, while Gliben is used as positive control. The resulting mixture is incubated at 25 °C within 15 min. The determination of free glucose is measured after the addition of 1 mL of the enzyme reagent (Glucose oxidase 15 kIU/L, peroxidase 1.5 kIU/L, phenol 77 mmol/L, 0.1 M

Potassium buffer pH 7.0.) at 505 nm.

$$\text{Free GC after treatment} = \frac{(Treated\ glucose\ OD * Concentration\ of\ untreated\ glucose)}{Untread\ Glucose\ OD}$$

### 2.2. *In silico* (Molecular Docking) studies

#### 2.2.1 *Protein preparation*

The crystal structure of the protein used in this study was retrieved from the Protein Data Bank with PDB ID 1GAL, and imported into chimera for visualizing the binding domain and identifying the amino acids in the binding pocket. The survey of the binding-site was carried out with the reference of amino acid residues in the binding domain, as previously reported (Anshika et al., 2017; Vidya et al., 2019). The hydrogen atoms were added to the protein in order to correct the ionization and tautomeric states of the amino acid residues. The water molecules were removed before the docking. Incomplete side chains were replaced using the Drunbrack rotamer library (Shapovalov & Dunbrack, 2011). In addition, the protein was subjected to energy minimization by applying the AMBER 14SB force field method, and AM1-BCC was used for other residues with a maximum number of 200 steps and gradient descent method at a RMS gradient of 0.02.

The optimized protein was saved in pdbqt format and imported to PyRx for molecular docking which was carried out by using Autodock Vina virtual screening tool (Trott & Olson, 2010).

#### 2.2.2 *Generation of ligand dataset*

The selected phytocompounds from *Lippia multiflora* derivatives from various literature resources (Arthur et al., 2011; Avlessi et al., 2011; Bagora et al., 2014; Bassole et al., 2003; Soro et al., 2016) were drawn using ACD/MarvinSketch (20.9). The 2D structures of the sketched ligands (compounds **1-9)** are shown in Figure 2. Further, ligands were imported into ChemDraw to obtain 3D from 2D. The 3D ligands were optimized using Mercury software by conformer generation.

### 2.3. *In vivo* anti-hyperglycemic activity

The study was performed on an animal model consisting of 15 Swiss mice subjected to temporary hyperglycemia by gavage of a glucose solution (200 mg/mL). These 15 mice were divided into three groups or batches as follows: the first group of 5 mice for the negative control (physiological water), the second group of 5 mice for the positive control (glibenclamide 10 mg/kg), and the third group of 5 mice for the test with the *L. multiflora* infusion at a dose of 250 mg/kg body weight. Blood glucose determination was performed using an SD check glucometer on whole blood collected from the tail.

## 3. RESULTS AND DISCUSSION

### 3.1. *In vitro* bioassay

Figure 3, 4, 5 and 6 show the evolution of the glucose level as a function of the drug dose (Gliben or plant extract).

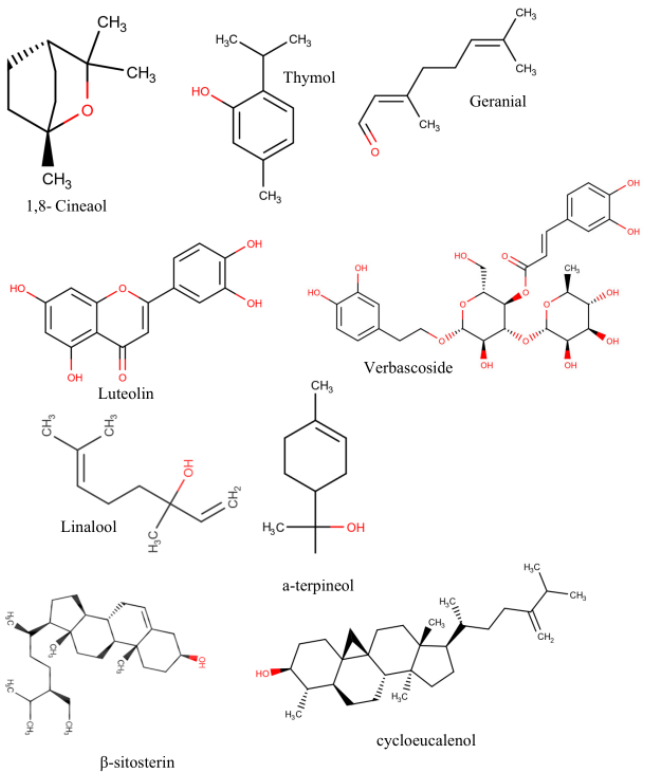


**Figure 2.** 2D structures of thesketched ligands (compounds1-9) derived from *Lippia multiflora*

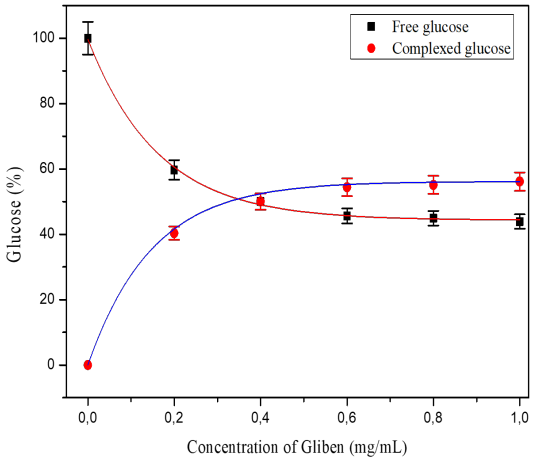


**Figure 3.** Effect of Glibenclamide (positive control) on glucose level *in vitro*

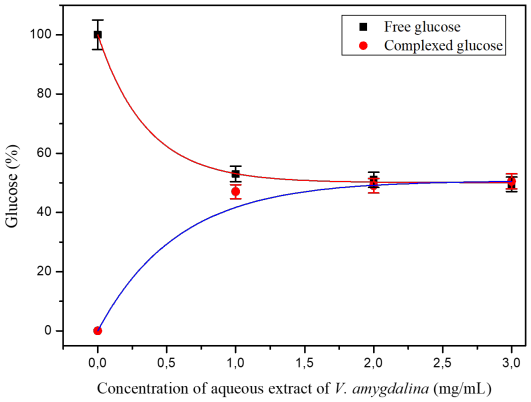


**Figure 4.** Effect of *V. amygdalina* aqueous extract on the glucose level *in vitro*

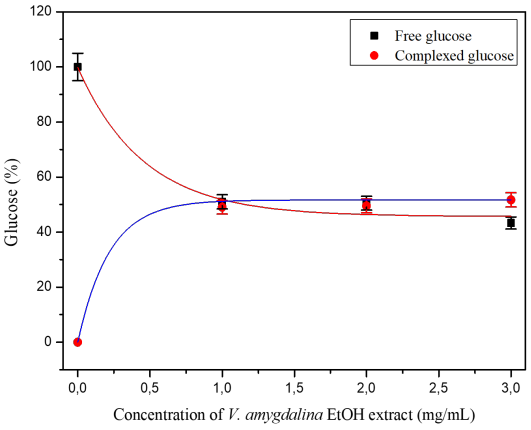


**Figure 5.** Effect of *V. amygdalina* ethanolic extract on the glucose level *in vitro*

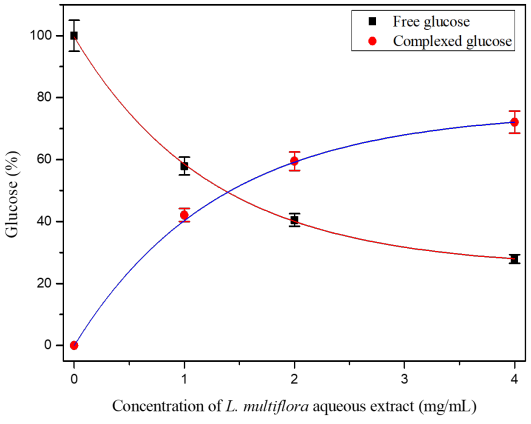


**Figure 6.** Effect of *L. multiflora* aqueous extract on the glucose level *in vitro*

These curves show that the drugs used exponentially reduce the concentration of free glucose *in vitro* (*in vitro* hypoglycemic effect); so these drugs have the effect of sequestering/complexing glucose *in vitro*.

The drug concentrations that reduce 50% of free glucose levels *in vitro* are shown in Table 1.

**Table 1**
Mean $IC_{50}$ values for anti-diabetic drugs

| Drugs | $IC_{50}$ (mg/mL) |
|---|---|
| Gliben (positive control) | 0.36 ± 0.04 |
| *V. amygdalina* (aqueous extract) | 3.00 ± 0.54 |
| *V. amygdalina* (ethanolic extract) | 2.00 ± 0.31 |
| *L. multiflora* (aqueous extract) | 1.36 ± 0.09 |

From this table, it can be seen that Gliben, a standard anti-diabetic agent, has shown a hypoglycemic effect *in vitro*. Thus, the perfect agreement between the calculated curves and the experimental points allows validating the used method. Moreover, the *in vitro* anti-hyperglycemic activity of *L. multiflora* and *V. amygdalina* confirms the *in vivo* antidiabetic activity of these medicinal plants as previously reported (Mgbeje et al., 2016; Ugoanyanwu et al., 2015). This allows the use of UV-visible spectrophotometry as a technique for screening anti-diabetic plants using GOX activity. The present study showed that *L. multiflora* is more active than *V. amygdalina* ($IC_{50}$: 1.36 ± 0.09 mg/mL Vs $IC_{50}$: 3.00 ± 0.54 mg/mL).

The Figure 7 shows the evolution of optical density as a function of glucose concentration.

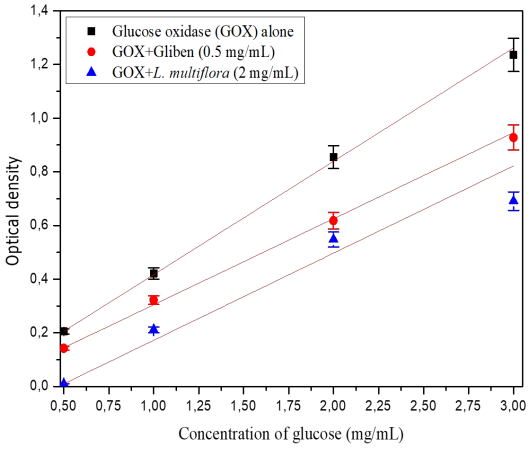


**Figure 7.** Effect of inhibitors on glucose oxidase activity *in vitro*

Figure 7 shows that Gliben and *L. multifora* reduce optical density, reflecting the inhibitory effect of these drugs on glucose oxidase *in vitro*. Thus, glucose oxidase inhibitory drugs have anti-glycemic properties (Njeri et al., 2017) .

The effect of *L. multiflora* on free glucose concentration as a function of time is given in Figure 8.

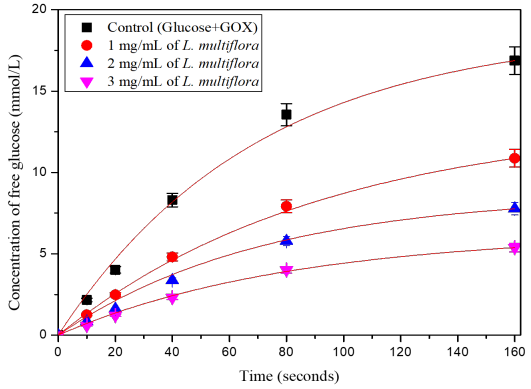


**Figure 8.** Effect of *L. multiflora* on free glucose concentration as a function of time

The Figure 8 shows that *L. multifora* has the effect of reducing the concentration of free glucose as a function of time. This hypoglycemic effect *in vitro* is dose-dependent.

The molar extinction coefficient of quinone imine, which is the oxidation product of the second substrate (chromogen), was determined by linear regression (Figure 10) and represents the slope of the line.

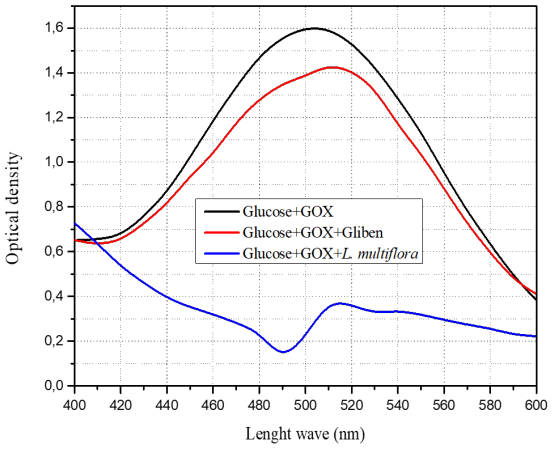


**Figure 9.** Glucose absorption spectrum (after enzymatic derivation) in the presence of Gliben (0.5 mg/mL) and *L. multiflora* (2 mg/mL)

Knowledge of the molar extinction coefficient allowed the Michaelis-Menten equations to be determined in the presence and absence of glucose oxidase inhibitors. After linearization according to Lineweaver and Burk, the kinetic parameters were calculated (Table 2).

Table 2 shows that Gliben (0.5 mg/mL) and *L. multiflora* (2 mg/mL) reduce both the rate of oxidation of glucose by glucose oxidase (catalytic power) and the affinity of this enzyme for its substrate (glucose) via biochemical mechanism so called mixed inhibition. The present study revealed that Gliben, *V. amygdalina*, and *L. multiflora* decrease the concentration of free

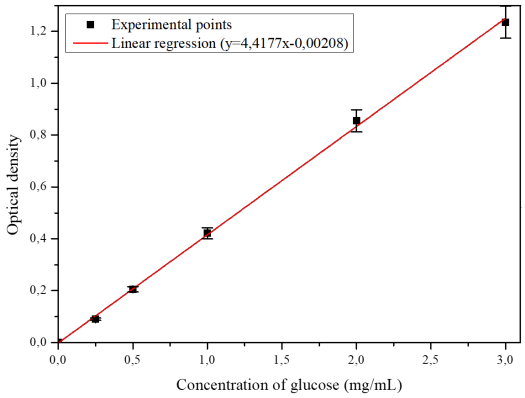


**Figure 10.** Calibration line for glucose in the presence of the enzyme reagent (Glucose oxidase 15 kUI/L, peroxidase 1,5 kUI/L, phenol 77 mmol/L, pH 7.0., Potassium phosphate buffer, temperature 25 °C, Wavelength: 505 nm)

**Table 2**
Mean values of enzyme kinetic parameters in the presence and absence of the inhibitors

| Glucose oxidase | Kinetic parameters | |
|---|---|---|
| | $v_{max}$ (mg*mL$^{-1}$*min$^{-1}$) | $K_M$ (mg/mL) |
| Absence of inhibitor | 2.86 ± 0.44 | 8.07 ± 1.96 |
| + Gliben (0.5 mg/mL) | 0.84 ± 0.11 | 15.11 ± 2.72 |
| + *L. multiflora* (2 mg/mL) | 1.72 ± 0.13 | 9.17 ± 1.56 |

($v_{max}$. maximal speed ; $K_M$. Michaelis-Menten constant)

glucose *in vitro*. This hypoglycemic effect *in vitro* is manifested by a decrease in light intensity (absorbance) at 505 nm. These data confirm the results of previous work on the anti-diabetic properties of Gliben and the tested plants, thus validating the GOX/HRP enzyme system as a biochemical model for the *in vitro* anti-diabetic screening of medicinal plants (Ibegbu et al., 2018). The inhibition of enzymatic oxidation of glucose *in vitro* (expressed as glucose sequestration by the enzyme-drug complex or as enzyme-glucose-drug complex) is dose-dependent (Sushil & Talambedu, 2012). Thus, if glucose was not sequestered as an ESI complex, a decrease in light intensity at 505 nm, the absorption wavelength of the chromogenic substrate in its oxidized form, would not be expected. This study shows that de Gliben, *V. amygdalina*, and *L. multiflora* inhibit GOX from converting glucose to gluconic acid by reducing the concentration of free glucose (hypoglycemic effect) *in vitro* in the form of a GOX-glucose-drug complex (Figure 10). As diabetes is a major public health problem, the population is increasingly turning to herbal medicines for its management (Tunga et al., 2020).

The inhibitors of GOX activity are secondary metabolites present in active plants including Lippia multiflora.

### 3.2. Molecular docking studies

To determine the binding affinities and key interactions between 1GAL and *L. multiflora* compounds, docking studies

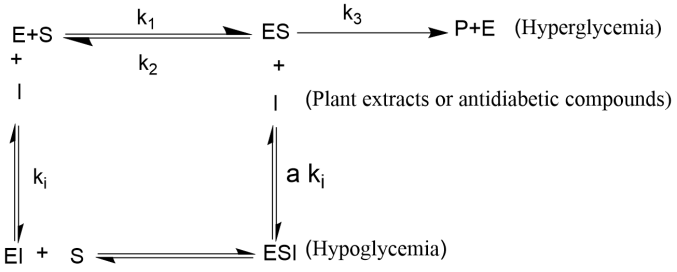


**Figure 11.** Proposed mechanism of action of anti-diabetic drugs *in vitro*

were performed using Autodock 4.2. The binding pocket of the target protein was decided using a grid size of 50 x 50 x 50 Å$^3$ with the help of an auto grid centered at x = 43.730, y = 12.702 and z = 59.371. Two important parameters were taken into account to select potential compounds from the given inputs: (i) the prediction of the binding energy of the best docking pose using the scores calculated by the Autodock scoring function and (ii) the details of the hydrogen bonds of the top ranked pose. The docking was run 10 times and average values for each compound were calculated. The summary of the docking information of the top ranked poses is presented in Table 3. To analyze the results, Glibenclamide was used reference molecule (positive control).

**Table 3**
Binding affinity energies of the tested phyto-compounds derived from *L. multiflora*

| Receptor pdb ID | Ligands | | Binding Affinity (ΔG in Kcal/mol) | Standard deviation |
|---|---|---|---|---|
| | Verbascoside | 8 | -10.1 | 0.0 |
| | Luteolin | 6 | -9.5 | 0.1 |
| | *β*-sitosterin | 3 | -8.3 | 0.0 |
| | **Glibenclamide** | **Reference** | -8.3 | 0.2 |
| | cycloeucalenol | 9 | -8.2 | 0.1 |
| 1GAL | Thymol | 2 | -7.1 | 0.1 |
| | 1,8-Cineaole | 1 | -6.7 | 0.0 |
| | a-terpineol | 7 | -6.6 | 0.0 |
| | Géraniale | 4 | -6.4 | 0.1 |
| | Linalool | 5 | -6 | 0.1 |
| | Nerol | 11 | -5.9 | 0.1 |

The Table 3 revealed that all the tested compounds form a thermodynamically stable complex with the GOX enzyme (1GAL). However, the best docked ligands are obtained with compounds **8** (-10.1±0.0 Kcal/mol), **6** (-9.5±0.1 Kcal/mol), **3** (-8.3±0.0 Kcal/mol), and **9** (-8.2±0.1 Kcal/mol). These results confirm the enzymatic inhibition mechanism proposed to explain the anti-diabetic activity of *Lippia multiflora*. However, it should be noted that only compounds **8** and **6** involve hydrogen bonds in the formation of the complex with the enzyme 1GAL: compound **8** forms five hydrogen bonds (**ASN514, ASP424,** ARG95, TYP68, LEU65) while compound **6** forms two hydrogen bonds (ASN514 and SER422).

However, no H-bonding interaction occurs in the complex that involves ligands **9** and **3** despite their high binding energy (-8.2±0.1 Kcal/mol and -8.3±0.0 Kcal/mol respectively). Similar results were also previously reported (Kasende et al., 2017; Mpiana et al., 2020). Indeed, several forces contribute to the stability of a complex between a receptor and a ligand, among them dispersion forces, mainly in the stacking interactions between aromatic systems, and van der Walls interactions (Scheiner, 2020) . The stabilization of complexes is strengthened by other interactions ($\pi$-cation, $\pi$-alkyl, $\pi$-sigma) as well (Matondo et al., 2021).

The results obtained in this study show that the GOX enzyme is inhibited by medicinal plant extracts according to the same mechanism of action as the enzymatic models traditionally used to evaluate the anti-diabetic activity of plants (Yusaka & Kuniyo, 2015). Thus, this enzyme can validly constitute a very good model for *in vitro* studies. The UV-visible spectroscopy used in this study is a physical method that allows high accuracy, and the standard spectrophotometer is an easy-to-handle device. Its relatively high purchase cost is compensated by the use of less expensive consumables. The use of this enzymatic test instead of animal models (mice, rats, guinea pigs) makes the method less expensive and quick for large-scale screening.

Indeed, diabetes is a real public health problem. According to the World Health Organization, about 80% of rural populations living in developing countries rely on Traditional Medicine to meet their health care needs. Hence, developing a simple but robust method for the scientific validation of herbs used for the management of diabetes may help to tackle the disease (Ngbolua, Rafatro, et al., 2011; Ngbolua, Rakotoarimanana, et al., 2011).

The results of the *in vitro* and *in silico* anti-diabetic activity using GOX enzyme as a model were validated by the *in vivo* study. Indeed, the *in vivo* study showed that *L. multiflora* reduced blood glucose levels very significantly in treated mice as shown in Figure 13.

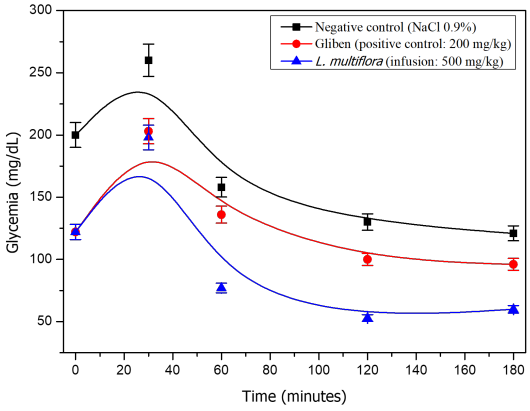


**Figure 13.** Effect of *L. multiflora* on glycemia rate in mice

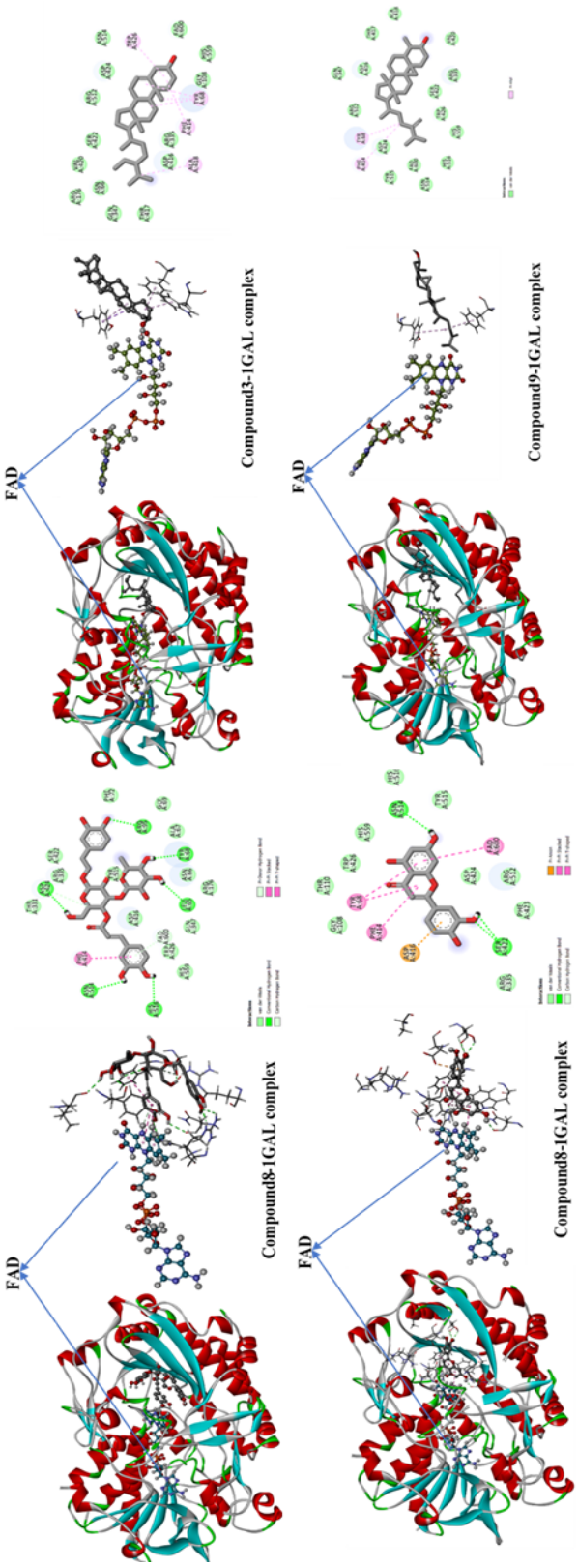


**Figure 12.** Complexes of the fourbest-docked ligands with GOX enzyme

The enzyme glucose oxidase plays an important role in basic research as a biosensor (Hassan et al., 2021; Hyo et al., 2020; Stefano et al., 2011). In this study, this enzyme was used to develop a UV-visible spectrophotometric method for screening plants with anti-diabetic properties.

## 4. CONCLUSION AND SUGGESTIONS

The present study aimed to create hyperglycemia *in vitro* in order to exploit biochemical pathways involving glucose oxidase as an alternative to screen by UV-visible spectrophotometry medicinal plants for their anti-diabetic activity. The *in vitro* and *in silico* results revealed that glucose oxidase can serve as a marker enzyme for *in vitro* anti-diabetic activity evaluation of medicinal plants. GOX displays similar pharmacological sensitivity such as standardized glucosidic metabolism enzyme alpha amylase towards anti-diabetic drugs/plants (Glibenclamide, *Lippia multiflora*, and Vernonia amygdalina) through mixed inhibition. We suggest the use of this *in vitro* technique for the scientific validation of the bioactivity of plants species traditionally used for the management of diabetes.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Conceptualization: K.N.N., V.M. and P.T.M.; Methodology: C.M.A. and A.T.M.; validation, D.S.T.T., J.K.T., A.M. and K.N.N.; Formal analysis: C.I.L., B.G.Z. and L.M.E.; Investigation: C.M.A. and A.T.M.; Resources: D.T.D.; Data curation: J.K.T., A.M. and D.S.T.T; Writing-original draft preparation: C.M.A., A.T.M.; Writing-review and editing, J.T.K. and K.N.N; Visualization: C.M.A. and A.T.M.; Supervision: K.N.N.; Project administration: P.T.P. and V.M. All authors have read and agreed to the published version of the manuscript.

## REFERENCES


Anshika, N.S., Meghna, M.B., Neeti, S., 2017. Structure based docking studies towards exploring the potential anti-androgen activity of selected phytochemicals against Prostate Cancer. Scientific Reports. 7, 1955–1955. https://doi.org/10.1038/s41598-017-02023-5

Arthur, H., Joubert, E., Beer, D., Malherbe, D., Witthuhn, C.J., C, R., 2011. Phenylethanoid glycosides as major antioxidants in Lippia multiflora herbal infusion and their stability during steam pasteurization of plant material. Food Chemistry. 127(2), 581–588. https://doi.org/10.1016/j.foodchem.2011.01.044

Avlessi, F., Alitonou, G., Sohounhloue, D.K., Menut, C., Bessiere, J.M., 2011. Aromatic plants of tropical West Africa. Part XIV:Chemical and biological investigation of the essential oil of Lippia multiflora Mold. from Benin. Journal of Essential Oil Research. 17, 405–407. https://doi.org/10.1080/10412905.2005.9698944

Bagora, B., Bassole, I., Gnoula, C., Nebie, R., Yonli, A., Morel, L., Figueredo, G., Nikiema, J.B., Lobaccaro, J.-M., Simpore, J., 2014. Chemical composition, antioxidant, anti-inflammatory and anti-proliferative activities of essential oils of plants from Burkina Faso. PLoS ONE. 9(3). https://doi.org/10.1371/journal.pone.0092122

Bassole, I., Ouattara, A.S., Nebieb, R., Ouattara, C., Kabore, Z.I., Traore, S.A., 2003. Chemical composition and antibacterial activities of essential oils of Lippia chevalieri and Lippia multiflora from Burkina Faso. Phytochemistry. 62, 209–212. https://doi.org/10.1016/s0031-9422(02)00477-6

Hassan, M.H., Vyas, C., Grieve, B., Bartolo, P., 2021. Recent Advances in Enzymatic and Non-Enzymatic Electrochemical Glucose Sensing. Sensors. 21, 4672–4672. https://doi.org/10.3390/s21144672

Hyo, Y.K., Ki, S.P., Hyun, G.P., 2020. Glucose oxidase-like activity of cerium oxide nanoparticles: use for personal glucose meter-based label-free target DNA detection. Theranostics. 10(10), 4507–4514. https://doi.org/10.7150/thno.41484

Ibegbu, M.D., Nnaemeka, E.J., Ikele, I.T., Nwachukwu, D.C., 2018. Anti-hyperglycaemic and anti-hyperlipidemic effect of aqueous leaf extract of Vernonia amygdalina in Wistar rats. African Journal of Pharmacy and Pharmacology. 12, 231–239. https://doi.org/10.5897/AJPP2018.4917

Kasende, O.E., Matondo, A., Muya, J.T., Scheiner, S., 2017. Interactions between temozolomide and guanine and its S and Se-substituted analogues. International Journal of Quantum Chemistry. 117, 157–169. https://doi.org/10.1002/qua.25294

Khang, W.O., Hsu, A., Lixia, S., Dejian, H., Kwong, B., Huat, T., 2011. Polyphenols-rich Vernonia amygdalina shows anti-diabetic effects in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology. 133, 598–607. https://doi.org/10.1016/j.jep.2010.10.046

Matondo, A., Kilembe, J.T., Ngoyi, E.M., Kabengele, C.N., Kasiama, G.N., Lengbiye, E.M., Mbadiko, C.M., Inkoto, C.L., Bongo, G.N., Gbolo, B.Z., Falanga, C.M., Mwanangombo, D.T., Opota, D.O., Tshibangu, D., Tshilanda, D.D., Ngbolua, D.D., Mpiana, P.T., 2021. Oleanolic Acid, Ursolic Acid and Apigenin from Ocimum basilicum as Potential Inhibitors of the SARS-CoV-2 Main Protease: A Molecular docking study. International Journal of Pathogen Research. 6(2), 1–16. https://doi.org/10.9734/ijpr/2021/v6i230156

Mgbeje, B., Terungwa, A.K., Ugoanyanwu, F.O., Ebong, P.E., 2016. Effect of phytochemical fractions of Vernonia amygdalina on liver

of STZ induced diabetic male Wistar Rats. International Journal of Current Microbiology and Applied Sciences. 5(11), 656–667. http://dx.doi.org/10.20546/ijcmas.2016.511.077

Mpiana, P.T., Ngbolua, K.N., Tshibangu, D., Kilembe, J., Gbolob, B.Z., Mwanangombo, D.T., Inkoto, L.C., Lengbiye, M.E., Mbadiko, M.C., Matondo, A., Bongo, N.G., Tshilanda, D.D., 2020. Identification of potential inhibitors of SARS-CoV-2 main protease from Aloe vera compounds: A molecular docking study. Chemical Physics Letters. 754, 137751–137751. https://doi.org/10.1016/j.cplett.2020.137751

Ngbolua, K.N., Rafatro, H., Rakotoarimanana, H., Urverg, R.S., Mudogo, V., Mpiana, P.T., Tshibangu, D., 2011. Pharmacological screening of some traditionally-used antimalarial plants from the Democratic Republic of Congo compared to its ecological taxonomic equivalence in Madagascar. International Journal of Biological and Chemical Sciences. 5(5), 1797–1804. https://doi.org/10.4314/ijbcs.v5i5.3

Ngbolua, K.N., Rakotoarimanana, H., Rafatro, H., Urverg, R.S., Mudogo, V., Mpiana, P.T., Tshibangu, D., 2011. Comparative antimalarial and cytotoxic activities of two Vernonia species: V. amygdalina from the Democratic Republic of Congo and V. cinerea subsp vialis endemic to Madagascar. International Journal of Biological and Chemical Sciences. 5(1), 345–353. https://doi.org/10.4314/ijbcs.v5i1.68111

Njeri, L.K., Eliud, N., Orinda, O.G., 2017. In vivo oral and intraperitoneal administration of extract from Vernonia Lasiopus. Medicinal and Aromatic Plants,. , pp. 6–6. http://dx.doi.org/10.4172/2167-0412.1000308

Scheiner, S., 2020. Understanding noncovalent bonds and their controlling forces. The Journal of Chemical Physics. 153, 140901. https://doi.org/10.1063/5.0026168

Shapovalov, M.S., Dunbrack, R.L., 2011. A smoothed backbone-dependent rotamer library for proteins derived from adaptive kernel density estimates and regressions. Structure. 19, 844–858. https://doi.org/10.1016/j.str.2011.03.019

Soro, L.C., Munier, S., Pelissier, Y., Grosmaire, L., Yada, R., Kitts, D., Atchibri, A., Guzman, C., Boudard, F., Menut, C., Robinson, J.C., Poucheret, P., 2016. Influence of geography, seasons and pedology on the chemical composition and anti-inflammatory activities of essential oils from Lippia multiflora Mold. leaves. Journal of Ethnopharmacology. 194, 587–594. https://doi.org/10.1016/j.jep.2016.10.047

Stefano, F., Katsuhiro, K., Koji, S., 2011. Review of Glucose Oxidases and Glucose Dehydrogenases: A Bird's Eye View of Glucose Sensing Enzymes. Journal of Diabetes Science and Technology. 5(5), 1068–1076. https://doi.org/10.1177/193229681100500507

Sushil, K.M., Talambedu, U., 2012. An in vitro new vista to identify hypoglycemic activity. International Journal of Fundamental & Applied Sciences. 1(2), 27–29.

Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry. 31, 455–461. https://doi.org/10.1002/jcc.21334

Tunga, K.A., Kilembe, J.T., Matondo, A., 2020. Computational analysis by molecular docking of thirty alkaloid compounds from medicinal plants as potent inhibitors of SARS-CoV-2 main protease. Journal of Computational Chemistry & Molecular Modeling. 4(4), 487–503. http://www.dx.doi.org/10.25177/JCCMM.4.4.RA.10699

Ugoanyanwu, F.O., Mgbeje, B., Igile, G.O., Ebong, P.E., 2015. The flavonoid-rich fraction of Vernonia amygdalina leaf extract reversed diabetes-induced hyperglycemia and pancreatic beta cell damage in albino Wistar Rats. World Journal of Pharmacy and Pharmaceutical Sciences. 4(10), 1788–1802.

Vidya, M., Madhu, S.S., Batoul, F., Mastan, M., Afroz, A., Ganj, P.N., 2019. Molecular docking of angiogenesis target protein HIF-1$\alpha$ and genistein in breast cancer. Gene. 701, 169–172. https://doi.org/10.1016/j.gene.2019.03.062

Yusaka, N., Kuniyo, I., 2015. Inhibition of porcine pancreas alpha amylase by chlorogenic acids from green coffee beans and cinnamic derivatives. Coffee in health and disease prevention, 757–763. http://dx.doi.org/10.1016/B978-0-12-409517-5.00084-X